

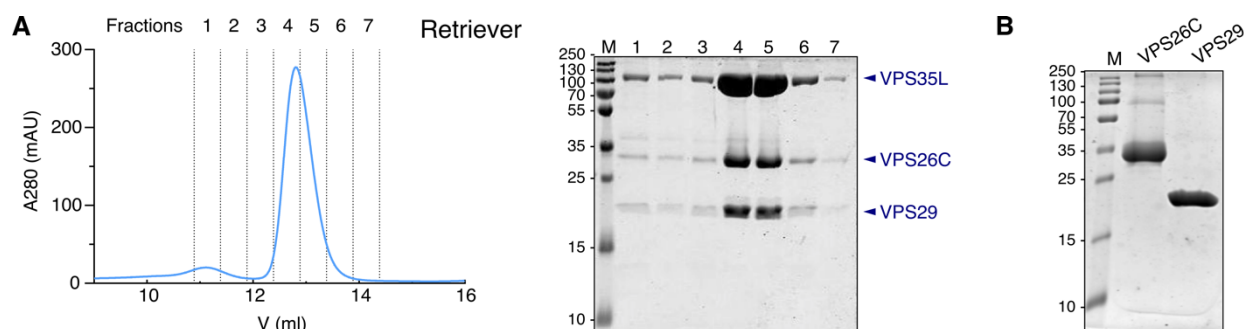
Selective cargo and membrane recognition by SNX17 regulates its interaction with Retriever

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Appendix

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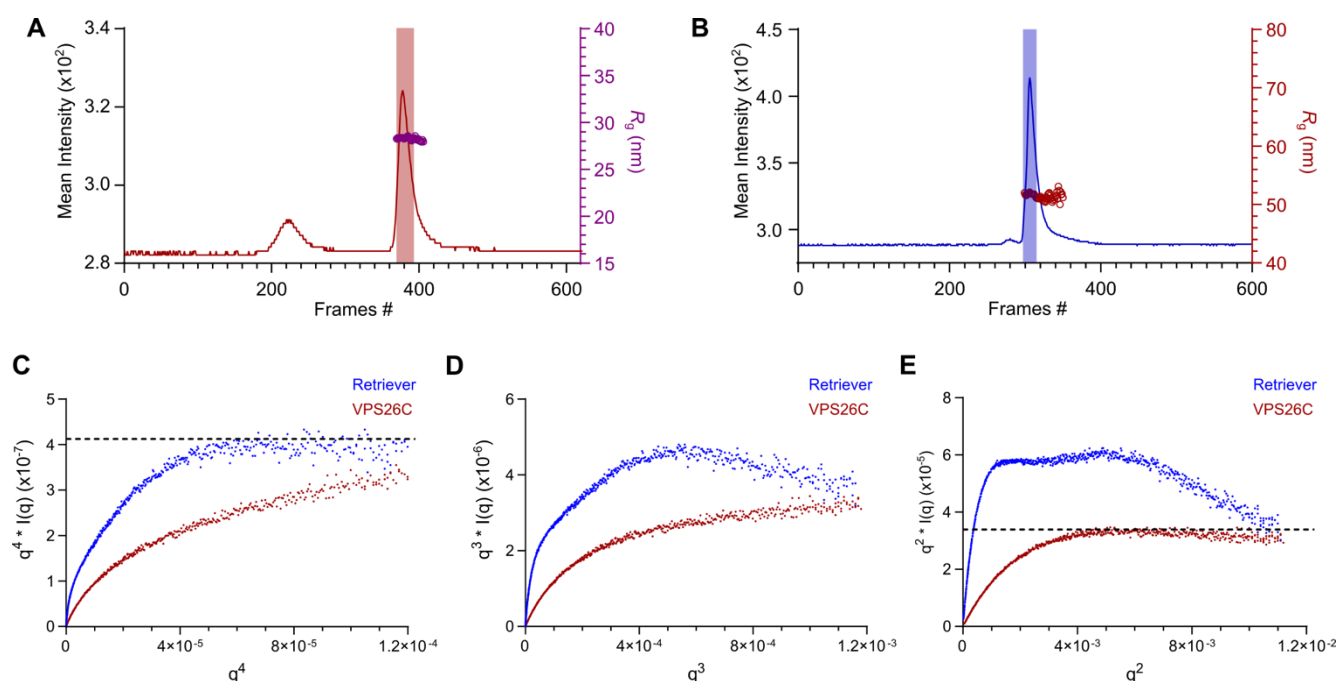
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Appendix Figure S1. Purification of the Retriever complex and its individual subunits.

(A) Coomassie-stained SDS-PAGE analysis of the purified Retriever complex composed of VPS35L, VPS26C, and VPS29 (right) from the fractions obtained in a Superdex 200 Increase 10/300 gel filtration chromatography (left). Two peaks containing the three proteins are observed, which correspond to the dimeric (first peak) and monomeric (second peak) forms of the complex. M, protein marker.

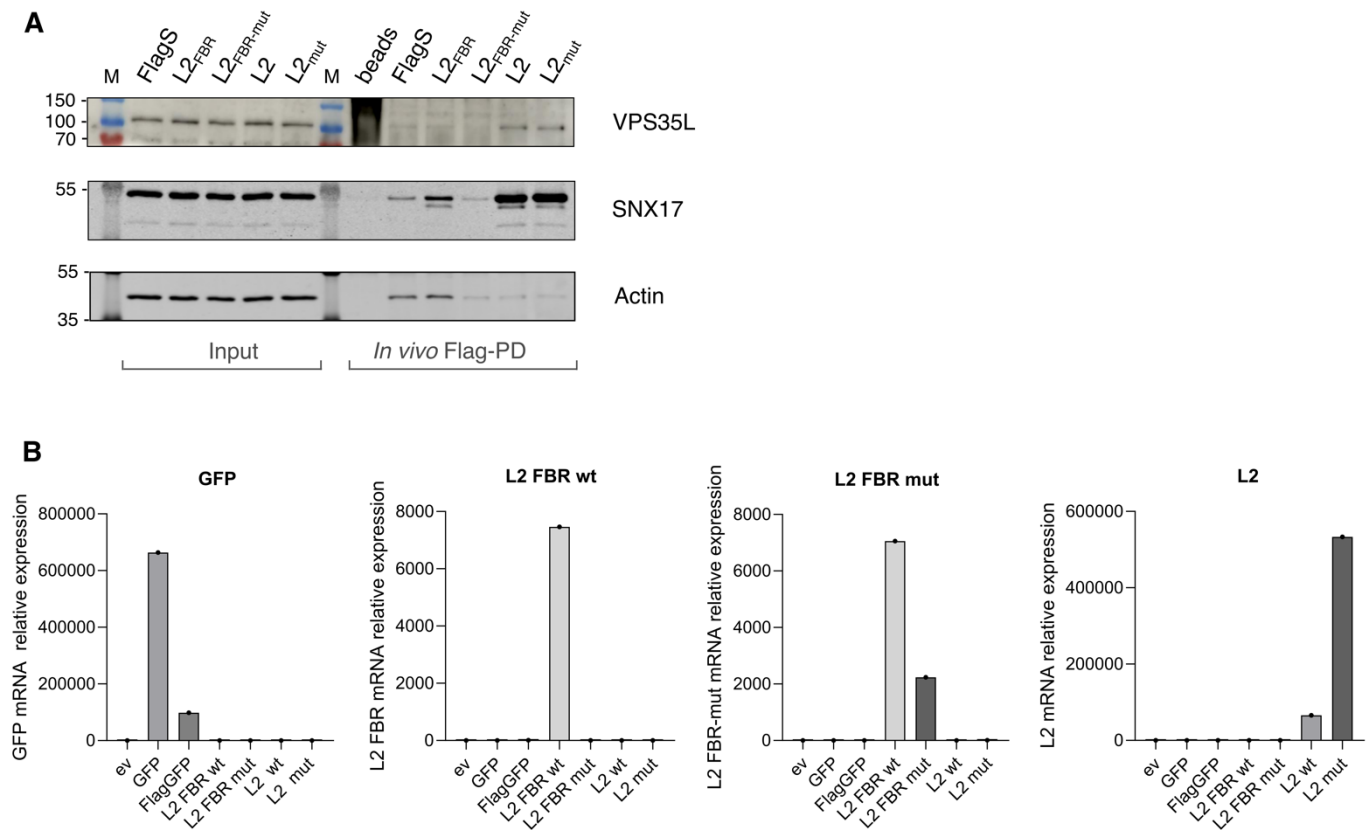
(B) Coomassie-stained SDS-PAGE gel of purified VPS26C and VPS29 subunits of the Retriever complex.



Appendix Figure S2. SAXS analysis of VPS26C and Retriever.

(A-B) SEC-SAXS elution profiles of VPS26C (A) and the Retriever complex (B). Frames are plotted as a function of the mean intensity. The selected range of frames to average the data, marked with a shaded area, exhibited a flat distribution of R_g values, indicating that the sample was monodisperse.

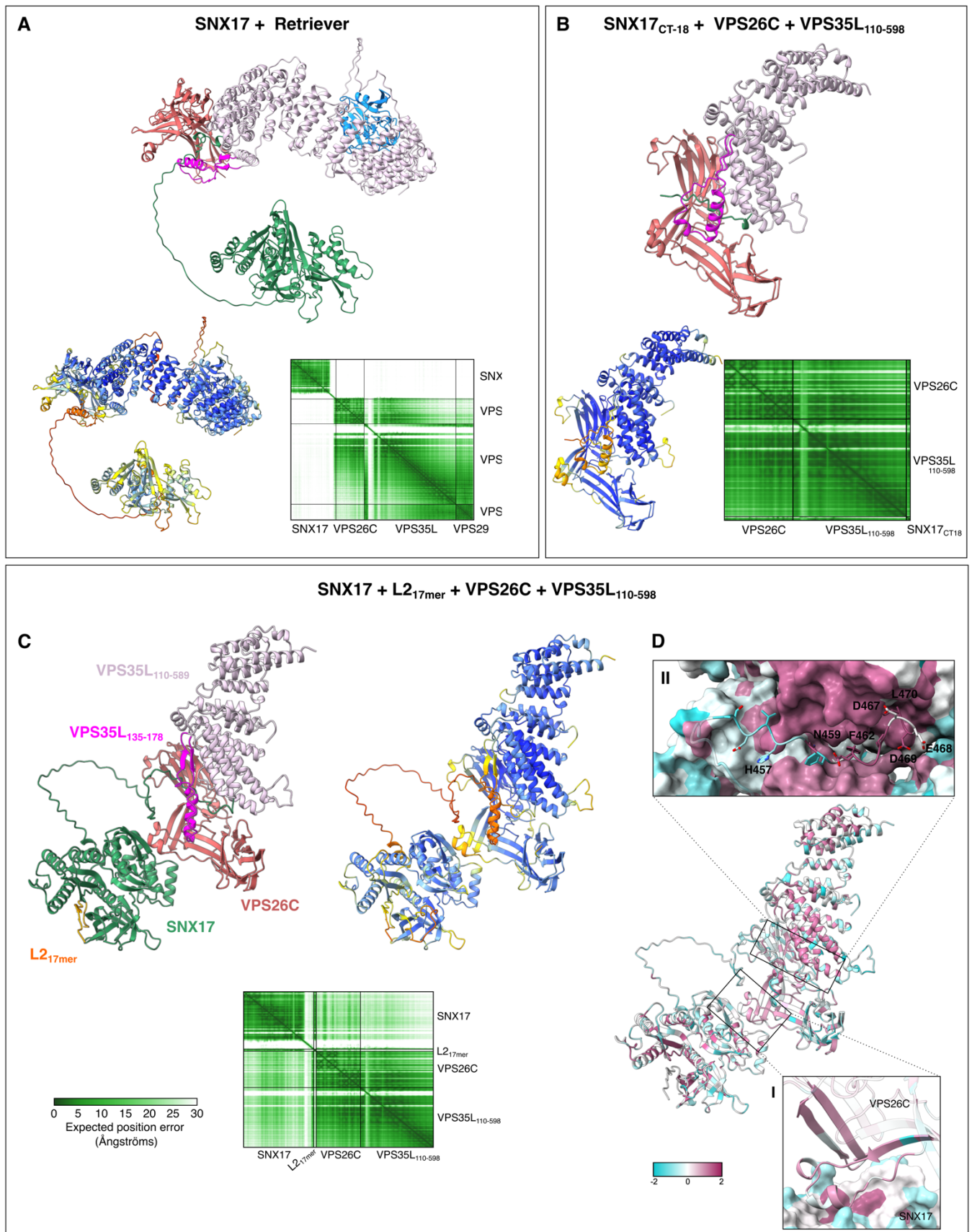
(C-E) Porod-Debye plots illustrating the highly flexible nature of VPS26C. Comparison of the changes in the Porod-Debye regions between $q^4 I(q)$ vs. q^4 (C), $q^3 I(q)$ vs. q^3 (D), $q^2 I(q)$ vs. q^2 (E) plots for both VPS26C and the Retriever complex. A discontinuous line has been drawn to indicate the asymptotic region.



Appendix Figure S3. Detection of VPS35L and SNX17 following Flag pull-down of overexpressed L2 proteins.

(A) HEK293T cells expressing either the wild-type or the mutant version (lacking the NPxY motif) of the Flag-tagged L2_{FBR} or L2_{FL} protein were lysed. The supernatants were incubated with M2 anti-Flag beads, followed by pull-down and immunoblot analysis using specific antibodies against VPS35L and SNX17. Cells expressing the empty vector served as a negative pull-down control. Actin was used as loading control for the input samples. Due to non-specific detection by the anti-Flag antibody, mRNA expression levels of the transfected plasmids were quantified by RT-qPCR.

(B) Relative transcript expression levels of the GFP vector control, wild-type, and mutant versions (lacking the NPxY motif) of the L2_{FBR} or L2_{FL} proteins were measured by quantitative reverse transcription PCR (RT-qPCR, n=1).



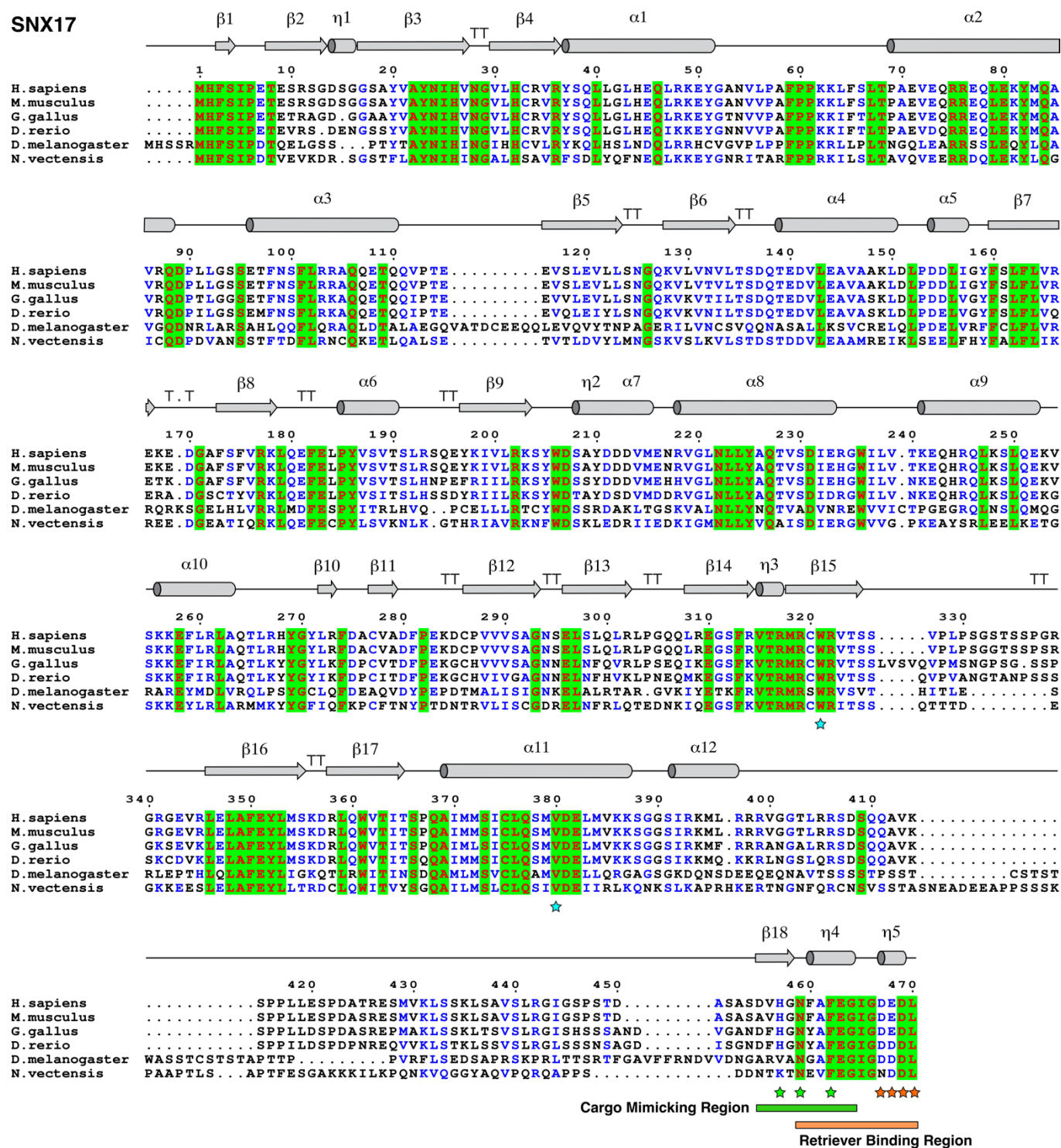
Appendix Figure S4. AlphaFold2-multimer prediction of Retriever-SNX17 interaction.

