

Supplementary Table 1: DNA constructs used in this study.

Name	Description	Source/reference	Identifier
Recombinant Protein preparation			
pAV5a empty vector	pAV5a empty vector for insect cell expression	Published ¹	pAV5a
pMalC2Tev empty vector	pMalC2Tev empty vector for bacterial expression of MBP-Tev-tagged proteins	Published ¹	pMalTev
pGexTev empty vector	pGexTev empty vector for bacterial expression of GST-Tev-tagged proteins	Published ¹	pGexTev
VPS35L	VPS35L (1-963, full-length, codon optimized for <i>E. coli</i> expression) in pAV5a vector	This study	pDB122
VPS35L point mutants	V205D R248M T276W N279L N279W W280Y W280D K283D V205D/R248M	This study This study This study This study This study This study This study This study This study	pDB155 pDB156 pDB139 pDB157 pDB138 pDB158 pDB137 pDB159 pDB136
VPS26C	VPS26C (1-297, full-length) in pAV5a vector	Published ⁵	pDB48
VPS26C point mutant	K14E	This study	pDB160
MBP-VPS26C	VPS26C (1-297, full-length, codon-optimized for <i>E. coli</i> expression) in pMalC2Tev vector	This study	pDB72
VPS29-His ₆	VPS29-Tev-(GGS) ₂ -His ₆ in pAV5a vector	Published ⁵	pDB47
MBP-CCDC22 NN-CH-VBD	MBP-Tev-CCDC22 (1-118)-(GGSK) ₆ -CCDC22 (436-727) in pMalC2Tev vector	Published ⁵	pDB79
MBP-CCDC93 VBD	MBP-Tev-hCCDC93 (442-631) in pMalC2Tev vector	Published ⁵	pDB80
GST-SNX17	GST-Tev-hSNX17 (1-470, full-length) in modified pET vector	Published ⁴	pDB77
GST-SNX17 truncations	Δ470 Δ467-470	This study This study	pDB176 pDB177
GST-SNX17 CT	GST-SNX17 (451-470) in modified pET vector	This study	pDB169
GST-SNX17 CT point mutants	I465L I465V G466A G466R G466L G466E E468A E468R E468L E468G L470V L470I L470G	This study This study	pDB179 pDB180 pDB181 pDB182 pDB183 pDB184 pDB185 pDB186 pDB187 pDB188 pDB189 pDB190 pDB191
GST-SNX31 CT	GST-SNX31 (421-440) in pGexTev vector	This study	pDB170
GST-LRMDA CT	GST-LRMDA (179-198) in pGexTev vector	This study	pDB171
GST-TIMM23 CT	GST-TIMM23 (190-209) in pGexTev vector	This study	pDB172
GST-PATE1 CT	GST-PATE1 (107-126) in pGexTev vector	This study	pDB173
GST-ARHGEF25 CT	GST-ARHGEF25 (561-580) in pGexTev vector	This study	pDB174

GST-HYOU1 CT	GST-HYOU1 (980-999) in pGexTev vector	This study	pDB175
Mammalian expression vectors			
pEBB	Empty vector	Published ²	EB006
pEBB-VPS35L-2xHA	Full length, also referred to as wild-type (WT)	Published ³	EB1758
pEBB-VPS35L-2xHA point mutations	N279W W280D V205D/R248M	This study	EB1919, EB1918, EB1917
pEBB-HA-VPS26C	Wild-type (WT)	This study	EB1354
pLVX	Empty vector	Takara	EB1611
pLVX-VPS35L-2xHA point mutations	Wild-type (WT) N279W W280D V205D/R248M	Published ³ This study	EB1778, EB1925, EB1924, EB1923
pCI2-GFP-FLAG-SNX17	Wild-type (WT)	This study	EB1915
pcDNA 3.1-FLAG-SNX31	Wild-type (WT)	This study	EB1931

References

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Supplementary Table 2: Sequences of recombinant proteins used in this study.

Only sequences in the final product (i.e., after protease cleavage to remove the affinity tag) are shown and are annotated by corresponding colors.