(A-C) Two cartoon representations of AF2-multimer predicted structures of SNX17:Retriever (A), SNX17_{CT-18}:VPS26C:VPS35L₁₁₀₋₅₉₈ (B), and SNX17:L2_{17mer}:VPS26C:VPS35L₁₁₀₋₅₉₈ (C). In the first representation, SNX17 is colored green, L2 orange, VPS26C red and VPS35L₁₁₀₋₅₉₈ pink. The hinge region (VPS35L₁₃₅₋₁₇₈) is highlighted in magenta. In the second representation, the models are colored according to the pLDDT confidence score (blue, very high confidence pLDDT ≥ 90 ; cyan, high confidence $70 \leq \text{pLDDT}$

< 90; yellow, low confidence $50 \leq \text{pLDDT} < 70$; orange, very low confidence $\text{pLDDT} < 50$). The graph at the bottom displays the corresponding PAE (predicted aligned error) plot. The color key of the PAE plot in panel C applies to panels A and B.

(D) Depiction of the complex SNX17:L2_{17mer}:VPS26C:VPS35L₁₁₀₋₅₉₈ in cartoon, colored according to the evolutionary conservation calculated by AL2CO (Pei & Grishin, 2001) implemented in ChimeraX using the alignments of SNX17 ([Appendix Fig. S5](#)), VPS26C ([Appendix Fig. S6](#)) and VPS35L ([Appendix Fig. S7](#)), with blue-to-purple color scale indicating variable-to-conserved positions. Zoomed-in view I focused on the interaction interface between VPS26C (in cartoon) and SNX17 (as surface). Zoomed-in view II shows the conservation of the SNX17_{CT-18} binding pocket, with SNX17 residues as sticks, VPS26C and VPS35L₁₁₀₋₅₉₈ as surface. The orientation is the same as in [Fig. 3B](#). To facilitate visualization of the binding pocket the residues 135-178 of VPS35L were omitted.

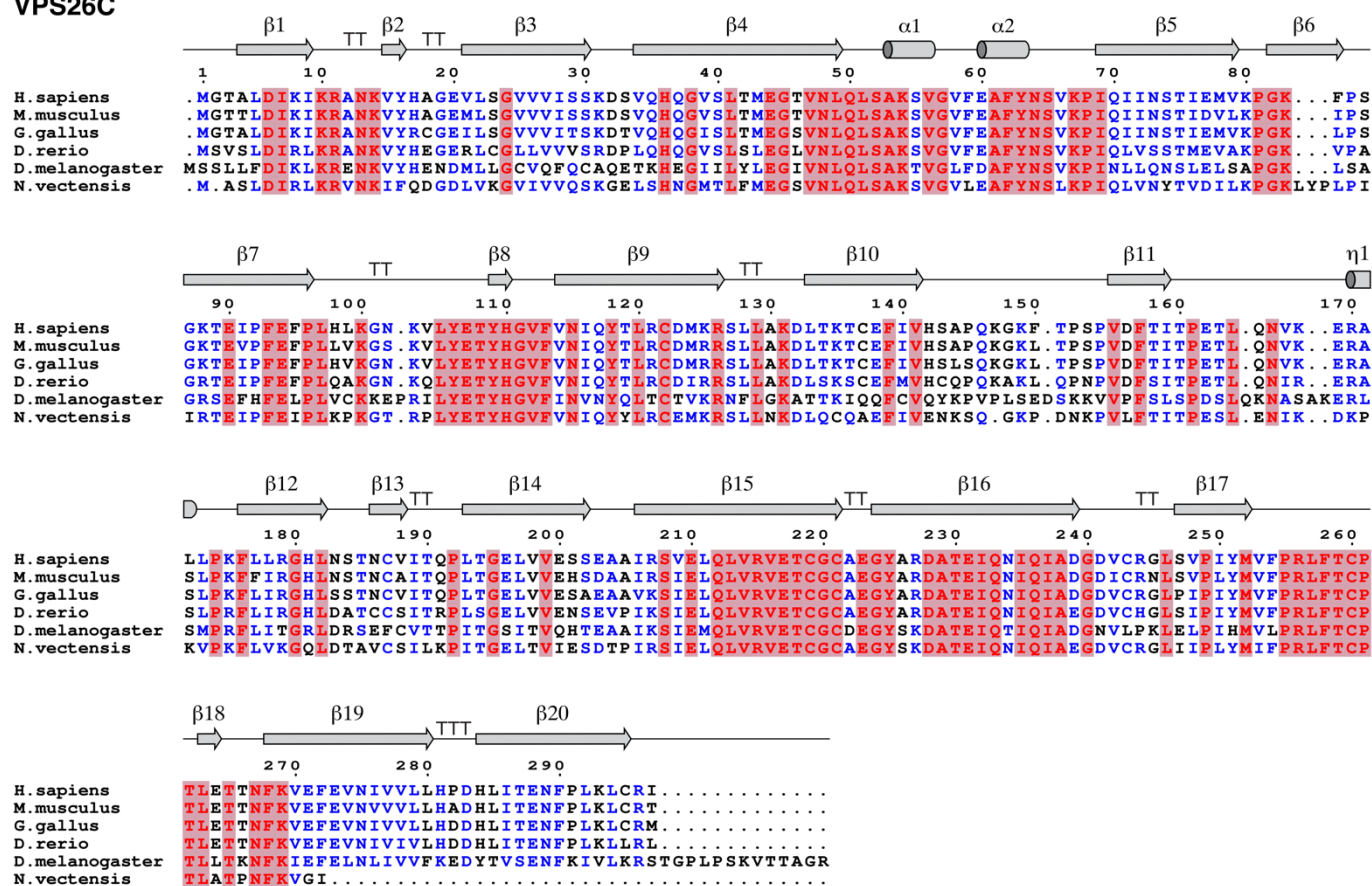
SNX17



Appendix Figure S5. Conservation of contact residues across SNX17 orthologs.

Structure-based sequence alignment of human SNX17 and orthologs generated with the PROMALS3D server and plotted with ESPrnt 3. The UniProt accession numbers of the AF2 models used in the sequence alignment are as follows: *Homo sapiens* (Q15036), *Mus musculus* (Q8BVL3), *Gallus gallus* (A0A1D5NVF3), *Danio rerio* (Q5RID7), *Drosophila melanogaster* (Q9VL28), and *Nematostella vectensis* (A7SGL2). The numbering of the residues and secondary structure annotations refer to SNX17 from *Homo sapiens*. α refers to alpha-helices and h to 3₁₀ helices (both represented as cylinders). β refers to beta-strands (represented as arrows) and TT to strict b-turns. Invariant residues are colored red on a green background, and conserved residues are in blue. Mutated residues implicated in the autoinhibition mechanism are marked with cyan stars (for the cargo-binding pocket) or green stars (for the cargo-mimicking region). Mutations within the Retriever-binding region are marked with orange stars.

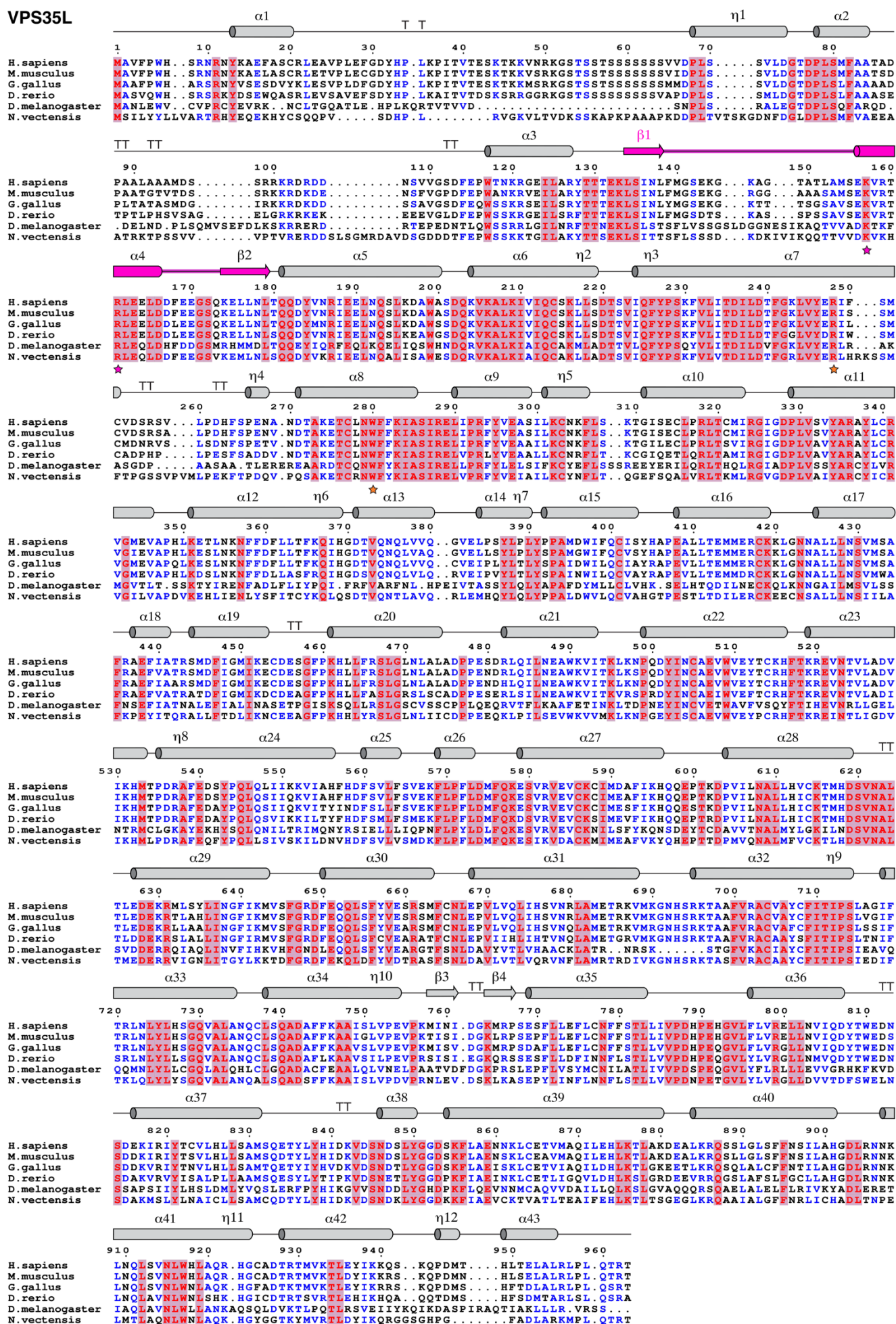
VPS26C



Appendix Figure S6. Conservation of contact residues across VPS26C orthologs.

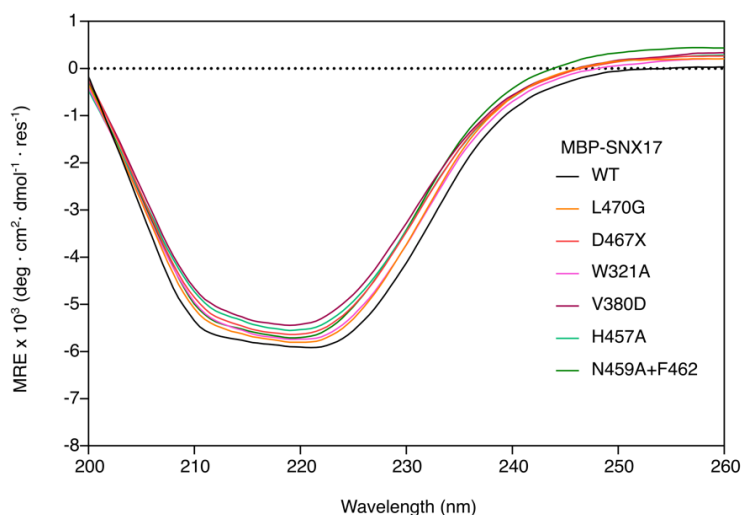
Structure-based sequence alignment of human VPS26C and orthologs. The alignment was generated as detailed in Appendix Fig. S5. The color code is the same as in Appendix Fig. S5, except for invariant residues, which are highlighted with a red background. The residue numbering and the secondary structure elements shown above the alignment correspond to human VPS26C. The UniProt accession numbers of the AF2 models used in the alignment are as follows: *Homo sapiens* (O14972), *Mus musculus* (O35075), *Gallus gallus* (E1BS11), *Danio rerio* (Q6DHL2), *Drosophila melanogaster* (Q9VPC3), and *Nematostella vectensis* (A7S8I4).

VPS35L

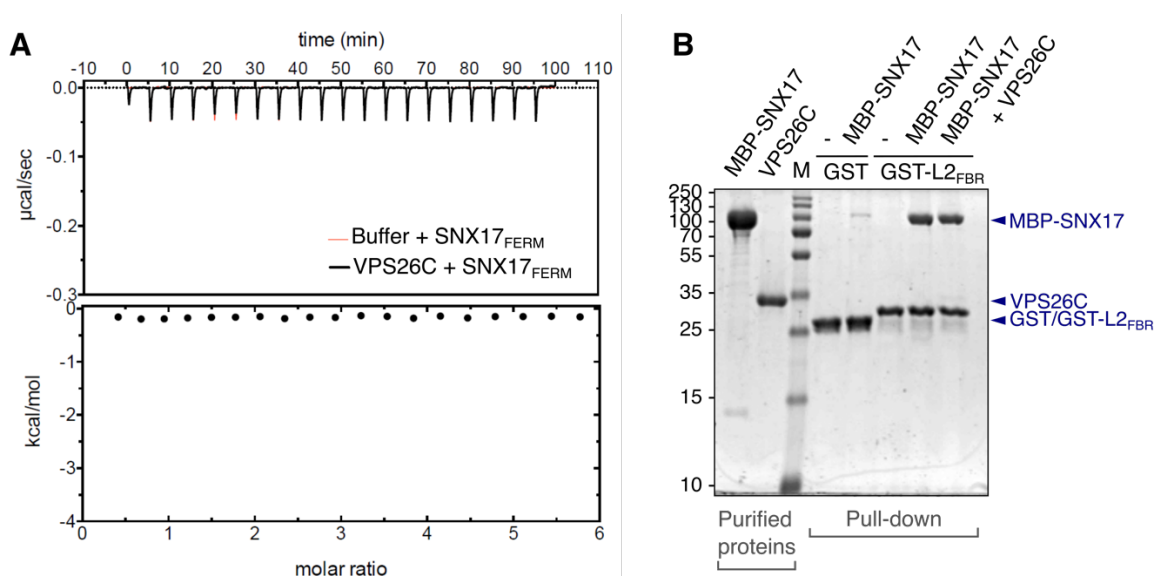


Appendix Figure S7. Conservation of contact residues across VPS35L orthologs.

Structure-based sequence alignment of human VPS35L with its orthologs. The alignment generation process and the color-coding scheme are the same as those described in [Appendix Fig. S5](#), except for invariant residues, which are highlighted with a pink background. The residue numbering and the secondary structure elements shown above the alignment correspond to human VPS35L. The UniProt accession numbers of the AF2 models used in the alignment are as follows: *Homo sapiens* (Q7Z3J2), *Mus musculus* (Q8BWQ6), *Gallus gallus* (A0A1D5PY39), *Danio rerio* (A4VCH4), *Drosophila melanogaster* (Q9VHM2), and *Nematostella vectensis* (XP_048575353). AF2 models were downloaded from the AlphaFold Protein Structure Database, with the exception of the model for *Nematostella vectensis*, which was obtained using ColabFold. The secondary structure elements of the hinge region are colored in magenta. Residues mutated in this work located in the SNX17 L470 binding pocket are marked with orange stars (R248 and W280), and those within the hinge region are marked with magenta stars (K157 and R161).

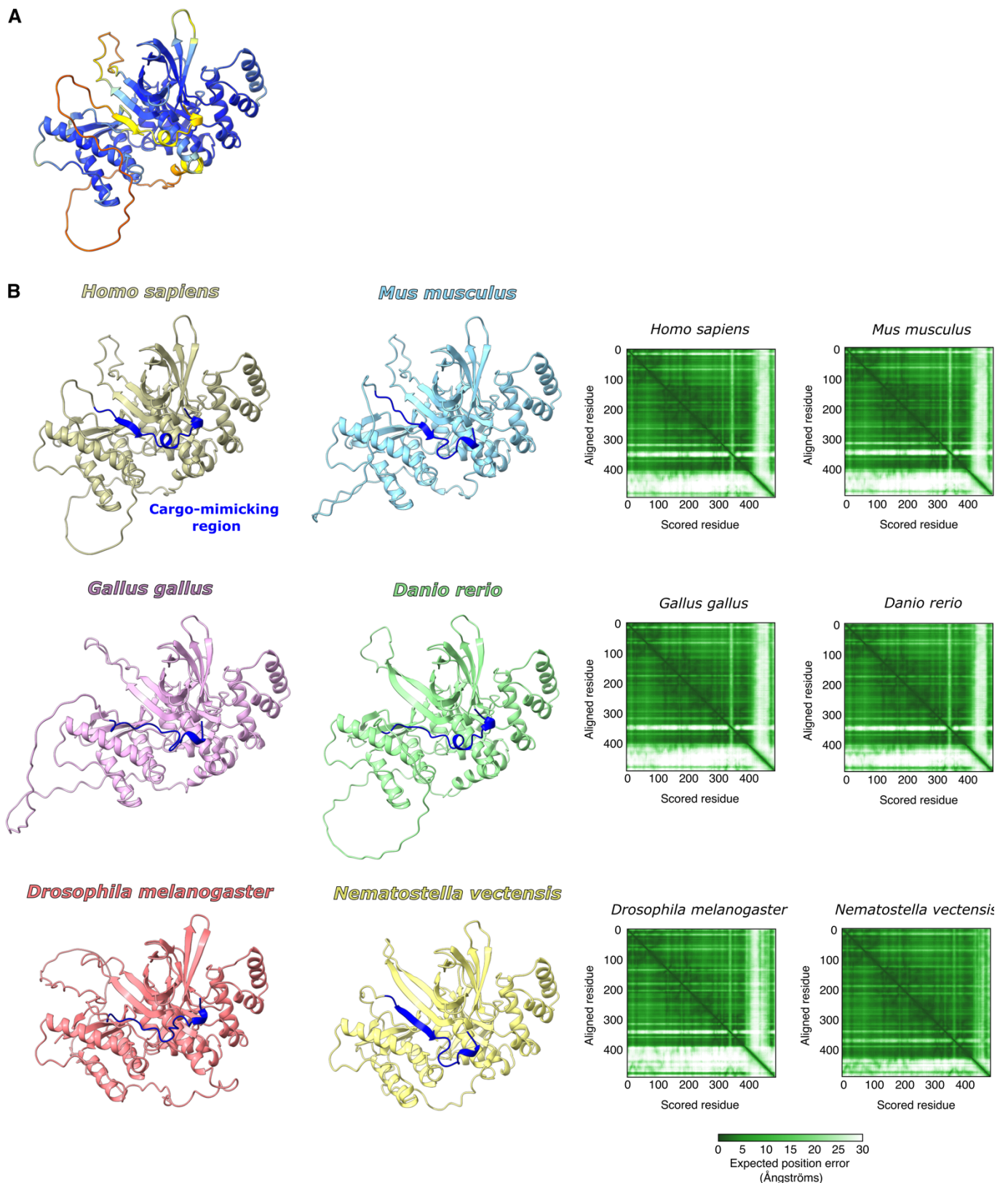
**Appendix Figure S8. Conformational properties of MBP-SNX17 mutants.**

Far-UV CD spectra of WT MBP-SNX17 and its mutants used in [Figs. 3](#) and [4](#). MRE, mean residual ellipticity.

**Appendix Figure S9. Analysis of the interaction between VPS26C and SNX17.**

(A) ITC assay of 30 μ M VPS26C and 300 μ M SNX17_{FERM-CT}. No significant interaction was detected, with values comparable to the negative control.

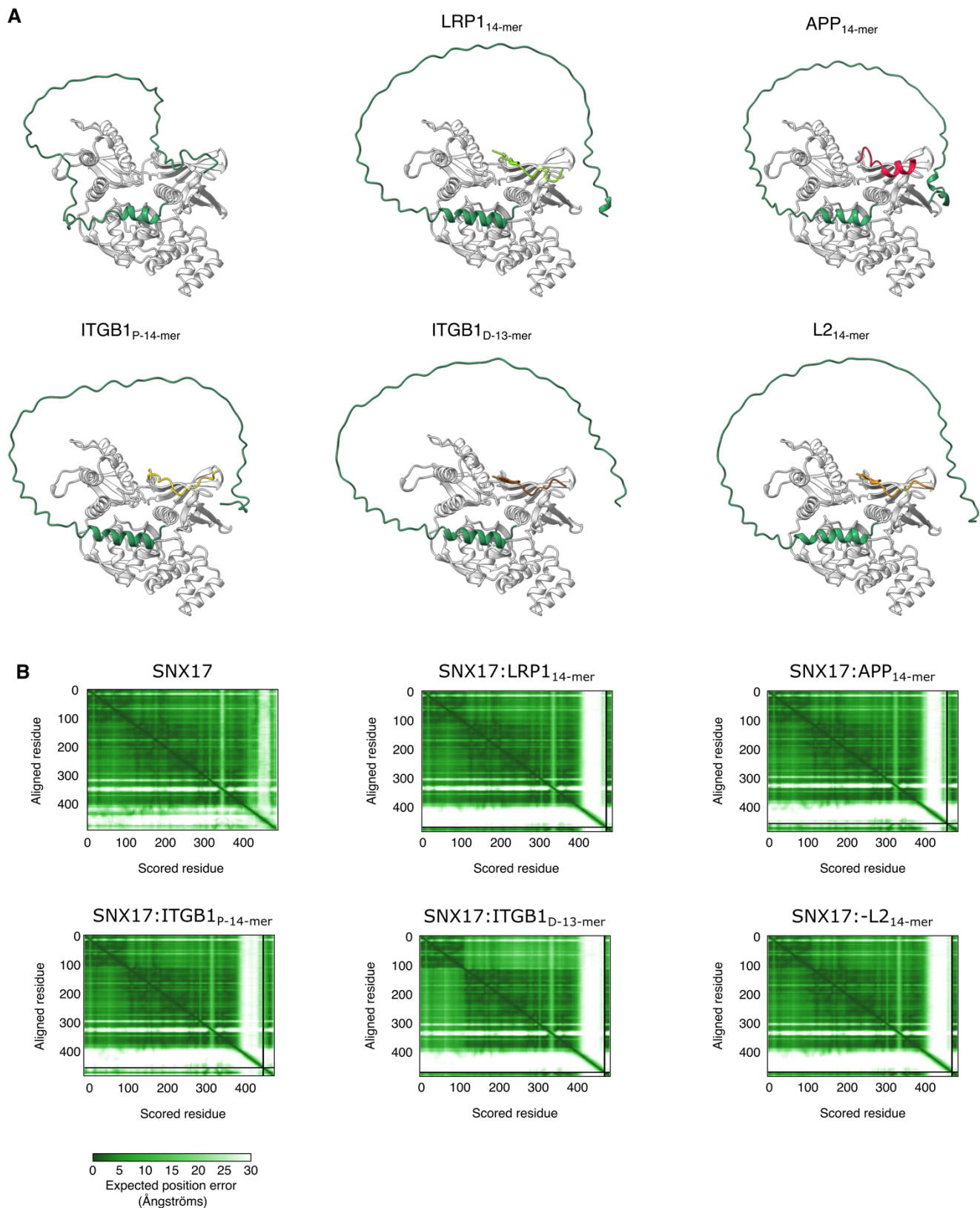
(B) VPS26C was incubated with MBP-SNX17 in the presence of GST-L2_{FBR} in GST pull-down assays. Non-fused GST protein was used as a negative control. Purified proteins and pull-down samples were separated by SDS-PAGE and visualized by Coomassie Blue staining. M, protein marker.



Appendix Figure S10. Conservation of the autoinhibitory mechanism across SNX17 orthologs.

(A) Cartoon representation of the AF2 model for human SNX17 (UniProt Q15036), colored by pLDDT confidence score. The prediction confidence color-coding is the same as in [Appendix Fig. S4](#).

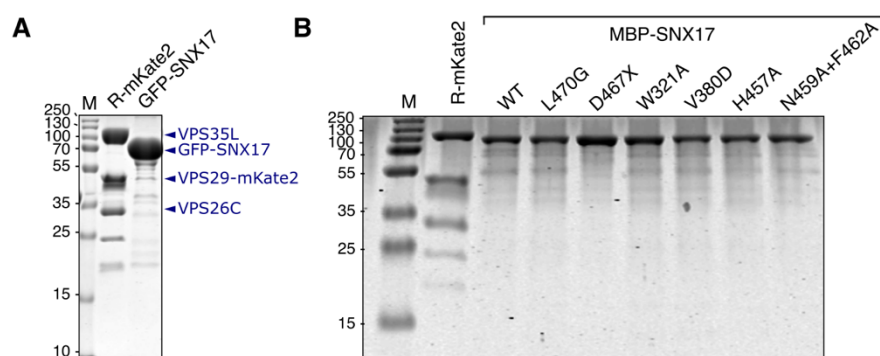
(B) AF2 models of human SNX17 and orthologs with the corresponding PAE plots. The cargo-mimicking region (from 453 to 470 in the human sequence) is highlighted in dark blue. Models were downloaded from the AlphaFold Protein Structure Database, with the exception of the *Nematostella vectensis* model, which was obtained using ColabFold. The UniProt accession numbers for these proteins are detailed in [Appendix Fig. S5](#).



Appendix Figure S11. Assessment of the disruption of SNX17 autoinhibited conformation through cargo binding using AF2-multimer modeling.

(A) AF2-multimer prediction of the complex between SNX17 (grey) and the cargo peptides used in fluorescence anisotropy assays: LRP1_{14-mer} (light green), APP_{14-mer} (red), ITGB1_{P-14-mer} (yellow), ITGB1_{D-13-mer} (brown), and L2_{14-mer} (orange). Note the displacement of the CT region (green) of SNX17 when cargo peptides are present.

(B) PAE plots of the models shown in A.



Appendix Figure S12. Purified proteins used for studying SNX17 and Retriever interaction with membranes using GUVs. Coomassie stained SDS-PAGE gels showing the purified proteins Retriever-mKate2 and GFP-SNX17 (**A**), as well as MBP-SNX17 wild-type and mutants (**B**), used for confocal microscopy assays.

Appendix Table S1. SAXS

	VPS26C	Retriever
Data collection parameters		
Instrument	B21 (Diamond Light Source, United Kingdom)	B21 (Diamond Light Source, United Kingdom)
Buffer	50 mM Tris pH 7.5, 150 mM NaCl, 1 mM TCEP	50 mM Tris pH 7.5, 200 mM NaCl, 1 mM TCEP
Beam size at sample (mm)	1.10 x 0.24	1.0 x 0.25
Wavelength (Å)	0.954	0.954
q range (Å ⁻¹)	0.0045 - 0.34	0.0045 - 0.34
Exposure Time (s/frame)	1.000	3.000
Exposure Temperature (°C)	25.000	15.000
Concentration used in gel filtration chromatography (mg/ml)	5.000	5.000
Structural Parameters		
Forward scattering intensity [$I(0)$] (from Guinier)	0.020	0.128
Forward scattering intensity [$I(0)$] [from $P(r)$]	0.020	0.129
R_g (Å) [from Guinier]	26.626	52.073
R_g (Å) [from $P(r)$]	27.340	53.550
D_{max} (Å)	94.000	215.000
Porod Volume estimate (Å ³) [from $P(r)$]	71164.694	434649.887
Porod Volume estimate corrected (Å ³) [from $P(r)$]	42379.132	241477.927
c^2 [from $P(r)$]	1.035	1.098
Total estimate [from $P(r)$]	0.801	0.614
Molecular weight determination (kDa)		
Theoretical molecular weight	33.010	169.830
From volume of correlation (V_c)	31.373	162.499
From Bayesian assessment [credibility interval], probability	33.1 [31.3,34.2], 94.80	169.625 [162.650,194.950], 52.970
From Shape&Size assessment	32.762	193.038
From Porod volume assessment	35.175	200.427
Software employed		
Data processing	BioXSTAS RAW 2.2.1	BioXSTAS RAW 2.2.1
<i>Ab initio</i> (analysis)	ATSAS-3.2.0	ATSAS-3.2.0
Validation, averaging and final refinement	DAMMIN refinement of DAMSTART from GASBOR	DAMMIN refinement of DAMSTART from GASBOR
3D structure prediction	AF2	AF2-multimer
Computation of model intensities	CRY SOL	CRY SOL
3D graphics representations	UCSF ChimeraX	UCSF ChimeraX

Appendix Table S2. DNA constructs for recombinant protein production used in this study.