> VPS35L ^{v205D}
MAVFPWHSRNRNYKAEFASCRLEAVPLEFGDYHPLKPITVTESKTKVNRKGSTSSTSSSSSVDPSSVLDGTDPLSMFAATADPAALAAAMDSSRRKRDRDDNSVV GSDFEPWTNKRGEILARYTTTEKLSINLFGMSEKGAGTATLAMSEKVRTRLEELDDFEEGSQKELLNLTQDYNRIEELNQSLKDAWASDQKVKALKIVIQCCKSLLSD TSVIQFYPKFVLITDILDFGKLVYERIFSMCVDERSVLPDHFSPEANANDTAKECLCNWFFKIASIRELI P RFYVEASILKCNFKLSKTGISECLPRLTCMIRGIDPL VSVYARAYLCRVGMEAVPHLKETLNKNFFDFLTFQIHGDTVNQLVVGVELPSYLPLYPAMDWIFQCISYHAPEALLTEMMERCKKLGNNALLNSVMSAFRAEFI ATRSMDFIGMIKECDESGFPKHLLFRSLGLNLALADPPESDRQLQILNEAWKVITKLKNPQDYINCAEVWVEYTKHFTKREVNTVLADVIKHMTPDRAFEDSYPLQLII KKVIAHFHDFSVLFSVEKFLPFLDMFQKESVRVEVKCIMDAFIKHQQEPTKDPVILNALLHVCKTMHDHSVNALEDEKRMILSYLINGFIKMVSFGRDFEQQLSFYVES RSMFCNLEPVVLVQLIHSVNRЛАМЕТРКВМКГНHSRКТААФVRACVAYCFITIPSLAGIFTRNLНLHSGQVALANQCLSQADAFFKAAISLVPEVPKMINIDGKMRPSES FLLEFLCNFFSTLLIVPDHPHEGVLFVRELLNVIQDYTWEDEKIRIYTCVLHLLSAMSQETYLYHIDKVDNSDLSYGGDSKFLAENNKLСЕТВМАQILEHLKTLAK DEALKRQSSLGLSFFNSILAHGDLRNNKLNQSLVNLWHLAQRHGCADTRTMVKLEYIKKQSKQPDMLHTELALRLPLQTRT
> VPS35L ^{R248M}
MAVFPWHSRNRNYKAEFASCRLEAVPLEFGDYHPLKPITVTESKTKVNRKGSTSSTSSSSSVDPSSVLDGTDPLSMFAATADPAALAAAMDSSRRKRDRDDNSVV GSDFEPWTNKRGEILARYTTTEKLSINLFGMSEKGAGTATLAMSEKVRTRLEELDDFEEGSQKELLNLTQDYNRIEELNQSLKDAWASDQKVKALKIVIQCCKSLLSD TSVIQFYPKFVLITDILDFGKLVYERIFSMCVDERSVLPDHFSPEANANDTAKECLCNWFFKIASIRELI P RFYVEASILKCNFKLSKTGISECLPRLTCMIRGIDPL VSVYARAYLCRVGMEAVPHLKETLNKNFFDFLTFQIHGDTVNQLVVGVELPSYLPLYPAMDWIFQCISYHAPEALLTEMMERCKKLGNNALLNSVMSAFRAEFI ATRSMDFIGMIKECDESGFPKHLLFRSLGLNLALADPPESDRQLQILNEAWKVITKLKNPQDYINCAEVWVEYTKHFTKREVNTVLADVIKHMTPDRAFEDSYPLQLII KKVIAHFHDFSVLFSVEKFLPFLDMFQKESVRVEVKCIMDAFIKHQQEPTKDPVILNALLHVCKTMHDHSVNALEDEKRMILSYLINGFIKMVSFGRDFEQQLSFYVES RSMFCNLEPVVLVQLIHSVNRЛАМЕТРКВМКГНHSRКТААФVRACVAYCFITIPSLAGIFTRNLНLHSGQVALANQCLSQADAFFKAAISLVPEVPKMINIDGKMRPSES FLLEFLCNFFSTLLIVPDHPHEGVLFVRELLNVIQDYTWEDEKIRIYTCVLHLLSAMSQETYLYHIDKVDNSDLSYGGDSKFLAENNKLСЕТВМАQILEHLKTLAK DEALKRQSSLGLSFFNSILAHGDLRNNKLNQSLVNLWHLAQRHGCADTRTMVKLEYIKKQSKQPDMLHTELALRLPLQTRT
> VPS35L ^{T276W}
MAVFPWHSRNRNYKAEFASCRLEAVPLEFGDYHPLKPITVTESKTKVNRKGSTSSTSSSSSVDPSSVLDGTDPLSMFAATADPAALAAAMDSSRRKRDRDDNSVV GSDFEPWTNKRGEILARYTTTEKLSINLFGMSEKGAGTATLAMSEKVRTRLEELDDFEEGSQKELLNLTQDYNRIEELNQSLKDAWASDQKVKALKIVIQCCKSLLSD TSVIQFYPKFVLITDILDFGKLVYERIFSMCVDERSVLPDHFSPEANANDTAKE W CWNWFFKIASIRELI P RFYVEASILKCNFKLSKTGISECLPRLTCMIRGIDPL VSVYARAYLCRVGMEAVPHLKETLNKNFFDFLTFQIHGDTVNQLVVGVELPSYLPLYPAMDWIFQCISYHAPEALLTEMMERCKKLGNNALLNSVMSAFRAEFI ATRSMDFIGMIKECDESGFPKHLLFRSLGLNLALADPPESDRQLQILNEAWKVITKLKNPQDYINCAEVWVEYTKHFTKREVNTVLADVIKHMTPDRAFEDSYPLQLII KKVIAHFHDFSVLFSVEKFLPFLDMFQKESVRVEVKCIMDAFIKHQQEPTKDPVILNALLHVCKTMHDHSVNALEDEKRMILSYLINGFIKMVSFGRDFEQQLSFYVES RSMFCNLEPVVLVQLIHSVNRЛАМЕТРКВМКГНHSRКТААФVRACVAYCFITIPSLAGIFTRNLНLHSGQVALANQCLSQADAFFKAAISLVPEVPKMINIDGKMRPSES FLLEFLCNFFSTLLIVPDHPHEGVLFVRELLNVIQDYTWEDEKIRIYTCVLHLLSAMSQETYLYHIDKVDNSDLSYGGDSKFLAENNKLСЕТВМАQILEHLKTLAK DEALKRQSSLGLSFFNSILAHGDLRNNKLNQSLVNLWHLAQRHGCADTRTMVKLEYIKKQSKQPDMLHTELALRLPLQTRT
> VPS35L ^{N279L}
MAVFPWHSRNRNYKAEFASCRLEAVPLEFGDYHPLKPITVTESKTKVNRKGSTSSTSSSSSVDPSSVLDGTDPLSMFAATADPAALAAAMDSSRRKRDRDDNSVV GSDFEPWTNKRGEILARYTTTEKLSINLFGMSEKGAGTATLAMSEKVRTRLEELDDFEEGSQKELLNLTQDYNRIEELNQSLKDAWASDQKVKALKIVIQCCKSLLSD TSVIQFYPKFVLITDILDFGKLVYERIFSMCVDERSVLPDHFSPEANANDTAKE L WFFKIASIRELI P RFYVEASILKCNFKLSKTGISECLPRLTCMIRGIDPL VSVYARAYLCRVGMEAVPHLKETLNKNFFDFLTFQIHGDTVNQLVVGVELPSYLPLYPAMDWIFQCISYHAPEALLTEMMERCKKLGNNALLNSVMSAFRAEFI ATRSMDFIGMIKECDESGFPKHLLFRSLGLNLALADPPESDRQLQILNEAWKVITKLKNPQDYINCAEVWVEYTKHFTKREVNTVLADVIKHMTPDRAFEDSYPLQLII KKVIAHFHDFSVLFSVEKFLPFLDMFQKESVRVEVKCIMDAFIKHQQEPTKDPVILNALLHVCKTMHDHSVNALEDEKRMILSYLINGFIKMVSFGRDFEQQLSFYVES RSMFCNLEPVVLVQLIHSVNRЛАМЕТРКВМКГНHSRКТААФVRACVAYCFITIPSLAGIFTRNLНLHSGQVALANQCLSQADAFFKAAISLVPEVPKMINIDGKMRPSES FLLEFLCNFFSTLLIVPDHPHEGVLFVRELLNVIQDYTWEDEKIRIYTCVLHLLSAMSQETYLYHIDKVDNSDLSYGGDSKFLAENNKLСЕТВМАQILEHLKTLAK DEALKRQSSLGLSFFNSILAHGDLRNNKLNQSLVNLWHLAQRHGCADTRTMVKLEYIKKQSKQPDMLHTELALRLPLQTRT
> VPS35L ^{N279W}
MAVFPWHSRNRNYKAEFASCRLEAVPLEFGDYHPLKPITVTESKTKVNRKGSTSSTSSSSSVDPSSVLDGTDPLSMFAATADPAALAAAMDSSRRKRDRDDNSVV GSDFEPWTNKRGEILARYTTTEKLSINLFGMSEKGAGTATLAMSEKVRTRLEELDDFEEGSQKELLNLTQDYNRIEELNQSLKDAWASDQKVKALKIVIQCCKSLLSD TSVIQFYPKFVLITDILDFGKLVYERIFSMCVDERSVLPDHFSPEANANDTAKE W FFKIASIRELI P RFYVEASILKCNFKLSKTGISECLPRLTCMIRGIDPL VSVYARAYLCRVGMEAVPHLKETLNKNFFDFLTFQIHGDTVNQLVVGVELPSYLPLYPAMDWIFQCISYHAPEALLTEMMERCKKLGNNALLNSVMSAFRAEFI ATRSMDFIGMIKECDESGFPKHLLFRSLGLNLALADPPESDRQLQILNEAWKVITKLKNPQDYINCAEVWVEYTKHFTKREVNTVLADVIKHMTPDRAFEDSYPLQLII KKVIAHFHDFSVLFSVEKFLPFLDMFQKESVRVEVKCIMDAFIKHQQEPTKDPVILNALLHVCKTMHDHSVNALEDEKRMILSYLINGFIKMVSFGRDFEQQLSFYVES RSMFCNLEPVVLVQLIHSVNRЛАМЕТРКВМКГНHSRКТААФVRACVAYCFITIPSLAGIFTRNLНLHSGQVALANQCLSQADAFFKAAISLVPEVPKMINIDGKMRPSES FLLEFLCNFFSTLLIVPDHPHEGVLFVRELLNVIQDYTWEDEKIRIYTCVLHLLSAMSQETYLYHIDKVDNSDLSYGGDSKFLAENNKLСЕТВМАQILEHLKTLAK DEALKRQSSLGLSFFNSILAHGDLRNNKLNQSLVNLWHLAQRHGCADTRTMVKLEYIKKQSKQPDMLHTELALRLPLQTRT
> VPS35L ^{W280Y}
MAVFPWHSRNRNYKAEFASCRLEAVPLEFGDYHPLKPITVTESKTKVNRKGSTSSTSSSSSVDPSSVLDGTDPLSMFAATADPAALAAAMDSSRRKRDRDDNSVV GSDFEPWTNKRGEILARYTTTEKLSINLFGMSEKGAGTATLAMSEKVRTRLEELDDFEEGSQKELLNLTQDYNRIEELNQSLKDAWASDQKVKALKIVIQCCKSLLSD TSVIQFYPKFVLITDILDFGKLVYERIFSMCVDERSVLPDHFSPEANANDTAKE Y FFKIASIRELI P RFYVEASILKCNFKLSKTGISECLPRLTCMIRGIDPL VSVYARAYLCRVGMEAVPHLKETLNKNFFDFLTFQIHGDTVNQLVVGVELPSYLPLYPAMDWIFQCISYHAPEALLTEMMERCKKLGNNALLNSVMSAFRAEFI ATRSMDFIGMIKECDESGFPKHLLFRSLGLNLALADPPESDRQLQILNEAWKVITKLKNPQDYINCAEVWVEYTKHFTKREVNTVLADVIKHMTPDRAFEDSYPLQLII KKVIAHFHDFSVLFSVEKFLPFLDMFQKESVRVEVKCIMDAFIKHQQEPTKDPVILNALLHVCKTMHDHSVNALEDEKRMILSYLINGFIKMVSFGRDFEQQLSFYVES RSMFCNLEPVVLVQLIHSVNRЛАМЕТРКВМКГНHSRКТААФVRACVAYCFITIPSLAGIFTRNLНLHSGQVALANQCLSQADAFFKAAISLVPEVPKMINIDGKMRPSES FLLEFLCNFFSTLLIVPDHPHEGVLFVRELLNVIQDYTWEDEKIRIYTCVLHLLSAMSQETYLYHIDKVDNSDLSYGGDSKFLAENNKLСЕТВМАQILEHLKTLAK DEALKRQSSLGLSFFNSILAHGDLRNNKLNQSLVNLWHLAQRHGCADTRTMVKLEYIKKQSKQPDMLHTELALRLPLQTRT
> VPS35L ^{W280D}