Identifier	Plasmid construct	Source/reference
	pET28-Sumo3	EMBL, Heidelberg
	pGST-P2	(Sheffield et al, 1999)
	pET28NStrep	KP Hopfner Lab (Gene Center)
	pCDH-CMV-fmkate2-EF1-puro	I. Varela Lab (IBBTEC)
	pLIB	(Weissmann et al, 2016)
	pBIG1a	(Weissmann et al, 2016)
	pHis-MBP-P4	(Sheffield et al, 1999)
	pBABE-Flag-S vector	(Gammoh et al, 2013)
pMLG30	pIA-His	This study
pMLG31	pIA-GST	This study
pMLG32	pIA-His-MBP	This study
pMLG34	pIA-Strep	This study
pMLG73	pIA-TwinStrep	This study
pMLG23	pMK-VPS26C (opt*)	Invitrogen GeneArt
pMLG21	pET28-His-Sumo3-VPS26C (opt)	This study
pMLG6	pGST-VPS29	(Romano-Moreno et al, 2017)
pMLG11	pECE-H2-SNX17	Addgene # 69811
pMLG13	pET28-His-Sumo3-SNX17	This study
pMLG135	pET28-His-Sumo3-SNX17 _{PX}	This study
pMLG1	pET28-His-Sumo3-SNX17 _{FERM-CT}	This study
pMLG14	pET28-His-Sumo3-EGFP-SNX17	This study
pMLG159	pIA-His-MBP-SNX17	This study
pMLG179	pIA-His-MBP-SNX17 _{D467X}	This study
pMLG178	pIA-His-MBP-SNX17 _{L470G}	This study
pMLG203	pIA-His-MBP-SNX17 _{W321A}	This study
pMLG204	pIA-His-MBP-SNX17 _{V380D}	This study
pMLG205	pIA-His-MBP-SNX17 _{H457A}	This study
pMLG206	pIA-His-MBP-SNX17 _{N459A+F462A}	This study
pMLG25	pMK-LRP1 _{ICD} (opt)	Invitrogen GeneArt
pMLG18	pIA-GST-LRP1 _{ICD} (opt)	This study
pMLG275	pIA-GST-LRP1 _{ICD-mut} (N4470A+Y4473A)	This study
pMLG124	pIA-GST-3C-His ₁₀ -LRP1 _{ICD} (opt)	This study
pMLG165	pIA-His-MBP-His ₁₀ -LRP1 _{ICD} (opt)	This study
pMLG173	pIA-MBP-His ₁₀ -LRP1 _{ICD} (opt)	This study
pMLG48	pGST-APP _{ICD}	A. Hierro Lab (CICbioGUNE)
pMLG49	pIA-GST-APP _{ICD}	This study
pMLG167	gBlock-ITGB1 _{ICD}	IDT gBlock
pMLG168	pIA-GST-ITGB1 _{ICD}	This study
pMLG28	pIA-GST-L2 _{FBR}	This study
pMLG276	pIA-GST-L2 _{FBR-mut} (N254A+Y257A)	This study
pMLG131	pIA-GST-3C-His ₁₀ -L2 _{FBR}	This study
pMLG22	pMK-VPS35L (opt)	Invitrogen GeneArt
pMLG44	pIA-His-VPS35L	This study
pMLG37	pLIB-His-VPS35L (opt)	This study
pMLG05	pET28-His-Sumo3-VPS26C	A. Hierro Lab (CICbioGUNE)
pMLG83	pIA-TwinStrep-VPS26C	This study
pMLG84	pLIB-TwinStrep-VPS26C (opt)	This study
pMLG7	pET28-Sumo3-VPS29	A. Hierro Lab (CICbioGUNE)
pMLG39	pLIB-VPS29	This study

pMLG85	pBIG1a-His-VPS35L-TwinStrep-VPS26C-VPS29	This study
pMLG121	pLIB-GST-HRV3C-VPS29	This study
pMLG122	pBIG1a-His-VPS35L-TwinStrep-VPS26C-GST-VPS29	This study
pMLG109	pIA-His-VPS35L ₁₁₀₋₉₆₃	This study
pMLG113	pLIB-His-VPS35L ₁₁₀₋₉₆₃	This study
pMLG118	pBIG1a-His-VPS35L ₁₁₀₋₉₆₃ -TwinStrep-VPS26C-VPS29	This study
pMLG107	pIA-His-VPS35L ₁₁₀₋₅₉₈	This study
pMLG111	pLIB-His-VPS35L ₁₁₀₋₅₉₈	This study
pMLG116	pBIG1a-His-VPS35L ₁₁₀₋₅₉₈ -TwinStrep-VPS26C-VPS29	This study
pMLG137	pLIB-His-VPS35L ₁₋₅₉₈	This study
pMLG138	pBIG1a-His-VPS35L ₁₋₅₉₈ -TwinStrep-VPS26C-VPS29	This study
pMLG141	pLIB-His-VPS35L ₁₋₄₃₆	This study
pMLG144	pBIG1a-His-VPS35L ₁₋₄₃₆ -TwinStrep-VPS26C-VPS29	This study
pMLG151	pBIG1a-His-VPS35L-GST-VPS29	This study
pMLG228	pLIB-HisVPS35L _{R248E+W280D}	This study
pMLG231	pBIG1a-HisVPS35L _{R248E+W280D} -TwinStrep-VPS26C-VPS29	This study
pMLG229	pLIB-HisVPS35L _{K157E+R161E}	This study
pMLG232	pBIG1a-HisVPS35L _{K157E+R161E} -TwinStrep-VPS26C-VPS29	This study
pMLG15	pET28-Sumo3-VPS29-GFP	This study
pMLG59	pET28-Sumo3-VPS29-mKate2	This study
pMLG115	pLIB-VPS29-mkate2	This study
pMLG120	pBIG1a-His-VPS35L-TwinStrep-VPS26C-VPS29-mKate2	This study
pMLG223	cDNA L2 (HPV16)	Invitrogen GeneArt
pMLG278	pMSCV-FlagS	This study
pMLG279	pMSCV-FlagS-EGFP	This study
pMLG284	pMSCV-FlagS-L2 _{FBR}	This study
pMLG285	pMSCV-FlagS-L2 _{FBR(N254A+Y257A)}	This study
pMLG286	pMSCV-FlagS-L2	This study
pMLG287	pMSCV-FlagS-L2 _(N254A+Y257A)	This study

*Opt: Optimized for *E. coli* expression.

Appendix Table S3. DNA oligos used in this study.

ID: pMLG Plasmid identification number.

ID	Amplification	Template	Primer Identifier and sequence, all 5' to 3'
30	His-TEV	pET28-Sumo3	088: CCGATGAAACGAGAGAGGATGCTCACGATACGGGTTACTG 071: GCCCTGGAAGTACAGGTTTTGCGCCGCTGCTGTGATGATGATGATGATG
	His-TEV	pET28-Sumo3	070: GGCGAAAACCTGTACTTCCAGGGCGTGAGCAAGGGCGAGGAGCTG 089: CAGTAACCCGTATCGTGAGCATCCTCTCTCGTTTCATCGG
31	Insert GST	pGST-P2	072: GAAGGAGATATACCATGGGCAGCAGCATGTCCCTATACTAGGTTATTGGAAAATTAAGGGCC 073: GGAAGTACAGGTTTTGCGCCGCTGCTTTTTGGAGGATGGTCGCCACCACC
	Vector	pIA-His	095: GCTGCTGCCATGGTATATCTCCTTCTTAAAGTTAAAC 096: AGCAGCGGCGAAAACCTGTACTTCCAGGGC
32	His-MBP-TEV	pHis-MBP-P4	088: CCGATGAAACGAGAGAGGATGCTCACGATACGGGTTACTG 074: GGAAGTACAGGTTTTGCGCCGCTGCTCCCGAGGTTGTTGTTATTGTTATTGTTGTTG
34	Strep	pET28NStre p	088: CCGATGAAACGAGAGAGGATGCTCACGATACGGGTTACTG 076: GGAAGTACAGGTTTTGCGCCGCTGCTAGCGCCTTTTTCGAACTGCGGGTGG
	Vector	pMLG30	096: AGCAGCGGCGAAAACCTGTACTTCCAGGGC 089: CAGTAACCCGTATCGTGAGCATCCTCTCTCGTTTCATCGG
73	Half of the vector	pMLG34	089: CAGTAACCCGTATCGTGAGCATCCTCTCTCGTTTCATCGG 150: CGGTGGATCAGGTGGAAGTGCATGGTCTCATCCTCAGTTTGAGAAAAGCAGCGGCGAAAACCTGTACTTCCAGG
	Half of the vector	pMLG34	088: CCGATGAAACGAGAGAGGATGCTCACGATACGGGTTACTG 151: GGATGAGACCATGCACCTCCACCTGATCCACCGCCAGAACCTCCGCCCTTTTTCGAACTGCGGGTGGCTCC
21	Insert VPS26C	pMLG23	053: CGTGTTCAGCAGCAGACCGGTGGAATGGGCACCGCACTGGATATCAAAATCAAACG 054: CTCAGTGGTGGTGGTGGTGGTGTAAATGCGGCACAGTTTCAGCGGAAAGTTTTTCGG
	Vector	pET28-Sumo3	002: CACCACCACCACCACCACTGAGATCCGGCTGCTAAC 001: TCCACCGGTCTGCTGCTGGAACACGTCGATGGTGTCTCG
13	Insert SNX17	pMLG11	032: CGACGTGTTCCAGCAGCAGACCGGTGGAATGCACCTTTTCCATTCCCGAAACCGAGTCCCGC 007: CCGGATCTCAGTGGTGGTGGTGGTGGTGTACAGATCCTCATCTCCAATGCCCTCGAAGG
	Vector	pET28-Sumo3	As in 21.
135	Insert SNX17 _{px}	pMLG11	032: CGACGTGTTCCAGCAGCAGACCGGTGGAATGCACCTTTTCCATTCCCGAAACCGAGTCCCGC 184: GATCTCAGTGGTGGTGGTGGTGGTGGTGTACTCCTGTTGTGCCGACGCAGGAAAC
	Vector	pET28-Sumo3	As in 21.
1	Insert SNX17 _{109C}	pMLG11	006: CGACGTGTTCCAGCAGCAGACCGGTGGAACACAGCAGGTCCCGACAGAGGAAGTG 007: CCGGATCTCAGTGGTGGTGGTGGTGGTGTACAGATCCTCATCTCCAATGCCCTCGAAGG
	Vector	pET28-Sumo3	As in 21.
14	Insert SNX17	pMLG13	007: CCGGATCTCAGTGGTGGTGGTGGTGGTGTACAGATCCTCATCTCCAATGCCCTCGAAGG 034: GGGATCACTCTCGGCATGGACGAGCTCTACAAGGGCGGCGGCGGCGAGCATGCACTTTTCCATTCCC GAAACCGAGTCCCGC
	Vector	pET28-Sumo3	002: CACCACCACCACCACCACTGAGATCCGGCTGCTAAC 036: CTTGTAGAGCTCGTCCATGCCGAGAGTGATCCCGGCG
159	Insert SNX17	pMLG11	007: CCGGATCTCAGTGGTGGTGGTGGTGGTGTACAGATCCTCATCTCCAATGCCCTCGAAGG 208: GACGCGCAGACTAATTCGAGCTCTATGCACCTTTTCCATTCCCGAAACCGAGTCC
	Insert MBP	pMLG32	203: GCGAAAACCTGTACTTCCAGGGCATGATCGAAGAAGGTAACTGGTAATCTGGATTAACG 204: AGAGCTCGAATTAGTCTGCGCGTCTTTCAG
	Vector	pMLG30	002: CACCACCACCACCACCACTGAGATCCGGCTGCTAAC 094: GCCCTGGAAGTACAGGTTTTGCGCCGCTGC
179	Insert MBP-SNX17 _{D467X}	pMLG159	203: GCGAAAACCTGTACTTCCAGGGCATGATCGAAGAAGGTAACTGGTAATCTGGATTAACG 240: GATCTCAGTGGTGGTGGTGGTGGTGTATCCAATGCCCTCGAAGGCGAAATTGC
	Vector	pMLG30	As in 159.
178	Insert MBP-SNX17 _{L470G}	pMLG159	203: GCGAAAACCTGTACTTCCAGGGCATGATCGAAGAAGGTAACTGGTAATCTGGATTAACG 239: GATCTCAGTGGTGGTGGTGGTGGTGTAAACCATCCTCATCTCCAATGCCCTCGAAGG
	Vector	pMLG30	As in 159.
203	pIA-HisMBP-SNX17 _{W321A_1}	pMLG159	246: GTCACCCGCATGCGATGCGCGCGGGTCACCTCCTCTGTAC 245: CAGTAACCCGTATCGTGAGCATCCTCTCTC
	pIA-HisMBP-SNX17 _{W321A_2}	pMLG159	244: GAGAGAGGATGCTCACGATACGGGTTACTG 247: GTACAGAGGAGGTGACCCGCGCGCATCGCATGCGGGTGAC
204	pIA-HisMBP-SNX17 _{V380D_1}	pMLG159	248: GCAGTCCATGGATGATGAAGTATGGTGAAGAAATCTGGC 245: CAGTAACCCGTATCGTGAGCATCCTCTCTC
	pIA-HisMBP-SNX17 _{V380D_2}	pMLG159	244: GAGAGAGGATGCTCACGATACGGGTTACTG 249: CACCATCAGTTTCATCATCCATGCAAGCAGATGCTC
205	pIA-HisMBP-SNX17 _{H457A_1}	pMLG159	250: TGCCAGTGATGTCGCCGGCAATTTGCGCTTCGAGG 245: CAGTAACCCGTATCGTGAGCATCCTCTCTC
	pIA-HisMBP-SNX17 _{H457A_2}	pMLG159	244: GAGAGAGGATGCTCACGATACGGGTTACTG 251: AAGGCGAAATTGCCGCGACATCACTGGCACTGGCATCTG
206	pIA-HisMBP-SNX17 _{N459A+F462A_1}	pMLG159	252: GGTGCTTTTCGCTGCTGAGGGCATTGGAGATGAGGATCTG 245: CAGTAACCCGTATCGTGAGCATCCTCTCTC

[illegible]

18

			131: GGAGCTGCCGCGCCGCC
	Vector	pMLG7	002: CACCACCACCACCACCTGAGATCCGGCTGCTAAC 143: GCTCGCTCACCATGGAGCTGCCGCGCCGCCAGGTTTTTTGTATTGATTGATTCTACTTTACATCA TCTCC
115	Insert VPS29 and mkate2	pMLG59	061: CCACCATCGGGCGCGGATCCAATGTTGGTGTTGGTATTAGGAGATCTGCAC 153: GGTATGGCTGATTATGATCCTCTAGTACTTCTCGACAAGCTTCGGATCTCAGTGGTGGTGGTGGT G
120	Insert VPS29-mkate2	pMLG115	059: AACCTAATGATGCCTGATGTTTAAATCGACCTACTCCGGAATATTAATAGATC 060: AACCCCGATTGAGATATAGATTTATTTAAATGGTTATGATAGTTATTGCTCAGCG
	Insert VPS35L	pMLG37	As in 85.
	Insert VPS26C	pMLG84	As in 85.
278	Vector	MSCV-puro BgIII-XhoI	
	Insert FlagS	pBABE- Flag-S vector	
279	Vector	pMLG278 XhoI-EcoRI	
	Insert EGFP		
284	Vector	pMLG278 XhoI+EcoRI	
	Insert L2 _{FBR}	pMLG28	361: GGACAGCGGAGGTGGAGGTACTAGTCCCGATCCGGCCTTTGTGACC 344: GAAAAGCGCCTCCCTACCCGGTAGTTAGAAGTACAGGGTGTTATCCACATC
285	Vector	pMLG278 XhoI+EcoRI	
	Insert L2 _{FBR(N254A+Y257A)}	pMLG276	As in 284.
286	Vector	pMLG278 XhoI+EcoRI	
	Insert L2	pMLG223	362: GGACAGCGGAGGTGGAGGTACTAGTCCCATGCGTCATAAACGCAGCGC 346: GAAAAGCGCCTCCCTACCCGGTAGTTAGGCAGCAAGAGAGACGTCTGAG
287	Vector	pMLG278 XhoI+EcoRI	
	Mutagenesis of L2 _{(N254A+Y257A)_1}	pMLG223	345: GGACAGCGGGGTGGAGGTACTAGTCATGCGTCATAAACGCAGCGC 348: CCCTTCAGCGGCTGGAGCATCATAGGTAATTAACCTGGTAGGAGTTG
	Mutagenesis of L2 _{(N254A+Y257A)_2}	pMLG223	347: CCTATGATGCTCCAGCCGCTGAAGGGATTGACGTTGATAACACC 346: GAAAAGCGCCTCCCTACCCGGTAGTTAGGCAGCAAGAGAGACGTCTGAG

Appendix Table S4. Sequences of recombinant proteins used in this work.

ID: This column lists the pMLG plasmid identification numbers.

The listed sequences correspond to the cloned proteins before the removal of the affinity tag by protease cleavage. The tag sequences are annotated in blue, the protease recognition sites in orange, the extra sequences resulting from cloning in green and mutations from the wild-type sequence in red.