MAVFPWHSRNRNYKAEFASCRLEAVPLEFGDYHPLKPITVTESKTKVNRKGSTSSTSSSSSVDPLOSSVLDGTDPLSMFAATADPAALAAAMDSSRRKRDRDDNSVV
GSDFPWPNKRGEILARYTTTEKLSINLFGMSEKGKAGTATLAMSEKVRTRLEELDDFEEGSQKELLNLTQDQDVNRIELNQSLKDAWASDQKVALKIVIQCCKSLLSD
TSVIQFYPKFVLITDILDTFGKLVYERIFSMCVDRSRSPDHFSOPENANDTAKETCLND**D**FFKIASIRELIPRFYVEASILKCNFKLSKTGISECLPRLTCMIRGIDPL
VSVYARAYLCRVMGEAVPHLKETLNKNFFDFLTFQIHGDTVQNLVVGQVELPSYLPLYPAMDWIFQCISYHAPEALLTEMMERCKKLGNNALLNSVMSAFRAEFI
ATRSMDFIGMIKECDGESGFPKHLLFRSLGLNLAADPPESDRQLQILNEAWKVITKLKNPQDYINCAEVWVEYTCFKHTKREVNTVLADVIKHMTPDRAFEDSYPLQLII
KKVIAHFHDFSVLFSVEKFLPFLDMFQKESVRVEVKCIMDAFIKHQQEPTKDPVILNALHVCKTMHDHSVNALEDEKRMISYLINGFIKMVSFGRDFEQQLSFYVES
RSMFCNLIEPVLVQLIHSVRLAMETRKVMGNHSRKTAFFRACVACYCFITIPSLAGIFTRNLNYLHSGQVALANQCLSQADAFFKAAISLVPEVPMINIDGKMRPSES
FLLEFLCNFSTLLIVPDHPHEGVLFVRELLNVIQDWTWEDNSDEKIRIYTCVLHLLSAMSQETYLYHIDKVDNSDLSYGGDSKFLAENNKLCTVMAQILEHLKTLAK
DEALKRQSSLGLSFFNSILAHGDLRNKNLQLSVNLWHLAQRHGCADTRTMVKLEYIKKQSKQPDMLTHTELALRLPLQTRT