ID	Sequence
31	> GST MGSSMSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYI ADKHNM LGGCPKERA EISMLEGAVLDIRYGVSR IAYSKDFETLKVD FLSKLP EMLKMFEDRLCHKTYLNGDHVTHPDF MLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSSKYIAWPLQG WQATFGGGDHPPK
21	> His-Sumo3-VPS26C MGSSHHHHHHHSSGLVPRGSHMNDHINLKVAGQDGSVVQFKIKRHTPLSKLMKAYCERQGLSMRQIRFRFDGQPINE TDTPAQLEMEDED TIDVFQQQTGGMG TALDIKIRANKVYHAGEVLSGVVVISKDSVQH QGVSLTMEGT VNLQLSAK SVGVFEAFYNSVKPIQIINSTIEMVKPGKFP SGKTEIPFEFPLHLKGNKVL YET YHG VFVNIQYTLRCDMKRSLLAKDLTK TCEFIVHSAPQKGKFTSPVDFTITPETLQNVKERALLPKFLLRGHLNSTNCVITQPLTGELVVESSEAAIRSVELQLVR VETCGCAEGYARDATEIQNIQIADGDVCRGLSVPIYMFVPRFLTCTLET TNFKVEFEVNIVLLHPDHLITENFPLKLCR I
6	> GST-TEV-VPS29 MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKH MLGGCPKERA EISMLEGAVLDIRYGVSR IAYSKDFETLKVD FLSKLP EMLKMFEDRLCHKTYLNGDHVTHPDFMLYDA LDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSSKYIAWPLQG WQATFGGGDHPPKIDT ENLYFQGGAMG SMLV LVLGDLHIPHRCNSLP AKFKLLVPGKIQHILCTGNLCTKESYDYLKTLAGDVHIVRGDFDENL NYPEQKVVTVGQFKIG LIHGHQVIPWGD MASLALLQRQFDV DILISGHTHKFEAFEHENKFYINPGSATGAYNALETNIIPSVFLMDIQASTVVTYV YQLIGDDVKVERIEYKKP*
13	> His-Sumo3-SNX17 MGSSHHHHHHHSSGLVPRGSHMNDHINLKVAGQDGSVVQFKIKRHTPLSKLMKAYCERQGLSMRQIRFRFDGQPINE TDTPAQLEMEDED TIDVFQQQTGGMHFSIPETESRSGDSGGSAYVAYNIHVNGVLHCRVRYSQLLGLHEQLRKEYGA NVLPAPFPKKL FSLTPAEVEQRREQLEKYMQAVRQDPLLGSSETFNSFLRRAQQETQQVPTEEVSLEVLLSNGQKVL VNVLTSDQTEDVLEA VAAKLDLPDDLIGYFSLFLVREKEDGAFS FVRKLQEFELPYVSVTSLRSQ EYKIVLRKSYWDSA YDDDMENRVGLNLLYAQTVSDIERGWILVTKEQHRQLKSLQEKVSKKEFLRLAQT LRHYGYLRFDACVADFEKDC PVVVSAGNSEL SLQLRLPGQQLREGSFRVTRMRCWRVTSSVPLPSGSTSSPGRGRGEVRLELA FEYLM SKDRLQW VTITSPQAIMMSICLQSMVDELMVKKSGGSIRKMLRRRVGGTLRRSDSQQAVKSPPLLESPDATRES MVKLSSKLSAV SLRGIGSPSTDASASDVHGNFAFEGIGDEDL
135	> His-Sumo3-SNX17 _{Px} MGSSHHHHHHHSSGLVPRGSHMNDHINLKVAGQDGSVVQFKIKRHTPLSKLMKAYCERQGLSMRQIRFRFDGQPINE TDTPAQLEMEDED TIDVFQQQTGGMHFSIPETESRSGDSGGSAYVAYNIHVNGVLHCRVRYSQLLGLHEQLRKEYGA NVLPAPFPKKL FSLTPAEVEQRREQLEKYMQAVRQDPLLGSSETFNSFLRRAQQE
1	> His-Sumo3-SNX17 _{109C} MGSSHHHHHHHSSGLVPRGSHMNDHINLKVAGQDGSVVQFKIKRHTPLSKLMKAYCERQGLSMRQIRFRFDGQPINE TDTPAQLEMEDED TIDVFQQQTGGTQQVPTEEVSLEVLLSNGQKVLVNVLTSDQTEDVLEA VAAKLDLPDDLIGYFSL FLVREKEDGAFS FVRKLQEFELPYVSVTSLRSQ EYKIVLRKSYWDSAYDDDMENRVGLNLLYAQTVSDIERGWILVT KEQHRQLKSLQEKVSKKEFLRLAQT LRHYGYLRFDACVADFEKDCPVVVSAGNSEL SLQLRLPGQQLREGSFRVTR MRCWRVTSSVPLPSGSTSSPGRGRGEVRLELA FEYLM SKDRLQWVTITSPQAIMMSICLQSMVDELMVKKSGGSIRK MLRRRVGGTLRRSDSQQAVKSPPLLESPDATRES MVKLSSKLSAVSLRGIGSPSTDASASDVHGNFAFEGIGDEDL
14	> His-Sumo3-EGFP-SNX17 MGSSHHHHHHHSSGLVPRGSHMNDHINLKVAGQDGSVVQFKIKRHTPLSKLMKAYCERQGLSMRQIRFRFDGQPINE TDTPAQLEMEDED TIDVFQQQTGGSVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLT LKFICTTGKL PVPWPTLVTTLT YGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRA EVKFEGDTLVNRIELKGIDFK EDGNILGHKLEYNYN SHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKD PNEKRDHMLVLEFVTAAGITLGMDELYKGGGSMHFSIPETESRSGDSGGSAYVAYNIHVNGVLHCRVRYSQLLGLH EQLRKEYGANVLPAPFPKKL FSLTPAEVEQRREQLEKYMQAVRQDPLLGSSETFNSFLRRAQQETQQVPTEEVSLEV LLSNGQKVLVNVLTSDQTEDVLEA VAAKLDLPDDLIGYFSLFLVREKEDGAFS FVRKLQEFELPYVSVTSLRSQ EYKIVL RKS YWDSAYDDDMENRVGLNLLYAQTVSDIERGWILVTKEQHRQLKSLQEKVSKKEFLRLAQT LRHYGYLRFDACV ADFPEKDCPVVVSAGNSEL SLQLRLPGQQLREGSFRVTRMRCWRVTSSVPLPSGSTSSPGRGRGEVRLELA FEYLM SKDRLQWVTITSPQAIMMSICLQSMVDELMVKKSGGSIRKMLRRRVGGTLRRSDSQQAVKSPPLLESPDATRES MVK LSSKLSAVSLRGIGSPSTDASASDVHGNFAFEGIGDEDL
159	> His-TEV-MBP-SNX17 MGSSHHHHHHHSSG ENLYFQGMIEEGKLVIWINGDKGYNGLA EVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGP DIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDK ELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGV DNAGAKAGLTFVLDIKNKHMNADTDYSIAEA AFNKGETAMTINGPWAWSNIDTSKVNYGVTLP TFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAV

	NKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIQMSAFWYAVRTAVINAASGRQTVDEALKDAQT^{NSSS} MHFSIPETESRSGDSGGSAYVAYNIHVNGVLHCRVRYSQLLGLHEQLRKEYGANVLPAPFPKKLFSLTPAEVEQRRE QLEKYMQAVRQDPLLGSSETFNSFLRRAQGETQQVPTTEEVSLLEVLLSNGQKVLNVNLTSDQTEDVLEAVAAKLDLPD DLIGYFSLFLVREKEDGAFSFRKLQEFELPYVSVTSLRSQEKIVLRKSYWDSAYDDVDMENRVGLNLLYAQTVSDIE RGWILVTKEQHRQLKSLQEKVSKKEFLRLAQTLRHYGYLRFDACVADFPEKDCPVVVSAGNSELSLQRLRPGQQLRE GSFRVTRMRCWRVTSSVPLPSGSTSSPGRGRGEVRLELAFEYLMKSDRLQWVTITSPQAIMMSICLQSMVDELMVK KSGGSIRKMLRRRVGGTLRRSDSQAVKSPPLLESPDATRESMVKLSSKLSAVSLRGIGSPSTDASASDVHGNFAFE GIGDEDL
179	> His-TEV-MBP-SNX17^{D467X} MGSSHHHHHHSSGENLYFQGMIEEGKLVWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGP DIIFWAHDFRGGYQAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDK ELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVNDAGAKAGLTFLVDLIKNKHMNADTDYSIAEA AFNKGETAMTINGPWAWSNIDTSKVNYGVTLPFTFGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAV NKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIQMSAFWYAVRTAVINAASGRQTVDEALKDAQT^{NSSS} MHFSIPETESRSGDSGGSAYVAYNIHVNGVLHCRVRYSQLLGLHEQLRKEYGANVLPAPFPKKLFSLTPAEVEQRRE QLEKYMQAVRQDPLLGSSETFNSFLRRAQGETQQVPTTEEVSLLEVLLSNGQKVLNVNLTSDQTEDVLEAVAAKLDLPD DLIGYFSLFLVREKEDGAFSFRKLQEFELPYVSVTSLRSQEKIVLRKSYWDSAYDDVDMENRVGLNLLYAQTVSDIE RGWILVTKEQHRQLKSLQEKVSKKEFLRLAQTLRHYGYLRFDACVADFPEKDCPVVVSAGNSELSLQRLRPGQQLRE GSFRVTRMRCWRVTSSVPLPSGSTSSPGRGRGEVRLELAFEYLMKSDRLQWVTITSPQAIMMSICLQSMVDELMVK KSGGSIRKMLRRRVGGTLRRSDSQAVKSPPLLESPDATRESMVKLSSKLSAVSLRGIGSPSTDASASDVHGNFAFE GIG
178	> His-TEV-MBP-SNX17^{L470G} MGSSHHHHHHSSGENLYFQGMIEEGKLVWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGP DIIFWAHDFRGGYQAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDK ELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVNDAGAKAGLTFLVDLIKNKHMNADTDYSIAEA AFNKGETAMTINGPWAWSNIDTSKVNYGVTLPFTFGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAV NKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIQMSAFWYAVRTAVINAASGRQTVDEALKDAQT^{NSSS} MHFSIPETESRSGDSGGSAYVAYNIHVNGVLHCRVRYSQLLGLHEQLRKEYGANVLPAPFPKKLFSLTPAEVEQRRE QLEKYMQAVRQDPLLGSSETFNSFLRRAQGETQQVPTTEEVSLLEVLLSNGQKVLNVNLTSDQTEDVLEAVAAKLDLPD DLIGYFSLFLVREKEDGAFSFRKLQEFELPYVSVTSLRSQEKIVLRKSYWDSAYDDVDMENRVGLNLLYAQTVSDIE RGWILVTKEQHRQLKSLQEKVSKKEFLRLAQTLRHYGYLRFDACVADFPEKDCPVVVSAGNSELSLQRLRPGQQLRE GSFRVTRMRCWRVTSSVPLPSGSTSSPGRGRGEVRLELAFEYLMKSDRLQWVTITSPQAIMMSICLQSMVDELMVK KSGGSIRKMLRRRVGGTLRRSDSQAVKSPPLLESPDATRESMVKLSSKLSAVSLRGIGSPSTDASASDVHGNFAFE GIGDEDG
203	> His-TEV-MBP-SNX17^{W321A} MGSSHHHHHHSSGENLYFQGMIEEGKLVWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGP DIIFWAHDFRGGYQAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDK ELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVNDAGAKAGLTFLVDLIKNKHMNADTDYSIAEA AFNKGETAMTINGPWAWSNIDTSKVNYGVTLPFTFGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAV NKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIQMSAFWYAVRTAVINAASGRQTVDEALKDAQT^{NSSS} MHFSIPETESRSGDSGGSAYVAYNIHVNGVLHCRVRYSQLLGLHEQLRKEYGANVLPAPFPKKLFSLTPAEVEQRRE QLEKYMQAVRQDPLLGSSETFNSFLRRAQGETQQVPTTEEVSLLEVLLSNGQKVLNVNLTSDQTEDVLEAVAAKLDLPD DLIGYFSLFLVREKEDGAFSFRKLQEFELPYVSVTSLRSQEKIVLRKSYWDSAYDDVDMENRVGLNLLYAQTVSDIE RGWILVTKEQHRQLKSLQEKVSKKEFLRLAQTLRHYGYLRFDACVADFPEKDCPVVVSAGNSELSLQRLRPGQQLRE GSFRVTRMRCWRVTSSVPLPSGSTSSPGRGRGEVRLELAFEYLMKSDRLQWVTITSPQAIMMSICLQSMVDELMVKK SGGSIRKMLRRRVGGTLRRSDSQAVKSPPLLESPDATRESMVKLSSKLSAVSLRGIGSPSTDASASDVHGNFAFEGI GDEDL
204	> His-TEV-MBP-SNX17^{V380D} MGSSHHHHHHSSGENLYFQGMIEEGKLVWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGP DIIFWAHDFRGGYQAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDK ELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVNDAGAKAGLTFLVDLIKNKHMNADTDYSIAEA AFNKGETAMTINGPWAWSNIDTSKVNYGVTLPFTFGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAV NKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIQMSAFWYAVRTAVINAASGRQTVDEALKDAQT^{NSSS} MHFSIPETESRSGDSGGSAYVAYNIHVNGVLHCRVRYSQLLGLHEQLRKEYGANVLPAPFPKKLFSLTPAEVEQRRE QLEKYMQAVRQDPLLGSSETFNSFLRRAQGETQQVPTTEEVSLLEVLLSNGQKVLNVNLTSDQTEDVLEAVAAKLDLPD DLIGYFSLFLVREKEDGAFSFRKLQEFELPYVSVTSLRSQEKIVLRKSYWDSAYDDVDMENRVGLNLLYAQTVSDIE RGWILVTKEQHRQLKSLQEKVSKKEFLRLAQTLRHYGYLRFDACVADFPEKDCPVVVSAGNSELSLQRLRPGQQLRE GSFRVTRMRCWRVTSSVPLPSGSTSSPGRGRGEVRLELAFEYLMKSDRLQWVTITSPQAIMMSICLQSM^DDELMVK KSGGSIRKMLRRRVGGTLRRSDSQAVKSPPLLESPDATRESMVKLSSKLSAVSLRGIGSPSTDASASDVHGNFAFE GIGDEDL
205	> His-TEV-MBP-SNX17^{H457A} MGSSHHHHHHSSGENLYFQGMIEEGKLVWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGP DIIFWAHDFRGGYQAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDK ELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVNDAGAKAGLTFLVDLIKNKHMNADTDYSIAEA AFNKGETAMTINGPWAWSNIDTSKVNYGVTLPFTFGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAV

	NKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIQMSAFWYAVRTAVINAASGRQTVDEALKDAQT^{NSSS} MHFSIPETESRSGDSGGSAYVAYNIHVNGVLHCRVRYSQLLGHEQLRKEYGANVLPAPFPKKLFSLTAPAEVEQRRE QLEKYMQAVRQDPLLGSETFNSFLRRAQGETQQVPTTEEVSLLEVLLSNGQKVLVNVLTSDQTEDVLEAVAAKLDLPD DLIGYFSLFLVREKEDGAFSFRKLQEFELPYVSVTSLRSQEKIVLRKSYWDSAYDDVDMENRVGLNLLYAQTVSDIE RGWILVTKEQHRQLKSLQEKVSKKEFLRLAQLTRHYGYLRFDACVADFEKDCPVVVSAGNSELSLQLRPLPGQQLRE GSFRVTRMRCWRVTSSVPLPSGSTSSPGRGRGEVRLELAFEYLMKSDRLQWVTITSPQAIMMSICLQSMVDELMVK KSGGSIRKMLRRRVGGTLRRSDSQQAVKSPPLLESPDATRESMVKLSSKLSAVSLRGIGSPSTDASASDV^{AGNFAE} GIGDEDL
206	> His-TEV-MBP-SNX17^{N459A+F462A} MGSSHHHHHHSSGENLYFQGMIEEGKLVWINGDKGYNGLAIEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGP DIIFWAHDFRGGYQAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDK ELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVNDAGAKAGLTFLVDLIKHKHMNADTDYSIAEA AFNKGETAMTINGPWAWSNIDTSKVNYGVTLPFTFGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAV NKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIQMSAFWYAVRTAVINAASGRQTVDEALKDAQT^{NSSS} MHFSIPETESRSGDSGGSAYVAYNIHVNGVLHCRVRYSQLLGHEQLRKEYGANVLPAPFPKKLFSLTAPAEVEQRRE QLEKYMQAVRQDPLLGSETFNSFLRRAQGETQQVPTTEEVSLLEVLLSNGQKVLVNVLTSDQTEDVLEAVAAKLDLPD DLIGYFSLFLVREKEDGAFSFRKLQEFELPYVSVTSLRSQEKIVLRKSYWDSAYDDVDMENRVGLNLLYAQTVSDIE RGWILVTKEQHRQLKSLQEKVSKKEFLRLAQLTRHYGYLRFDACVADFEKDCPVVVSAGNSELSLQLRPLPGQQLRE GSFRVTRMRCWRVTSSVPLPSGSTSSPGRGRGEVRLELAFEYLMKSDRLQWVTITSPQAIMMSICLQSMVDELMVK KSGGSIRKMLRRRVGGTLRRSDSQQAVKSPPLLESPDATRESMVKLSSKLSAVSLRGIGSPSTDASASDVHG^{AF}AE GIGDEDL
18	> GST-TEV-LRP1^{ICD} MGSSMSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYI ADKHNMLGGCPKERAIEISMLEGAVLDIRYGVSRISYKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDF MLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGWQATFGGGDHPPK^{SSGENLYFQGYK} RRVQGAQGFQHQRTNGAMNVEIGNPTYKMYEGGEPDDVGGLLDADFALDPDKPTNFTNPVYATLYMGGHGSRHS LASTDEKRELLGRGPEDEIGDPLA
275	>GST-TEV-LRP1^{ICD-mut (N4470A+Y4473A)} MGSSMSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYI ADKHNMLGGCPKERAIEISMLEGAVLDIRYGVSRISYKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDF MLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGWQATFGGGDHPPK^{SSGENLYFQGYK} RRVQGAQGFQHQRTNGAMNVEIG^{APT}AKMYEGGEPDDVGGLLDADFALDPDKPTNFTNPVYATLYMGGHGSRHS LASTDEKRELLGRGPEDEIGDPLA
173	> MBP-TEV-His₁₀-LRP1^{ICD} MSYYMIEEGKLVWINGDKGYNGLAIEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIIFWAHDFRGGYQAQSG LLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEP YFTWPLIAADGGYAFKYENGKYDIKDVGVNDAGAKAGLTFLVDLIKHKHMNADTDYSIAEAFAFNKGETAMTINGPWAWS SNIDTSKVNYGVTLPFTFGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEEL AKDPRIAATMENAQKGEIMPNIQMSAFWYAVRTAVINAASGRQTVDEALKDAQT^{ENLYFQGH}HHHHHHHHHHHYKRR VQGAQGFQHQRTNGAMNVEIGNPTYKMYEGGEPDDVGGLLDADFALDPDKPTNFTNPVYATLYMGGHGSRHSLA STDEKRELLGRGPEDEIGDPLA
49	> GST-TEV-APP^{ICD} MGSSMSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYI ADKHNMLGGCPKERAIEISMLEGAVLDIRYGVSRISYKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDF MLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGWQATFGGGDHPPK^{SSGENLYFQGGKKK} QYTSIHGVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN
168	> GST-TEV-ITGB1^{ICD} MGSSMSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYI ADKHNMLGGCPKERAIEISMLEGAVLDIRYGVSRISYKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDF MLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGWQATFGGGDHPPK^{SSGENLYFQGGKLL} MIHHRREFAKFEKEKMNAKWDGTGENPIYKSAVTTVVNPKYEGK
28	> GST-TEV-L2^{FBR} MGSSMSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYI ADKHNMLGGCPKERAIEISMLEGAVLDIRYGVSRISYKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDF MLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGWQATFGGGDHPPK^{SSGENLYFQGGDP} AFVTTPTKLITYDNPAYEGIDVDNTLYF
276	>GST-TEV-L2^{FBR-mut (N254A+Y257A)} MGSSMSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYI ADKHNMLGGCPKERAIEISMLEGAVLDIRYGVSRISYKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDF MLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGWQATFGGGDHPPK^{SSGENLYFQGGDP} AFVTTPTKLITYD^{APAA}EGIDVDNTLYF
131	> GST-HRV3C-His₁₀-L2^{FBR} MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHN MLGGCPKERAIEISMLEGAVLDIRYGVSRISYKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDA

	LDVVLYMDPMCLDAFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSSGLEVLFOGPHHHHHH HHHHHPAFVTTPTKLITYDNPAYEGIDVDNTLYF
85	> His-TEV-VPS35L-TwinStrep-TEV-VPS26C-VPS29 MGSSHHHHHHSSGENLYFQGMVFPWHSRNRNYKAEFASCRLEAVPLEFGDYHPLKPITVTESKTKKVNRRKGSTSS TSSSSSSSVVDPLSSVLDGTDPLSMFAATADPAALAAAMDSSRRKRDRDDNSVVGSDFEFPTNKRGEILARYTTTEK LSINLFMGSEKKGAGTATLAMSEKVRTRLEELDDFEEGSQKELLNLTQQDYVNRIEELNQSLKDAWASDQKVKALKIVI QCSKLLSDTSVIQFYPSKFVLITDILDTFGKLVYERIFSMCVDSRSVLPDHFSPENANDTAKETCLNWWFKIASIRELIPRF YVEASILKCNKFLSKTGISECLPRLTCMIRGIGDPLVSVYARAYLCRVGMEVAPHLKETLNKNFFDFLLTFKQIHGDTVQ NQLVVQGVLPSPYLPYPPAMDWIFQCISYHAPEALLTEMMERCKKLGNALLNSVMSAFRAEFIASTRSMDFIGMIKE CDESGFPKHLLFRSLGLNLALADPPESDRLQILNEAWKVITKLKNPQDYINCAEVWVEYTCCKHFTKREVNTVLADVIKH MTPDRAFEDSYQQLQLIKKVIAHFHDFSFLSVFSVEKFLPFLDMFQKESVRVEVCKCIMDAFIKHQQEPTKDPVILNALLH VCKTMHDSVNALTLEDEKRMVSYLINGFIKMFVSGRDFEQQLSFYVESRSMFCNLEPVLVQLIHSVNRLAMETRKMVK GNHSRKTAAFVRACVAYCFITIPSLAGIFTRNLNLYLHSGQVALANQCLSQADAFFKAAISLVPEVPKMINIDGKMRPSES FLEFLCNFFSTLLIVPDHPEHGVFLVRELLNVIQDYTWEDNSDEKIRIYTCVLHLLSAMSQETYLYHIDKVDNSDSL YGGDSKFLAENNKLCETVMAQILEHLKTLAKDEALKRQSSGLSFFNSILAHGDLRNNKLNQLSVNLWHLAQRHGCADTR TMVKTLEYIKKQSKQPDMLTHLTELALRLPLQTRT MASWSHPQFEKGGGSGGGSGGSAWSHPQFEKSSGENLYFQGMGTALDIKIRANKVYHAGEVLSGVVVISSKDSV QHQQVSLTMEGTVNQLSASVGVFEAFYNSVKPIQIINSTIEMVKPGKFPSPGKTEIPFEFPLHLKGNKVLVYET YHGVFVNIQYTLRCDMKRSLAKDLTKTCEFIVHSAPQKGKFTSPVDFTITPETLQNVKERALLPKFLLRGHLNSTNCVITQPLT GELVVSSEAAIRSVELQLVRVETCGCAEGYARDATEIQNIQIADGDVCRGLSVPIYMFPRFLTCTPTLETTNFKVEFEV NIVLLHPDHLITENFPLKLCRI MLVLVLGDLHIPHRCNSLPKFKKLLVPGIKIHILCTGNLCTKESYDYLKTLAGDVHIVRGDFDENLNYPEQKVVTVGQ FKIGLIHGHQVIPWGDMAALLQRQFDVILISGHTHKFEAFEHENKFYINPGSATGAYNALETNIIPSFVLMIDIQASTV VTYVYQLIGDDVKVERIEYKKP
122	> His-TEV-VPS35L-TwinStrep-TEV-VPS26C-GST-HRV3C-VPS29 MGSSHHHHHHSSGENLYFQGMVFPWHSRNRNYKAEFASCRLEAVPLEFGDYHPLKPITVTESKTKKVNRRKGSTSS TSSSSSSSVVDPLSSVLDGTDPLSMFAATADPAALAAAMDSSRRKRDRDDNSVVGSDFEFPTNKRGEILARYTTTEK LSINLFMGSEKKGAGTATLAMSEKVRTRLEELDDFEEGSQKELLNLTQQDYVNRIEELNQSLKDAWASDQKVKALKIVI QCSKLLSDTSVIQFYPSKFVLITDILDTFGKLVYERIFSMCVDSRSVLPDHFSPENANDTAKETCLNWWFKIASIRELIPRF YVEASILKCNKFLSKTGISECLPRLTCMIRGIGDPLVSVYARAYLCRVGMEVAPHLKETLNKNFFDFLLTFKQIHGDTVQ NQLVVQGVLPSPYLPYPPAMDWIFQCISYHAPEALLTEMMERCKKLGNALLNSVMSAFRAEFIASTRSMDFIGMIKE CDESGFPKHLLFRSLGLNLALADPPESDRLQILNEAWKVITKLKNPQDYINCAEVWVEYTCCKHFTKREVNTVLADVIKH MTPDRAFEDSYQQLQLIKKVIAHFHDFSFLSVFSVEKFLPFLDMFQKESVRVEVCKCIMDAFIKHQQEPTKDPVILNALLH VCKTMHDSVNALTLEDEKRMVSYLINGFIKMFVSGRDFEQQLSFYVESRSMFCNLEPVLVQLIHSVNRLAMETRKMVK GNHSRKTAAFVRACVAYCFITIPSLAGIFTRNLNLYLHSGQVALANQCLSQADAFFKAAISLVPEVPKMINIDGKMRPSES FLEFLCNFFSTLLIVPDHPEHGVFLVRELLNVIQDYTWEDNSDEKIRIYTCVLHLLSAMSQETYLYHIDKVDNSDSL YGGDSKFLAENNKLCETVMAQILEHLKTLAKDEALKRQSSGLSFFNSILAHGDLRNNKLNQLSVNLWHLAQRHGCADTR TMVKTLEYIKKQSKQPDMLTHLTELALRLPLQTRT MASWSHPQFEKGGGSGGGSGGSAWSHPQFEKSSGENLYFQGMGTALDIKIRANKVYHAGEVLSGVVVISSKDSV QHQQVSLTMEGTVNQLSASVGVFEAFYNSVKPIQIINSTIEMVKPGKFPSPGKTEIPFEFPLHLKGNKVLVYET YHGVFVNIQYTLRCDMKRSLAKDLTKTCEFIVHSAPQKGKFTSPVDFTITPETLQNVKERALLPKFLLRGHLNSTNCVITQPLT GELVVSSEAAIRSVELQLVRVETCGCAEGYARDATEIQNIQIADGDVCRGLSVPIYMFPRFLTCTPTLETTNFKVEFEV NIVLLHPDHLITENFPLKLCRI MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLQSMARIYIADKH NMLGGCPKERAISMLEGAVLDIRYGVSRISYKDFETLKVDFLSKLPKEMLFEDRLCHKTYLNGDHVTHPDFMLYDA LDVVLYMDPMCLDAFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSSGLEVLFOGPMMLVLVLGD LHIPHRCNSLPKFKKLLVPGIKIHILCTGNLCTKESYDYLKTLAGDVHIVRGDFDENLNYPEQKVVTVGQFKIGLIHGH QVIPWGDMAALLQRQFDVILISGHTHKFEAFEHENKFYINPGSATGAYNALETNIIPSFVLMIDIQASTVVTYVYQLIG DDVKVERIEYKKP
118	> His-TEV-VPS35L¹¹⁰⁻⁹⁶³-TwinStrep-TEV-VPS26C-VPS29 MGSSHHHHHHSSGENLYFQGVGSDFEFPTNKRGEILARYTTTEKLSINLFMGSEKKGAGTATLAMSEKVRTRLEELD DFEEGSQKELLNLTQQDYVNRIEELNQSLKDAWASDQKVKALKIVIQCSKLLSDTSVIQFYPSKFVLITDILDTFGKLVY ERIFSMCVDSRSVLPDHFSPENANDTAKETCLNWWFKIASIRELIPRFYVEASILKCNKFLSKTGISECLPRLTCMIRGIGD PLVSVYARAYLCRVGMEVAPHLKETLNKNFFDFLLTFKQIHGDTVQNQLVVQGVLPSPYLPYPPAMDWIFQCISYHA PEALLTEMMERCKKLGNALLNSVMSAFRAEFIASTRSMDFIGMIKECDESGFPKHLLFRSLGLNLALADPPESDRLQI LNEAWKVITKLKNPQDYINCAEVWVEYTCCKHFTKREVNTVLADVIKHMTDRAFEDSYQQLQLIKKVIAHFHDFSFLSVFS VEKFLPFLDMFQKESVRVEVCKCIMDAFIKHQQEPTKDPVILNALLHVCKTMHDSVNALTLEDEKRMVSYLINGFIKMFV SGRDFEQQLSFYVESRSMFCNLEPVLVQLIHSVNRLAMETRKMVKGNHSRKTAAFVRACVAYCFITIPSLAGIFTRLN LYLHSGQVALANQCLSQADAFFKAAISLVPEVPKMINIDGKMRPSESFLEFLCNFFSTLLIVPDHPEHGVFLVRELLN VIQDYTWEDNSDEKIRIYTCVLHLLSAMSQETYLYHIDKVDNSDSL YGGDSKFLAENNKLCETVMAQILEHLKTLAKDEALKRQSSGLSFFNSILAHGDLRNNKLNQLSVNLWHLAQRHGCADTR TMVKTLEYIKKQSKQPDMLTHLTELALRLPLQTRT MASWSHPQFEKGGGSGGGSGGSAWSHPQFEKSSGENLYFQGMGTALDIKIRANKVYHAGEVLSGVVVISSKDSV QHQQVSLTMEGTVNQLSASVGVFEAFYNSVKPIQIINSTIEMVKPGKFPSPGKTEIPFEFPLHLKGNKVLVYET YHGVFVNIQYTLRCDMKRSLAKDLTKTCEFIVHSAPQKGKFTSPVDFTITPETLQNVKERALLPKFLLRGHLNSTNCVITQPLT