>**VPS35I**^{K28D}

MAVFPWHSRNRNYKAEFASCRLEAVPLEFGDYHPLKPITVTESKTKVNRKGSTSSTSSSSSVDPLOSSVLDGTDPLSMFAATADPAALAAAMDSSRRKRDRDDNSVV
GSDFPWPNKRGEILARYTTTEKLSINLFGMSEKGKAGTATLAMSEKVRTRLEELDDFEEGSQKELLNLTQDQDVNRIELNQSLKDAWASDQKVALKIVIQCCKSLLSD
TSVIQFYPKFVLITDILDTFGKLVYERIFSMCVDRSRSPDHFSOPENANDTAKETCLNW**F**DIASIRELIPRFYVEASILKCNFKLSKTGISECLPRLTCMIRGIDPL
VSVYARAYLCRVMGEAVPHLKETLNKNFFDFLTFQIHGDTVQNLVVGQVELPSYLPLYPAMDWIFQCISYHAPEALLTEMMERCKKLGNNALLNSVMSAFRAEFI
ATRSMDFIGMIKECDGESGFPKHLLFRSLGLNLAADPPESDRQLQILNEAWKVITKLKNPQDYINCAEVWVEYTCFKHTKREVNTVLADVIKHMTPDRAFEDSYPLQLII
KKVIAHFHDFSVLFSVEKFLPFLDMFQKESVRVEVKCIMDAFIKHQQEPTKDPVILNALHVCKTMHDHSVNALEDEKRMISYLINGFIKMVSFGRDFEQQLSFYVES
RSMFCNLIEPVLVQLIHSVRLAMETRKVMGNHSRKTAFFRACVACYCFITIPSLAGIFTRNLNYLHSGQVALANQCLSQADAFFKAAISLVPEVPMINIDGKMRPSES
FLLEFLCNFSTLLIVPDHPHEGVLFVRELLNVIQDWTWEDNSDEKIRIYTCVLHLLSAMSQETYLYHIDKVDNSDLSYGGDSKFLAENNKLCTVMAQILEHLKTLAK
DEALKRQSSLGLSFFNSILAHGDLRNKNLQLSVNLWHLAQRHGCADTRTMVKLEYIKKQSKQPDMLTHTELALRLPLQTRT

>**VPS35I**^{V205D/R248M}

MAVFPWHSRNRNYKAEFASCRLEAVPLEFGDYHPLKPITVTESKTKVNRKGSTSSTSSSSSVDPLOSSVLDGTDPLSMFAATADPAALAAAMDSSRRKRDRDDNSVV
GSDFPWPNKRGEILARYTTTEKLSINLFGMSEKGKAGTATLAMSEKVRTRLEELDDFEEGSQKELLNLTQDQDVNRIELNQSLKDAWASDQKVALKIVIQCCKSLLSD
TSVIQFYPKFVLITDILDTFGKLVYER**M**IFSMCVDRSRSPDHFSOPENANDTAKETCLNW**F**FKIASIRELIPRFYVEASILKCNFKLSKTGISECLPRLTCMIRGIDPL
VSVYARAYLCRVMGEAVPHLKETLNKNFFDFLTFQIHGDTVQNLVVGQVELPSYLPLYPAMDWIFQCISYHAPEALLTEMMERCKKLGNNALLNSVMSAFRAEFI
ATRSMDFIGMIKECDGESGFPKHLLFRSLGLNLAADPPESDRQLQILNEAWKVITKLKNPQDYINCAEVWVEYTCFKHTKREVNTVLADVIKHMTPDRAFEDSYPLQLII
KKVIAHFHDFSVLFSVEKFLPFLDMFQKESVRVEVKCIMDAFIKHQQEPTKDPVILNALHVCKTMHDHSVNALEDEKRMISYLINGFIKMVSFGRDFEQQLSFYVES
RSMFCNLIEPVLVQLIHSVRLAMETRKVMGNHSRKTAFFRACVACYCFITIPSLAGIFTRNLNYLHSGQVALANQCLSQADAFFKAAISLVPEVPMINIDGKMRPSES
FLLEFLCNFSTLLIVPDHPHEGVLFVRELLNVIQDWTWEDNSDEKIRIYTCVLHLLSAMSQETYLYHIDKVDNSDLSYGGDSKFLAENNKLCTVMAQILEHLKTLAK
DEALKRQSSLGLSFFNSILAHGDLRNKNLQLSVNLWHLAQRHGCADTRTMVKLEYIKKQSKQPDMLTHTELALRLPLQTRT

>**VPS26C**

MGTALDIKIKRANKVYHAGEVLSGVVVISSKDSVQHQGVSLTMGETVNLQLSAKSVGFEAFYNSVKPIQIINSTIEMVKPGKFPSGKTEIPFEFPLHLKGKVNLYETYH
GVFVNQIYTLRCDMKRSLLAKDLTKTCEFIVHSAPQKGKFTPSVDPFTITPETLQNVRERALLPKFLRGHLNSTNCVITQPLTGELEVSEAAIRSVELQLVRVETCG
CAEGYARDATEIQNIQIADGDVCVRGLSVPYIMVFPRLTCPLETTNFKVEFEVNIVVLLHPDHЛИЕНFPLKLCRI

>**VPS26C**^{K14}

MGTALDIKIKRANKVYHAGEVLSGVVVISSKDSVQHQGVSLTMGETVNLQLSAKSVGFEAFYNSVKPIQIINSTIEMVKPGKFPSGKTEIPFEFPLHLKGKVNLYETYH
GVFVNQIYTLRCDMKRSLLAKDLTKTCEFIVHSAPQKGKFTPSVDPFTITPETLQNVRERALLPKFLRGHLNSTNCVITQPLTGELEVSEAAIRSVELQLVRVETCG
CAEGYARDATEIQNIQIADGDVCVRGLSVPYIMVFPRLTCPLETTNFKVEFEVNIVVLLHPDHЛИЕНFPLKLCRI

>**MBP-Tev-VPS26C**

MKIEEGKLVIWINGDKGYNGLAEVGKFKEDTGIVKTVVEHPDKLEEKFPQVAATGDGPDIIFWAHDREFGGYAQSGLLAEITPDKAQDKLYPFTDAVRYNGKLIAYPIA
VEALSLIYNKDLLPNPPKTWEIIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFVLVDLICKNKHMNADTDYSIAEAAFNK
GETAMTINGPWAWSNIDTSKVNQGVTLVPLTFKGQPSKPFVGVLASAGINAASPNKELAKEFLENYLLTDEGLEAVNKKDPLGAVALKSYYEEELAKDPIRAATMENAQKGEI
MPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSNNNNNNNNNLGIEGRISEFENLYFQGH**MGTALDIKIKRANKVYHAGEVLSGVVVISSKDSVQHQGVSL**
TMGETVNLQLSAKSVGFEAFYNSVKPIQIINSTIEMVKPGKFPSGKTEIPFEFPLHLKGKVNLYETYHGVFVNQIYTLRCDMKRSLLAKDLTKTCEFIVHSAPQKGKFT
PSPVDFITPETLQNVRERALLPKFLRGHLNSTNCVITQPLTGELEVSEAAIRSVELQLVRVETCGCAEGYARDATEIQNIQIADGDVCVRGLSVPYIMVFPRLTCP
TLETTNFKVEFEVNIVVLLHPDHЛИЕНFPLKLCRI