	GELVVESSEAAIRSVLQLVRVETCGCAEGYARDATEIQNIQIADGDVCRGLSVPIYMFPRFLTCTPTLETTNFKVEFEV NIVLLHPDHLITENFPLKLCRI MLVLVLGDLHIPHRCNSLPAKFKLLVPGKIQHILCTGNLCTKESYDYLKTLAGDVHIVRGDFDENLNYPEQKVVTVGQ FKIGLIHGHQVIPWGDMA SLALLQRQFDVILISGHTHKFEAFEHENKFYINPGSATGAYNALETNIIPSFVLMDIQASTV VTYVYQLIGDDVKVERIEYKKP
116	> His-TEV-VPS35L ¹¹⁰⁻⁵⁹⁸ -TwinStrep-TEV-VPS26C-VPS29 MGSSHHHHHHSSGENLYFQGMASVDFEPWTNKRGEILARYTTTEKLSINLFMGSEKKGAGTATLAMSEKVRTRLEELD DFEEGSQKELLNLTQQDYVNRIEELNQSLKDAWASDQVKALKIVIQCSKLLSDTSVIQFYPSKFVLITDILDTFGKLVYE RIFSMCVDSSRSVLPDHFSPENANDTAKETCLNWWFFKIASIRELIPRFYVEASILKCNKFLSKTGISECLPRLTCMIRGIGD PLVSVYARAYLCRVGM EVAPHLKETLNKNFFDFLLTFKQIHGDTVQNQLVVGVELPSYLYPPAMDWIFQCISYHA PEALLTEMMERCKKLGNNALLNSVMSAFRAEFIA TRSMDFIGMIKECDESGFPHLLFRSLGLNLALADPPESDRLQI LNEAWKVITKLKNPQDYINCAEVWVEYTCKHFTKREVNTVLADVIKHMTDPRAFEDSYPLQLIHKVIAHFHDFSVLFS VEKFLPFLDMFQKESVRVEVCKCIMDAFIKHQQ MASW SHPQFEKGGGSGGGSGGSAWSHPQFEKSSGENLYFQGMGTALDIKIRANKVYHAGEVLSGVVVISSKDSV QHQQVSLTMEGTVNQLSASVGVFEAFYNSVKPIQIINSTIEMVKPGKFPSGKTEIPFEFPLHLKGNKVLYETYHGVFV NIQYTLRCMDMKRSLAKDLTKTCEFIVHSAPQKGKFTSPVDFTITPETLQNVKERALLPKFLLRGHLNSTNCVITQPLT GELVVESSEAAIRSVLQLVRVETCGCAEGYARDATEIQNIQIADGDVCRGLSVPIYMFPRFLTCTPTLETTNFKVEFEV NIVLLHPDHLITENFPLKLCRI MLVLVLGDLHIPHRCNSLPAKFKLLVPGKIQHILCTGNLCTKESYDYLKTLAGDVHIVRGDFDENLNYPEQKVVTVGQ FKIGLIHGHQVIPWGDMA SLALLQRQFDVILISGHTHKFEAFEHENKFYINPGSATGAYNALETNIIPSFVLMDIQASTV VTYVYQLIGDDVKVERIEYKKP
138	> His-TEV-VPS35L ¹⁻⁵⁹⁸ -TwinStrep-TEV-VPS26C-VPS29 MGSSHHHHHHSSGENLYFQGMMAVFPWHSRNRNYKAEFASCRLEAVPLEFGDYHPLKPITVTESKTKKVNRRKGSTSS TSSSSSSSVVDPLSSVLDGTDPLSMFAATADPAALAAAMDSSRRKRDRDDNSVVGSDFEFWTNKRGEILARYTTTEK LSINLFMGSEKKGAGTATLAMSEKVRTRLEELDDFEEGSQKELLNLTQQDYVNRIEELNQSLKDAWASDQVKALKIVI QCSKLLSDTSVIQFYPSKFVLITDILDTFGKLVYERIFSMCVDSSRSVLPDHFSPENANDTAKETCLNWWFFKIASIRELIPRF YVEASILKCNKFLSKTGISECLPRLTCMIRGIGDPLVSVYARAYLCRVGM EVAPHLKETLNKNFFDFLLTFKQIHGDTVQ NQLVVGVELPSYLYPPAMDWIFQCISYHAPEALLTEMMERCKKLGNNALLNSVMSAFRAEFIA TRSMDFIGMIKE CDESGFPHLLFRSLGLNLALADPPESDRLQILNEAWKVITKLKNPQDYINCAEVWVEYTCKHFTKREVNTVLADVIKH MTPDRAFEDSYPLQLIHKVIAHFHDFSVLFSVEKFLPFLDMFQKESVRVEVCKCIMDAFIKHQQ MASW SHPQFEKGGGSGGGSGGSAWSHPQFEKSSGENLYFQGMGTALDIKIRANKVYHAGEVLSGVVVISSKDSV QHQQVSLTMEGTVNQLSASVGVFEAFYNSVKPIQIINSTIEMVKPGKFPSGKTEIPFEFPLHLKGNKVLYETYHGVFV NIQYTLRCMDMKRSLAKDLTKTCEFIVHSAPQKGKFTSPVDFTITPETLQNVKERALLPKFLLRGHLNSTNCVITQPLT GELVVESSEAAIRSVLQLVRVETCGCAEGYARDATEIQNIQIADGDVCRGLSVPIYMFPRFLTCTPTLETTNFKVEFEV NIVLLHPDHLITENFPLKLCRI MLVLVLGDLHIPHRCNSLPAKFKLLVPGKIQHILCTGNLCTKESYDYLKTLAGDVHIVRGDFDENLNYPEQKVVTVGQ FKIGLIHGHQVIPWGDMA SLALLQRQFDVILISGHTHKFEAFEHENKFYINPGSATGAYNALETNIIPSFVLMDIQASTV VTYVYQLIGDDVKVERIEYKKP
144	> His-TEV-VPS35L ¹⁻⁴³⁶ -TwinStrep-TEV-VPS26C-VPS29 MGSSHHHHHHSSGENLYFQGMMAVFPWHSRNRNYKAEFASCRLEAVPLEFGDYHPLKPITVTESKTKKVNRRKGSTSS TSSSSSSSVVDPLSSVLDGTDPLSMFAATADPAALAAAMDSSRRKRDRDDNSVVGSDFEFWTNKRGEILARYTTTEK LSINLFMGSEKKGAGTATLAMSEKVRTRLEELDDFEEGSQKELLNLTQQDYVNRIEELNQSLKDAWASDQVKALKIVI QCSKLLSDTSVIQFYPSKFVLITDILDTFGKLVYERIFSMCVDSSRSVLPDHFSPENANDTAKETCLNWWFFKIASIRELIPRF YVEASILKCNKFLSKTGISECLPRLTCMIRGIGDPLVSVYARAYLCRVGM EVAPHLKETLNKNFFDFLLTFKQIHGDTVQ NQLVVGVELPSYLYPPAMDWIFQCISYHAPEALLTEMMERCKKLGNNALLNSVMSAFR MASW SHPQFEKGGGSGGGSGGSAWSHPQFEKSSGENLYFQGMGTALDIKIRANKVYHAGEVLSGVVVISSKDSV QHQQVSLTMEGTVNQLSASVGVFEAFYNSVKPIQIINSTIEMVKPGKFPSGKTEIPFEFPLHLKGNKVLYETYHGVFV NIQYTLRCMDMKRSLAKDLTKTCEFIVHSAPQKGKFTSPVDFTITPETLQNVKERALLPKFLLRGHLNSTNCVITQPLT GELVVESSEAAIRSVLQLVRVETCGCAEGYARDATEIQNIQIADGDVCRGLSVPIYMFPRFLTCTPTLETTNFKVEFEV NIVLLHPDHLITENFPLKLCRI MLVLVLGDLHIPHRCNSLPAKFKLLVPGKIQHILCTGNLCTKESYDYLKTLAGDVHIVRGDFDENLNYPEQKVVTVGQ FKIGLIHGHQVIPWGDMA SLALLQRQFDVILISGHTHKFEAFEHENKFYINPGSATGAYNALETNIIPSFVLMDIQASTV VTYVYQLIGDDVKVERIEYKKP
151	> His-TEV-VPS35L-GST-HRV3C-VPS29 MGSSHHHHHHSSGENLYFQGMMAVFPWHSRNRNYKAEFASCRLEAVPLEFGDYHPLKPITVTESKTKKVNRRKGSTSS TSSSSSSSVVDPLSSVLDGTDPLSMFAATADPAALAAAMDSSRRKRDRDDNSVVGSDFEFWTNKRGEILARYTTTEK LSINLFMGSEKKGAGTATLAMSEKVRTRLEELDDFEEGSQKELLNLTQQDYVNRIEELNQSLKDAWASDQVKALKIVI QCSKLLSDTSVIQFYPSKFVLITDILDTFGKLVYERIFSMCVDSSRSVLPDHFSPENANDTAKETCLNWWFFKIASIRELIPRF YVEASILKCNKFLSKTGISECLPRLTCMIRGIGDPLVSVYARAYLCRVGM EVAPHLKETLNKNFFDFLLTFKQIHGDTVQ NQLVVGVELPSYLYPPAMDWIFQCISYHAPEALLTEMMERCKKLGNNALLNSVMSAFRAEFIA TRSMDFIGMIKE CDESGFPHLLFRSLGLNLALADPPESDRLQILNEAWKVITKLKNPQDYINCAEVWVEYTCKHFTKREVNTVLADVIKH MTPDRAFEDSYPLQLIHKVIAHFHDFSVLFSVEKFLPFLDMFQKESVRVEVCKCIMDAFIKHQQEPTKDPVILNALLH VCKTMHDSVNALTLEDEKRM LSYLINGFIKMVSFGRDFEQQLSFYVESRSMFCNLEPVLVQLIHSVNRLAMETRKMVK GNHSRKTA AFVRACVAYCFITIPSLAGIFTRLNLYLHSGQVALANQCLSQADAFFKAAISLVPEVPKMINIDGKMRPSES FLLEFLCNFFSTLLIVPDHPEHGVFLVRELLNVIQDYTWEDNSDEKIRIYTCVLHLLSAMSQETYLHYIDKVDSDNSLYG

	GDSKFLAENNNKLCETVMAQILEHLKTLAKDEALKRQSSLGLSFFNSILAHGDLRNNKLNQLSVNLWHLAQRHGCADTR TMVKTLEYIKKQSKQPDMDTHLTELALRLPLQTRT MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMALIRYIADKHN MLGGCPKERAEISMLEGAVLDIRYGVSRAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDA LDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIAWPLQGWQATFGGGDHPKPSLEVLFFQGPMLVLVLGD LHIPHRCNSLPKAFKKLLVPGIKIHILCTGNLCTKESYDYLKTLAGDVHIVRGDFDENLNYPEQKVVTVGQFKIGLIHGH QVIPWGDMAALLQRFQDVLISGHTHKFEAFEHENKFYINPGSATGAYNALETNIIPSFVLMDIQASTVVTVVYQLIG DDVKVERIEYKKP
231	> His-TEV-VPS35L^{R248E+W280D}-TwinStrep-TEV-VPS26C-VPS29 MGSSHHHHHHSSGENLYFQGMVAFWPWHSRNRNYKAEFASCRLEAVPLEFGDYHPLKPITVTESKTKKVNRRKGSTSS TSSSSSSSVVDPLSSVLDGTDPLSMFAATADPAALAAAMDSSRRKRDRDDNSVVGSDFFEPWTNKRGEILARYTTTEK LSINLFMGSEKGKAGTATLAMSEKVRTRLEELDDFEESQKELLNLTQQDYVNRIEELNQSLKDAWASDQVKALKIVI QCSKLLSDTSVIQFYPSKFVLITDILDTFGKLVYEEIFSMCVDSRSVLPDHFSPENANDTAKETCLNDFFKIASIRELIPRF YVEASILKCNKFLSKTGISECLPRLTCMIRGIGDPLVSVYARAYLCRVGMEVAPHLKETLNKNFFDFLLTFKQIHGDTVQ NQLVVQGVVELPSYLPYPPAMDWIFQCISYHAPEALLTEMMERCKKLGNNALLNSVMSAFRAEFIASTRSMDFIGMIKE CDESGFPKHLLFRSLGLNLALADPPESDRLQILNEAWKVITKLKNPQDYINCAEVWVEYTCKHFTKREVNTVLADVIKH MTPDRAFEDSYPLQLIHKVIAHFHDFSVLFSVEKFLPFLDMFQKESVRVEVCKCIMDAFIKHQQEPTKDPVILNALLH VCKTMHDSVNALTLEDEKRMLSYLINGFIKMSVSGRDFEQQLSFYVESRSMFCNLEPVLVQLIHSVNRLAMETRKMVK GNHSRKTAAFVRACVAYCFITIPSLAGIFTRLNLYLHSGQVALANQCLSQADAFFKAAISLVPEVPKMINIDGKMRPSES FLLEFLCNFFSTLLIVPDHPEHGVFLVRELLNVIQDYTWEDNSDEKIRIYTCVLHLLSAMSQETYLYHIDKVDSDNSLYG GDSKFLAENNNKLCETVMAQILEHLKTLAKDEALKRQSSLGLSFFNSILAHGDLRNNKLNQLSVNLWHLAQRHGCADTR TMVKTLEYIKKQSKQPDMDTHLTELALRLPLQTRT MASWSPQFEKGGGSGGGSGGSAWSPQFEKSSGENLYFQGMGTALDIKIRANKVYHAGEVLSGVVVISKDSV QHQQVSLTMEGTVNQLSAKSVGVFEAFYNSVKPIQIINSTIEMVKPGKFPSPGKTEIPFEFPLHLKGNKVLYETYHGVFV NIQYTLRCDMKRSLAKDLTKTCEFIVHSAPQKGKFTSPVDFTITPETLQNVKERALLPKFLLRGHLNSTNCVITQPLT GELVVESSAAIRSVELQLVRVETCGCAEGYARDATEIQNIQIADGDVCRGLSVPIYMFPRFLTCTPTLETTNFKVEFEV NIVLLHPDHLITENFPLKLCRI MLVLVLGDLHIPHRCNSLPKAFKKLLVPGIKIHILCTGNLCTKESYDYLKTLAGDVHIVRGDFDENLNYPEQKVVTVGQ FKIGLIHGHQVIPWGDMAALLQRFQDVLISGHTHKFEAFEHENKFYINPGSATGAYNALETNIIPSFVLMDIQASTV VTYVYQLIGDDVKVERIEYKKP
232	> His-TEV-VPS35L^{K157E+R161E}-TwinStrep-TEV-VPS26C-VPS29 MGSSHHHHHHSSGENLYFQGMVAFWPWHSRNRNYKAEFASCRLEAVPLEFGDYHPLKPITVTESKTKKVNRRKGSTSS TSSSSSSSVVDPLSSVLDGTDPLSMFAATADPAALAAAMDSSRRKRDRDDNSVVGSDFFEPWTNKRGEILARYTTTEK LSINLFMGSEKGKAGTATLAMSEVVRTLEELDDFEESQKELLNLTQQDYVNRIEELNQSLKDAWASDQVKALKIVI QCSKLLSDTSVIQFYPSKFVLITDILDTFGKLVYERIFSMCVDSRSVLPDHFSPENANDTAKETCLNWNFFKIASIRELIPRF YVEASILKCNKFLSKTGISECLPRLTCMIRGIGDPLVSVYARAYLCRVGMEVAPHLKETLNKNFFDFLLTFKQIHGDTVQ NQLVVQGVVELPSYLPYPPAMDWIFQCISYHAPEALLTEMMERCKKLGNNALLNSVMSAFRAEFIASTRSMDFIGMIKE CDESGFPKHLLFRSLGLNLALADPPESDRLQILNEAWKVITKLKNPQDYINCAEVWVEYTCKHFTKREVNTVLADVIKH MTPDRAFEDSYPLQLIHKVIAHFHDFSVLFSVEKFLPFLDMFQKESVRVEVCKCIMDAFIKHQQEPTKDPVILNALLH VCKTMHDSVNALTLEDEKRMLSYLINGFIKMSVSGRDFEQQLSFYVESRSMFCNLEPVLVQLIHSVNRLAMETRKMVK GNHSRKTAAFVRACVAYCFITIPSLAGIFTRLNLYLHSGQVALANQCLSQADAFFKAAISLVPEVPKMINIDGKMRPSES FLLEFLCNFFSTLLIVPDHPEHGVFLVRELLNVIQDYTWEDNSDEKIRIYTCVLHLLSAMSQETYLYHIDKVDSDNSLYG GDSKFLAENNNKLCETVMAQILEHLKTLAKDEALKRQSSLGLSFFNSILAHGDLRNNKLNQLSVNLWHLAQRHGCADTR TMVKTLEYIKKQSKQPDMDTHLTELALRLPLQTRT MASWSPQFEKGGGSGGGSGGSAWSPQFEKSSGENLYFQGMGTALDIKIRANKVYHAGEVLSGVVVISKDSV QHQQVSLTMEGTVNQLSAKSVGVFEAFYNSVKPIQIINSTIEMVKPGKFPSPGKTEIPFEFPLHLKGNKVLYETYHGVFV NIQYTLRCDMKRSLAKDLTKTCEFIVHSAPQKGKFTSPVDFTITPETLQNVKERALLPKFLLRGHLNSTNCVITQPLT GELVVESSAAIRSVELQLVRVETCGCAEGYARDATEIQNIQIADGDVCRGLSVPIYMFPRFLTCTPTLETTNFKVEFEV NIVLLHPDHLITENFPLKLCRI MLVLVLGDLHIPHRCNSLPKAFKKLLVPGIKIHILCTGNLCTKESYDYLKTLAGDVHIVRGDFDENLNYPEQKVVTVGQ FKIGLIHGHQVIPWGDMAALLQRFQDVLISGHTHKFEAFEHENKFYINPGSATGAYNALETNIIPSFVLMDIQASTV VTYVYQLIGDDVKVERIEYKKP
120	> His-TEV-VPS35L-TwinStrep-TEV-VPS26C-VPS29-mKate2 MGSSHHHHHHSSGENLYFQGMVAFWPWHSRNRNYKAEFASCRLEAVPLEFGDYHPLKPITVTESKTKKVNRRKGSTSS TSSSSSSSVVDPLSSVLDGTDPLSMFAATADPAALAAAMDSSRRKRDRDDNSVVGSDFFEPWTNKRGEILARYTTTEK LSINLFMGSEKGKAGTATLAMSEKVRTRLEELDDFEESQKELLNLTQQDYVNRIEELNQSLKDAWASDQVKALKIVI QCSKLLSDTSVIQFYPSKFVLITDILDTFGKLVYERIFSMCVDSRSVLPDHFSPENANDTAKETCLNWNFFKIASIRELIPRF YVEASILKCNKFLSKTGISECLPRLTCMIRGIGDPLVSVYARAYLCRVGMEVAPHLKETLNKNFFDFLLTFKQIHGDTVQ NQLVVQGVVELPSYLPYPPAMDWIFQCISYHAPEALLTEMMERCKKLGNNALLNSVMSAFRAEFIASTRSMDFIGMIKE CDESGFPKHLLFRSLGLNLALADPPESDRLQILNEAWKVITKLKNPQDYINCAEVWVEYTCKHFTKREVNTVLADVIKH MTPDRAFEDSYPLQLIHKVIAHFHDFSVLFSVEKFLPFLDMFQKESVRVEVCKCIMDAFIKHQQEPTKDPVILNALLH VCKTMHDSVNALTLEDEKRMLSYLINGFIKMSVSGRDFEQQLSFYVESRSMFCNLEPVLVQLIHSVNRLAMETRKMVK GNHSRKTAAFVRACVAYCFITIPSLAGIFTRLNLYLHSGQVALANQCLSQADAFFKAAISLVPEVPKMINIDGKMRPSES FLLEFLCNFFSTLLIVPDHPEHGVFLVRELLNVIQDYTWEDNSDEKIRIYTCVLHLLSAMSQETYLYHIDKVDSDNSLYG GDSKFLAENNNKLCETVMAQILEHLKTLAKDEALKRQSSLGLSFFNSILAHGDLRNNKLNQLSVNLWHLAQRHGCADTR TMVKTLEYIKKQSKQPDMDTHLTELALRLPLQTRT