>**VPS29-Tev-(GGS)₂-His₆** (corresponding to Q9UBQ0-2, isoform 2 in Uniprot)

MAGHRLVVLVLDLHPRHCNSLPAKFKLLVPGKQIHLICLGNLCTKESYDYLKTLAGDVHIVRGDFDENLNPYEQKVVTVQFKIGLIGHQVIPWGDMASLALLQRFQF
DVDILISGHTHKFEAFEHENKFYINPGSATGAYNALETNIIPSFVLMDIQASTVVTYVYQLIGDDVVKVERIEYKKPENLYFQGGSGSGHHHHHH

>**MBP-Tev-CCDC22 NN-CH (1-118)-(GGSK)₆-CCDC22 VBD (436-727)**

MKIEEGKLVIWINGDKGYNGLAEVGKFKEDTGIVKTVVEHPDKLEEKFPQVAATGDGPDIIFWAHDREFGGYAQSGLLAEITPDKAQDKLYPFTDAVRYNGKLIAYPIA
VEALSLIYNKDLLPNPPKTWEIIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFVLVDLICKNKHMNADTDYSIAEAAFNK
GETAMTINGPWAWSNIDTSKVNQGVTLVPLTFKGQPSKPFVGVLASAGINAASPNKELAKEFLENYLLTDEGLEAVNKKDPLGAVALKSYYEEELAKDPIRAATMENAQKGEI
MPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSNNNNNNNNNLGIEGRISEFENLYFQGH**MGTALDIKIKRANKVYHAGEVLSGVVVISSKDSVQHQGVSL**
LRVINPAVGSGLSPLLPLAMSARFLRMLSLAQACMDLGYPLELGQNFLYPSPEPDLRLLLFLAERLPTDASEDADQGGSKGGSKGGSKGGSKRKLQDCREL
ESSRRLAEIQLHQSVRAAEEARKEEYQKLMSELETLRPLDVRSLRAYTQRILEIVGNIRKQKEEITKILSDTKELKQKEINSLSGKLDRTFAVTDLFVKDAKDDAVR
KAYKYLAAHENCSQLIQTIEDTGTIMREVRDLEQIETELGKKTLSNLEKIREDYRALRQENAGLLGRVREA

>**MBP-Tev-CCDC93 VBD (442-631)**

MKIEEGKLVIWINGDKGYNGLAEVGKFKEDTGIVKTVVEHPDKLEEKFPQVAATGDGPDIIFWAHDREFGGYAQSGLLAEITPDKAQDKLYPFTDAVRYNGKLIAYPIA
VEALSLIYNKDLLPNPPKTWEIIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFVLVDLICKNKHMNADTDYSIAEAAFNK
GETAMTINGPWAWSNIDTSKVNQGVTLVPLTFKGQPSKPFVGVLASAGINAASPNKELAKEFLENYLLTDEGLEAVNKKDPLGAVALKSYYEEELAKDPIRAATMENAQKGEI
MPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSNNNNNNNNNLGIEGRISEFENLYFQGH**MGTALDIKIKRANKVYHAGEVLSGVVVISSKDSVQHQGVSL**
HRKIDEVPSRAELIQYQKRFELYRQISAVHKETQKFFTLYLDDKKVYLEKEISLLNSIHENFSQAMASPAARDQFLRQMEQIVEGIKQSRMKMEKKQENKMRDQL
NDQYLELLEKQRLYFKTVKEEGRKNEMLLSKVKAKAS

>**GST-Tev-SNX17**

MSPILGYWKIGLVPQPTRLLLEEKYEEHYERDEGDKWRNKKFELGLEFFNLPYYIDGDVKLTQSMAIIYIADKHNMLGGCPKERAIEISMLEGAVIDIRYGSRIA
YSKDFETLKVDFLSKLFPEMLKMFEDRCLCHTYLNGDHVTHPDFMLYDALDVLYMDPMCLDAFPKLCFKKRIEAPIQIDKYLSSKYIAWPLQGWQATFGGGDHPKAS
LVPRESENLYFQGSMHFSI**PETESRSGDGGSAVAYNIHVNGLHCRVRYSQLLGLHEQLRKEYGANVLPAPFPKKLFLSTP**AEEVQREQLEKYMQAVRQDPILLSSET
FNSFLRRAQQTQQPVTEEVSLLEVLLSNGQKVLNVNLTSQDTEVDLEAVAAKLDPDDLLIGYFSLFLVREKEDGAFSFVRKLQEFEPLYVSVTSLSRQSEYKIVLRKSYWD
SAYDDDVMENRVGLNLLYAQTVDIERGWLVTKEQHRLQKSLQEKVSKKEFLRLAQTQRLHYGYLRFDACVADPFPEKDCPVVVSAGNSELQIQLRLPGQQLREGSFRVTR
MRCWRVTSSVPLPSGSTSSPGRGRGEVRLAFAFEYILMSKDLQWVTTITSPQAIMMSICLQSMVDELMVKSGGSIRKMLRRVGGTLRSDSQQAQVKSPPLESPTARE
SMVKLSSKLSAVSLRGIGSPSTDASASDVHGNFAFEGIGDEDL

>**GST-Tev-SNX17 Δ470 (1-469)**

MSPILGYWKIGLVPQPTRLLLEEKYEEHYERDEGDKWRNKKFELGLEFFNLPYYIDGDVKLTQSMAIIYIADKHNMLGGCPKERAIEISMLEGAVIDIRYGSRIA
YSKDFETLKVDFLSKLFPEMLKMFEDRCLCHTYLNGDHVTHPDFMLYDALDVLYMDPMCLDAFPKLCFKKRIEAPIQIDKYLSSKYIAWPLQGWQATFGGGDHPKAS
LVPRESENLYFQGSMHFSI**PETESRSGDGGSAVAYNIHVNGLHCRVRYSQLLGLHEQLRKEYGANVLPAPFPKKLFLSTP**AEEVQREQLEKYMQAVRQDPILLSSET
FNSFLRRAQQTQQPVTEEVSLLEVLLSNGQKVLNVNLTSQDTEVDLEAVAAKLDPDDLLIGYFSLFLVREKEDGAFSFVRKLQEFEPLYVSVTSLSRQSEYKIVLRKSYWD
SAYDDDVMENRVGLNLLYAQTVDIERGWLVTKEQHRLQKSLQEKVSKKEFLRLAQTQRLHYGYLRFDACVADPFPEKDCPVVVSAGNSELQIQLRLPGQQLREGSFRVTR

MRCWRVTSSVPLPSGSTSSPGRGRGEVRELAFAYLMSKDRQLWVTTSPQAIMMSICLQSMVDELMVKSGGSIRKMLRRVGTLRRSDSQAVKSPPLLESPDATRE SMVKLSSKLSAVSLRGIGSPSTDASASDHGNFAFEGIGDED
>GST-Tev-SNX17 Δ467-470 (1-466) MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRSENLFYFQGSMSASDHGNFAFEGIGDED
>GST-Tev-SNX17 CT (451-470) MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRSENLFYFQGSMSASDHGNFAFEGIGDED
>GST-Tev-SNX17 CT (451-470) ^{I465L} MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRSENLFYFQGSMSASDHGNFAFEGIGDED
>GST-Tev-SNX17 CT (451-470) ^{I465V} MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRSENLFYFQGSMSASDHGNFAFEGIGDED
>GST-Tev-SNX17 CT (451-470) ^{G466A} MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRSENLFYFQGSMSASDHGNFAFEGIGDED
>GST-Tev-SNX17 CT (451-470) ^{G466R} MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRSENLFYFQGSMSASDHGNFAFEGIGDED
>GST-Tev-SNX17 CT (451-470) ^{G466L} MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRSENLFYFQGSMSASDHGNFAFEGIGDED
>GST-Tev-SNX17 CT (451-470) ^{G466E} MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRSENLFYFQGSMSASDHGNFAFEGIGDED
>GST-Tev-SNX17 CT (451-470) ^{E468A} MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRSENLFYFQGSMSASDHGNFAFEGIGDED
>GST-Tev-SNX17 CT (451-470) ^{E468R} MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRSENLFYFQGSMSASDHGNFAFEGIGDED
>GST-Tev-SNX17 CT (451-470) ^{E468L} MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRSENLFYFQGSMSASDHGNFAFEGIGDED
>GST-Tev-SNX17 CT (451-470) ^{E468G} MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRSENLFYFQGSMSASDHGNFAFEGIGDED
>GST-Tev-SNX17 CT (451-470) ^{I470V} MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRSENLFYFQGSMSASDHGNFAFEGIGDED
>GST-Tev-SNX17 CT (451-470) ^{I470I} MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRSENLFYFQGSMSASDHGNFAFEGIGDED
>GST-Tev-SNX17 CT (451-470) ^{I470G} MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRSENLFYFQGSMSASDHGNFAFEGIGDED
>GST-Tev-SNX31 CT (421-440) MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRSENLFYFQGHMSK1KIAKDDCVFGNIKEEDL
>GST-Tev-IRMDA CT (179-198) MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRGSENLYFQGHMRYYYYGKNSEGNRFIRDDQL
>GST-Tev-TIMM23 CT (190-209) MSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIIRYIADKHNLGGCPKERAIEISMLEGAVLDIRYGVRIA YSKDFETLKVDLSSLKPEMLKMFEDRCLCHKTLYNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFFKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKAS LVPRGSENLYFQGHMLAYLYNNWEHMKGSSLQSL

>**GST-Tev-PATE1** CT (107-126)

MSPILGYWKIKGLVQPTRLLEEKYEEHYERDEGDKWRNKKFELGLEFFPNLPYYIDGDVKLTQSMAIIRYIADKHNLGGCPKERAEIFSMLEGAVLDIRYGVSRIA
YSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTBPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKSD
LVPRGSENL~~YFQGHMSVYLVNFRCRSHDLCNEDL~~

>**GST-Tev-ARHGEF25** CT (561-580)

MSPILGYWKIKGLVQPTRLLEEKYEEHYERDEGDKWRNKKFELGLEFFPNLPYYIDGDVKLTQSMAIIRYIADKHNLGGCPKERAEIFSMLEGAVLDIRYGVSRIA
YSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTBPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKSD
LVPRGSENL~~YFQGHMPTPKTPPCQARIAKLDDEL~~

>**GST-Tev-HYOU1** CT (980-999)

MSPILGYWKIKGLVQPTRLLEEKYEEHYERDEGDKWRNKKFELGLEFFPNLPYYIDGDVKLTQSMAIIRYIADKHNLGGCPKERAEIFSMLEGAVLDIRYGVSRIA
YSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTBPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAPIQIDKYLKSSKYIAWPLQGWQATFGGDHPPKSD
LVPRGSENL~~YFQGHMEPEQKEQSTGQKRPLKNDEL~~

Supplementary Table 3: DNA oligos used in this study.