	MASWSPHQFEKGGGSGGGSGGSAWSPHQFEKSSGENLYFQGMGTALDIKIKRANKVYHAGEVLSGVVVISSKDSV QHQQVSLTMEGTVNLQLSAKSVGVFEAFYNSVKPIQIINSTIEMVKPGKFPSGKTEIPFEFPLHLKGNKVLYETYHGVFV NIQYTLRCDMKRSLAKDLTKTCEFIVHSAPQKGKFTSPVDFTITPETLQNVKERALLPKFLLRGHLNSTNCVITQPLT GELVVESSEAAIRSVELQLVRVETCGCAEGYARDATEIQNIQIADGDVCRGLSVPIYMFVPRFLTCTPTLETTNFKVEFEV NIVVLLHPDHLITENFPLKLCRI MLVLVLGDLHIPHCNSLPAKFKLLVPGKIQHILCTGNLCTKESYDYKTLAGDVHIVRGDFDENLNYPEQKVVTVGQ FKIGLIHGHPWGDMSLALLQRQFDVDILISGHTHKFEAFEHENKFYINPGSATGAYNALETNIIPSFVLMIDIQASTV VTYVYQLIGDDVKVERIEYKKPGGGSSMVSELIKENMHMKLYMEGTVNNHHFKCTSEGEKPYEGTQTMRIKAVEG GPLPFAFDILATSFMYGSKTFINHTQGIPDFFKQSFPEGFTWERVTYEDGGVLTATQDTSLDGGLIYNVKIRGVNFP SNGPVMQKKTGWEASTETLYPADGGLEGRADMALKLVGGGHLICNLKTTYRSKKPAKNLKMGPVYVDRRLERIKE ADKETYVEQHEVAVARYCDLPSKLGHR
279	> FlagS-EGFP MDYKDDDDKKETA^{AAK}FERQHMDSGGGGTSGLEMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLT LKFICTTGKLPVPWPTLVTTLTGYVQCFSRYPDHMKQHDFKFSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVN RIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKNGIKVNFKIRHNIEDGSQLADHYQQNTPIGDGPVLLPDNHYL STQSALS KDPNEKRDHMLLEFVTAAGITLGMDELYK
284	> FlagS-L2^{FBR} MDYKDDDDKKETA^{AAK}FERQHMDSGGGGTSPDPAFVTTPTKLITYDNPAYEGIDVDNTLYF
285	> FlagS-L2^{FBR(N254A+Y257A)} MDYKDDDDKKETA^{AAK}FERQHMDSGGGGTSPDPAFVTTPTKLITYD^{APAA}EGIDVDNTLYF
286	> FlagS-L2 MDYKDDDDKKETA^{AAK}FERQHMDSGGGGTSPMRHKRSARTRKRASATQLYKTCKQAGTCPPDIIPKVEGKTIAEQIL QYGSMDGVFFGGLGIGTGSGTGGRTGYIPLGTRPPTATDTLAPVRPPLTVDPVGPSPDSIVSLVEETSFIDAGAPTSVPS IPPDVSGFSITTSTDTTPAILDINNTVTTVTTHNNPTFTDPSVLQPPTPAETGGHFTLSSTISTHNYEEIPMDTFIVSTNP NTVTSSTPIPGSRPVARLGLYSRTTQQVKVVDPAFVTTPTKLITYDNPAYEGIDVDNTLYFSSNDNSINIAPDPDFLDIVA LHRPALTSTRRTGIRYSRIGNKQTLRTRSGKSIGAKVHYYYDLSTIDPAEEIELQTITPSTYTTTSHAASPTSINNGLYDIYA DDFITDTSTTPVPSVPSTSLSGYIPANTTIPFGGAYNIPLVSGPDIPINITDQAPSLIIVPGSPQYTIADAGDFYLHPSYY MLRKRRKRLPYFFSDVSLAA
287	> FlagS-L2^(N254A+Y257A) MDYKDDDDKKETA^{AAK}FERQHMDSGGGGTSPMRHKRSARTRKRASATQLYKTCKQAGTCPPDIIPKVEGKTIAEQIL QYGSMDGVFFGGLGIGTGSGTGGRTGYIPLGTRPPTATDTLAPVRPPLTVDPVGPSPDSIVSLVEETSFIDAGAPTSVPS IPPDVSGFSITTSTDTTPAILDINNTVTTVTTHNNPTFTDPSVLQPPTPAETGGHFTLSSTISTHNYEEIPMDTFIVSTNP NTVTSSTPIPGSRPVARLGLYSRTTQQVKVVDPAFVTTPTKLITYD^{APAA}EGIDVDNTLYFSSNDNSINIAPDPDFLDIVA LHRPALTSTRRTGIRYSRIGNKQTLRTRSGKSIGAKVHYYYDLSTIDPAEEIELQTITPSTYTTTSHAASPTSINNGLYDIYA DDFITDTSTTPVPSVPSTSLSGYIPANTTIPFGGAYNIPLVSGPDIPINITDQAPSLIIVPGSPQYTIADAGDFYLHPSYY MLRKRRKRLPYFFSDVSLAA

Appendix Table S5. Steps for protein purification for each construct.

Identifier	Protein construct	Steps	Final buffer
pMLG31	GST	GST ₁ , TEV in roller, GST ₂ , S200 16/60	Hp150+TCEP
pMLG21	His-Sumo3-VPS26C	Ni ₁ , SenP2 + dialysis, Ni ₂ , Q, S75 10/300	Hp150+TCEP
pMLG6	GST-VPS29	GST ₁ , TEV + dialysis, GST ₂ , Q y S75 10/300	Hp150+TCEP
pMLG13	His-Sumo3-SNX17 _{FL}	Ni ₁ , SenP2 + dialysis, Ni ₂ y Q	Hp300+TCEP
pMLG135	His-Sumo3-SNX17 _{N108}	Ni ₁ , SenP2 + dialysis, Ni ₂ , Q, S200 16/60	Hp300+TCEP
pMLG1	His-Sumo3-SNX17 _{FERM-CT}	Ni ₁ , SenP2 + dialysis, Ni ₂ , Q, S200 16/60	Hp300+TCEP
pMLG14	His-Sumo3-EGFP-SNX17	Ni ₁ , SenP2 + dialysis, Ni ₂ , Q, S200 16/60	Hp300+TCEP
pMLG159	His-MBP-SNX17	Ni ₁ , TEV + dialysis, Ni ₂ , S200 16/60	Hp300+TCEP
pMLG179	His-MBP-SNX17 _{D467X}	Ni ₁ , TEV + dialysis, Ni ₂ , S200 16/60	Hp300+TCEP
pMLG178	His-MBP-SNX17 _{L470G}	Ni ₁ , TEV + dialysis, Ni ₂ , S200 16/60	Hp300+TCEP
pMLG203	His-MBP-SNX17 _{W321A}	Ni ₁ , TEV + dialysis, Ni ₂ , S200 16/60	Hp300+TCEP
pMLG204	His-MBP-SNX17 _{V380D}	Ni ₁ , TEV + dialysis, Ni ₂ , S200 16/60	Hp300+TCEP
pMLG205	His-MBP-SNX17 _{H457A}	Ni ₁ , TEV + dialysis, Ni ₂ , S200 16/60	Hp300+TCEP
pMLG206	His-MBP-SNX17 _{N459A+F462A}	Ni ₁ , TEV + dialysis, Ni ₂ , S200 16/60	Hp300+TCEP
pMLG18	GST-LRP1 _{ICD}	GST ₁ , S200 16/60	Hp150+TCEP
pMLG275	GST-TEV-LRP1 _{ICD-mut (N4470A+Y4473A)}	GST ₁ , S200 16/60	Hp150+TCEP
pMLG173	MBP-His10-LRP1 _{ICD}	Ni ₁ , TEV + dialysis, Ni ₂ , Q, S200 16/60	Hp200+TCEP
pMLG49	GST-APP _{ICD}	GST ₁ , S200 16/60	Hp150+TCEP
pMLG168	GST-TEV-ITGB1 _{ICD}	GST ₁ , S200 16/60	Hp150+TCEP
pMLG28	GST-L2 _{FBR}	GST ₁ , S200 16/60	Hp150+TCEP
pMLG276	GST-TEV-L2 _{FBR-mut (N254A+Y257A)}	GST ₁ , S200 16/60	Hp150+TCEP
pMLG131	GST-3C-His10-L2 _{FBR}	GST ₁ , HRV 3C in roller, GST ₂ , Ni ₁ , S75 10/300	Hp200+TCEP
pMLG85	His-VPS35L-TwinStrep-VPS26C-VPS29	Ni ₁ , Strept ₁ , TEV in roller, Ni ₂ , Strept ₂ , S200 10/300	Hp300+TCEP
pMLG122	His-VPS35L-TwinStrep-VPS26C-GST-VPS29	Ni ₁ , TEV + dialysis, GST ₁ , S200 16/60	Hp300+TCEP
pMLG118	His-VPS35L ₁₁₀₋₉₆₃ -TwinStrep-VPS26C-VPS29	Strept ₁ , Ni ₁ , TEV + dialysis/in roller, Ni ₂ , S200 10/300	Hp300+TCEP
pMLG116	His-VPS35L ₁₁₀₋₅₉₈ -TwinStrep-VPS26C-VPS29	Strept ₁ , Ni ₁ , TEV + dialysis, Ni ₂ , S200 10/300	Hp300+TCEP
pMLG138	His-VPS35L ₁₋₅₉₈ -TwinStrep-VPS26C-VPS29	Strept ₁ , Ni ₁ , TEV + dialysis, Ni ₂ , S200 10/300	Hp200+TCEP
pMLG144	His-VPS35L ₁₋₄₃₆ -TwinStrep-VPS26C-VPS29	Strept ₁ , Ni ₁ , TEV + dialysis, Ni ₂ , S200 10/300	Hp200+TCEP
pMLG151	His-VPS35L-GST-VPS29	Ni ₁ , TEV + dialysis, Ni ₂ , GST ₁ , HRV 3C in roller, GST ₂ , S200 10/300	Hp200+TCEP
pMLG231	His-VPS35L _{R248E+W280D} -TwinStrep-VPS26C-VPS29	Ni ₁ , Strept ₁ , TEV in roller, Ni ₂ , S200 10/300	Hp300+TCEP
pMLG232	His-VPS35L _{K157E+R161E} -TwinStrep-VPS26C-VPS29	Ni ₁ , Strept ₁ , TEV in roller, Ni ₂ , S200 10/300	Hp300+TCEP
pMLG120	His-VPS35L-TwinStrep-VPS26C-VPS29-mKate2	Ni ₁ , Strept ₁ , TEV in roller, Strept ₂ , S200 10/300	Hp200+TCEP

Ni₁: Nickel-based affinity chromatography of His-tagged-protein.

Ni₂: Nickel-based affinity chromatography of His-tagged-protein after protease incubation.

Strept₁: Strep-Tactin XT-based affinity chromatography of TwinStrep-tagged-protein.

Strept₂: Strep-Tactin XT-based affinity chromatography of TwinStrep-tagged-protein after protease incubation.

GST₁: Glutathione S-transferase (GST)-based affinity chromatography of GST-tagged-protein.

GST₂: GST-based affinity chromatography of GST-tagged-protein after protease incubation.

Amylose₁: Amylose-based affinity chromatography of Maltose-Binding Protein (MBP)-tagged-protein.

TEV: Incubation with Tobacco Etch Virus (TEV) protease.

Senp2: Incubation with Sentrin-specific protease 2 (Senp2) protease.

HRV 3C: Incubation with Human Rhinovirus (HRV) 3C protease.

Q: Ion exchange chromatography using a gradient of NaCl.

S75 10/300: Size exclusion chromatography with HiLoad 10/300 Superdex 75 column.
 S200 10/300: Size exclusion chromatography with HiLoad 10/300 Superdex 200 column.
 S200 16/60: Size exclusion chromatography with HiLoad 16/60 Superdex 200 column
 Hp200+TCEP: 25 mM Hepes pH 7.5, 200 mM NaCl and 1mM TCEP
 Hp300+TCEP: 25 mM Hepes pH 7.5, 300 mM NaCl and 1mM TCEP
 Tr200+TCEP: 50 mM Tris pH 7.5, 200 mM NaCl and 1mM TCEP

Appendix Table S6. DNA oligos used for RT-PCR.

RNA	Identifier	Sequence
L2 _{FBR}	369	AAATTCGAACGCCAGCACAT
	370	ACATCAATGCCTTCGTACGC
L2 _{FBR(N254A+Y257A)}	371	ACAAGAAAGAAACCGCTGCT
	372	ATCAGTTTGGTCGGGGTGG
L2 / L2 _{N254A+Y257A}	373	ACACCGTTACTAGCAGCACT
	374	GCAGCGCTACGATATCAAGG
EGFP	375	AAGGGCATCGACTTCAAGGA
	376	CTTCTCGTTGGGGTCTTTGC
Actin	ACTB Fw	CATGTACGTTGCTATCCAGGC
	ACTB Rv	CTCCTTAATGTCACGCACGAT

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