Purpose	Identifier and sequence, all 5' to 3'
VPS35L	<pre> GAACTCCTAAAAACCGCCACC oDB220120-14, GGTGGCGTTTTAGGAGTTC, reverse primer to open pAV5a oDB220120-15, TAATCTAGACGCTGCAGTCGAG, forward primer to open pAV5a cbyo-230217-3, CTCGGTCCGAACTCCTAAAAACCGCCACCATGGCAGTTTCCGTGGCATAG, SLIC VPS35Lopti into untagged pAV5a fw CGCTGCAGACCCGTACCTAATCTAGAGCCTGCAGTCTCGAGGCATGC cbyo-230217-4, GCATGCCTCGAGACTGCAGGCTTAGATTAGGTACGGGTCTGCAGCG, SLIC VPS35Lopti into untagged pAV5a bw </pre>
VPS35L ^{V205D}	<pre> djbo230823-1, ATAAAGCACTGAAAATTGTGATCCAGTG , Aliblunt for VPS35L V205D FW CATGGCAAGCGATCAGAAAG djbo230823-2, CTTCTGATCGCTTGCCCCATG , Aliblunt for VPS35L V205D BW </pre>
VPS35L ^{R248M}	<pre> djbo230823-3, ATGATTTTAGCATGTGTGTTGATAGCCGTAG , Aliblunt for VPS35L R248M FW GGATACCTTGGTAAACTGGTGTATGAA djbo230823-4, TTCATACACCAGTTTACCAAAGGTATCC, Aliblunt for VPS35L R248M BW </pre>
VPS35L ^{T276W}	<pre> Cbyo-230927-5, TGGTGTCTGAACTGGTTTCAAAATTGCCAG, Aliblunt for VPS35Lopti T276W fw GGAAAATGCAAATGATAACGCCAAAGAA Cbyo-230927-6, TTCTTGGCGGTATCATTGCATTTCC, Aliblunt for VPS35Lopti T276W bw </pre>
VPS35L ^{N279L}	<pre> djbo230823-5, CTGTGGTTTCAAAATTGCCAGCATTC , Aliblunt for VPS35L N279L FW GATACGCCAAAGAAACCTGTCTG djbo230823-6, CAGACAGGTTCTTGGCGGTATC , Aliblunt for VPS35L N279L BW </pre>
VPS35L ^{N279W}	<pre> Cbyo-230927-3, TGGTGGTTTCAAAATTGCCAGCATTG , Aliblunt for VPS35Lopti N279W fw GATACGCCAAAGAAACCTGTCTG Cbyo-230927-4, CAGACAGGTTCTTGGCGGTATC, Aliblunt for VPS35Lopti N279W bw </pre>
VPS35L ^{W280Y}	<pre> djbo230823-7, ATTTCCTCAAAATTGCCAGCATTGTCTG , Aliblunt for VPS35L W280Y FW GATACGCCAAAGAAACCTGTCTGAACT djbo230823-8, AGTCAGACAGGTTCTTGGCGGTATC, Aliblunt for VPS35L W280Y BW </pre>
VPS35L ^{W280D}	<pre> Cbyo-230927-1, GATTTTTCAAAATTGCCAGCATTGTGAAC, Aliblunt for VPS35Lopti W280D fw GATACGCCAAAGAAACCTGTCTGAAAC Cbyo-230927-2, GTTCAGACAGGTTCTTGGCGGTATC, Aliblunt for VPS35Lopti W280D bw </pre>
VPS35L ^{K283D}	<pre> djbo230823-9, GATATTGCCAGCATTGTGAAC , Aliblunt for VPS35L K283D FW CAAAGAAACCTGTCTGAACTGGTTTTC djbo230823-10, GAAAACCAAGTTCAGACAGGTTCTTTG, Aliblunt for VPS35L K283D BW </pre>
VPS26C ^{K14E}	<pre> djbo230824-1, GAAGTTTACGCCGGGGAAAGTG, Aliblunt for VPS26C K14E FW CCTGGACATCAAGATAAAAGAGCGAAT djbo230824-2, ATTCTGCTTTAATCTGATGTCCAGG, Aliblunt for VPS26C K14E BW </pre>
MBP-Tev-VPS26C	<pre> oDB220725-3, ATATTACATATGGCACCGACTGGATATC, Forward primer to add NdeI to VPS26Copti geneblock GCTGAAACTGTGCCGATTTAAGGATCCTAATAA oDB220725-4, TTATTAGGATCCTAAATGCCGACAGTTTCAGC, Reverse primer to add BamHI to VPS26Copti geneblock </pre>
GST-Tev-SNX17 Δ470 (1-469)	<pre> >djbo230715-1, taaCTCGAGCACCACCAAC, forward primer to truncate SNX17 L470 cgaggccattggagatgaggat >djbo230715-2, atcctcatctccaatgcctcg, reverse primer to truncate SNX17 L470 </pre>
GST-Tev-SNX17 Δ467-470 (1-466)	<pre> >djbo230715-1, taaCTCGAGCACCACCAAC, forward primer to truncate SNX17 467-470 <u>caatttcgccattcgagggcatttgg</u> >djbo230715-3, tccaatgcctcgaaaggcatttg, backward primer to truncate SNX17 467-470 </pre>
GST-Tev-SNX17 CT (451-470)	<pre> >djbo230715-4, <u>gccagtgcactgtatgtccac</u>, forward primer to truncate 1-450 <u>GATCTGAAACACTGTATTTCAGGGATCCatg</u> >djbo230715-5, catGGATCCCTGAAAATACAGGTTTCAGATC, reverse primer to truncate 1-450 </pre>
GST-Tev-SNX17 CT (451-470) ^{I465L}	<pre> >djbo-240209-1, CTGGGAGATGAGGATCTGTAACTCGAGC, FW primer to make I465L CAATTCGCTTCGAGGGC >djbo240209-2, <u>GCCCTCGAAGGCAGAAATTG</u>, RV primer to make I465L </pre>
GST-Tev-SNX17 CT (451-470) ^{I465V}	<pre> >djbo-240209-3, GTGGGAGATGAGGATCTGTAACTCGAGC, FW primer to make I465V >use djbo240209-2 as RV primer </pre>
GST-Tev-SNX17 CT (451-470) ^{G466A}	<pre> >djbo-240209-4, GCCGATGAGGATCTGTAACTCGAGCACC, FW primer to make G466A CAATTCGCTTCGAGGGCATT >djbo240209-5, AATGCCCTCGAAGGCAGAAATTG, RV primer to make G466A </pre>
GST-Tev-SNX17 CT (451-470) ^{G466R}	<pre> >djbo240209-6, CGTGATGAGGATCTGTAACTCGAGCACC, FW primer to make G466R >use djbo240209-5 as RV primer </pre>
GST-Tev-SNX17 CT (451-470) ^{G466L}	<pre> >djbo240209-7, CTGGATGAGGATCTGTAACTCGAGCACC, FW primer to make G466L >use djbo240209-5 as RV primer </pre>
GST-Tev-SNX17 CT (451-470) ^{G466E}	<pre> >djbo240209-8, GAGGTGAGGATCTGTAACTCGAGCACC, FW primer to make G466E >use djbo240209-5 as RV primer </pre>

GST-Tev-SNX17 CT (451-470) ^{E468A}	>djbo240209-9, CGCGATCTG TAA CTCGAGCACCACC, FW primer to make E468A CCTTCGAGGGCATTGGAGAT >djbo240209-10, ATCTCCAATGCCCTCGAAGG, RV primer to make E468A
GST-Tev-SNX17 CT (451-470) ^{E468R}	>djbo240209-11, CGTGATCTG TAA CTCGAGCACCACC, FW primer to make E468R >use djbo240209-10 as RV primer
GST-Tev-SNX17 CT (451-470) ^{E468L}	>djbo240209-12, CTGGATCTG TAA CTCGAGCACCACC, FW primer to make E468L >use djbo240209-10 as RV primer
GST-Tev-SNX17 CT (451-470) ^{E468G}	>djbo240209-13, GGCGATCTG TAA CTCGAGCACCACC, FW primer to make E468G >use djbo240209-10 as RV primer
GST-Tev-SNX17 CT (451-470) ^{L470V}	>djbo240209-14, GTGT AAC TCGAGCACCACAC, FW primer to make L470V GAGGGCATTGGAGATGAGGAT >djbo240209-15, ATCTCATCTCCAATGCCCTC, RV primer to make L470V
GST-Tev-SNX17 CT (451-470) ^{L470I}	>djbo240209-16, ATT TTAAC TCGAGCACCACACC, FW primer to make L470I >use djbo240209-15 as RV primer
GST-Tev-SNX17 CT (451-470) ^{L470G}	>djbo230715-1, GGCTaaCTCGAGCACCACACCAC, forward primer to mutate SNX17 L470G cttcgagggcattggagatgaggat >djbo230715-2, atcctatctccaatgccctcgaaag, reverse primer to mutate SNX17 L470G
GST-Tev-SNX31 CT (421-440)	>djbo240131-1, GTTTTCGGTAACATCAAAGAGGAGGACCTTAAATCGTGA CTGACTGACTGACGATCTGC , FW primer to aliblunt SNX31 tail into pGex CtgaaaacctgtatttcagggccatatgAGCAAGATTAAATGCAAAGGATGACTGT >djbo240131-2, ACAGTCATC TTGCAATTAAATCTGCT catatggccctgaaaatacaggtttcaG, RV primer to aliblunt SNX31 tail into pGex
GST-Tev-LRMDA CT (179-198)	>djbo240131-3, GGTAA TCGTTTATCCGTGACGATCAATTATAATCGTGA CTGACTGACGATCTGC, FW primer to aliblunt LRMDA tail into pGex CtgaaaacctgtatttcagggccatatgCGCTACGTGACTACGGAAAAATAGTGAA >djbo240131-4, TTCACTATTTCGCCGTAGTACACGTAGCGcatatggccctgaaaatacaggtttcaG, RV primer to aliblunt LRMDA tail into pGex
GST-Tev-TIMM23 CT (190-209)	>djbo240131-5, ATGAAAGGGTCTCTGTTACA ACAGAGTCTGTAATCGTGA CTGACTGACGATCTGC, FW primer to aliblunt TIMM23 tail into pGex Ctgaaaacctgtatttcagggccatatg TGTAACGCGCTGTACAATAATTGGGAGCAT >djbo240131-6, ATGCTCCAA TATTGTACAGCGCGTACatatggccctgaaaatacaggtttcaG , RV primer to aliblunt TIMM23 tail into pGex
GST-Tev-PATE1 CT (107-126)	>djbo240131-7, CGTTCGCACGATCTTGCAC ACGAGGACCTTAAATCGTGA CTGACTGACGATCTGC, FW primer to aliblunt PATE1 tail into pGex Ctgaaaacctgtatttcagggccatatg TCGGTATATTAGTGAATTTCGTTGCTGT >djbo240131-8, ACAGAACGAAA ATTCACTAAATATACCGAcata tggccctgaaaatacaggtttcaG, RV primer to aliblunt PATE1 tail into pGex
GST-Tev-ARHGEF25 CT (561-580)	>djbo240131-9, CGCTTGGCGAA ACTTGATGAAGATGAGCTGTAATCGTGA CTGACTGACGATCTGC, FW primer to aliblunt ARHGEF25 tail into pGex Ctgaaaacctgtatttcagggccatatg CCGACCCCCAAACTCCGCCATGCCAGGCC >djbo240131-10, GGCCTGGCATGGCGGAGTTGGGGGTCGGcatatggccctgaaaatacaggtttcaG, RV primer to aliblunt ARHGEF25 tail into pGex
GST-Tev-HYOU1 CT (980-999)	>djbo240131-11, CAGAACGCC CCCTGAAAAATGATGAGTTATAAATCGTGA CTGACTGACGATCTGC, FW primer to aliblunt HYOU1 tail into pGex Ctgaaaacctgtatttcagggccatatg GAGCCCGAGCAAAGGAGCAATCCACGGGC >djbo240131-12, GCCCGTGGATTGCTC TTTGTCTCGGGCTC catatggccctgaaaatacaggtttcaG, RV primer to aliblunt HYOU1 tail into pGex

Supplementary Table 4: Antibodies used in this study.

Primary antibodies				
WB, western blot; IF, immunofluorescence staining; FC, Flow Cytometry.				
Target	Source (host species)	Catalog, Clone (References)	Application (dilution)	Validation information
CCDC22	ProteinTech Group (rabbit)	16636-1-AP	WB (1:1000)	Phillips-Krawczak et al., 2015 ⁴
CCDC93	ProteinTech Group (rabbit)	20861-1-AP	WB (1:1000)	Phillips-Krawczak et al., 2015 ⁴
COMMD1	ProteinTech Group (rabbit)	11938-1-AP	WB (1:1000)	Manufacturer validation using various tissues and IF
DENND10	Custom made, Cocalico Biologicals (rabbit)	95-110 (Singla et al., 2019 ⁶)	WB (1:500)	Singla et al., 2019 ⁶
FAM21	Custom made, Cocalico Biologicals (rabbit)	MC2188 (Gomez and Billadeau, 2009 ⁷)	IF (1:1000)	Gomez and Billadeau, 2009 ⁷
FLAG	Sigma (mouse)	F1804, M2	WB (1:500)	Manufacturer validation with overexpressed proteins
HA	Biolegend (mouse)	901502, 16B12	WB (1:500), IF (1:100)	Manufacturer validation with overexpressed proteins
HA	Cell Signaling (mouse)	2999S, 6E2	WB (1:1000)	Manufacturer validation with overexpressed proteins
Integrin- α 5	BD Biosciences (mouse)	555615, IIA1	IF (1:100)	Manufacturer validation in FACS using isotype control
Integrin- β 1	Santa Cruz (mouse)	sc-53711, TS2/16	IF (1:100)	Manufacturer validation in FACS using isotype control
LRMDA	Abcam (rabbit)	150986	WB (1:500)	Manufacturer validation with overexpressed proteins
TIMM23	Proteintech Group (rabbit)	11123-1-AP	WB (1:500)	Manufacturer validation with immunoprecipitation
VPS26C	Millipore (rabbit)	ABN87	WB (1:5000)	Singla et al., 2019 ⁶
VPS29	GeneTex (rabbit)	GTX104768	WB (1:500)	Singla et al., 2019 ⁶
Secondary antibodies used for immunofluorescence				
Fluorophore	Source (target species)	Catalog number (dilution)		Validation
Alexa 488	Invitrogen (mouse)	A11029 (1:500)		Manufacturer validation in IF using no primary antibody controls
Alexa 555	Invitrogen (rabbit)	A21428 (1:500)		

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