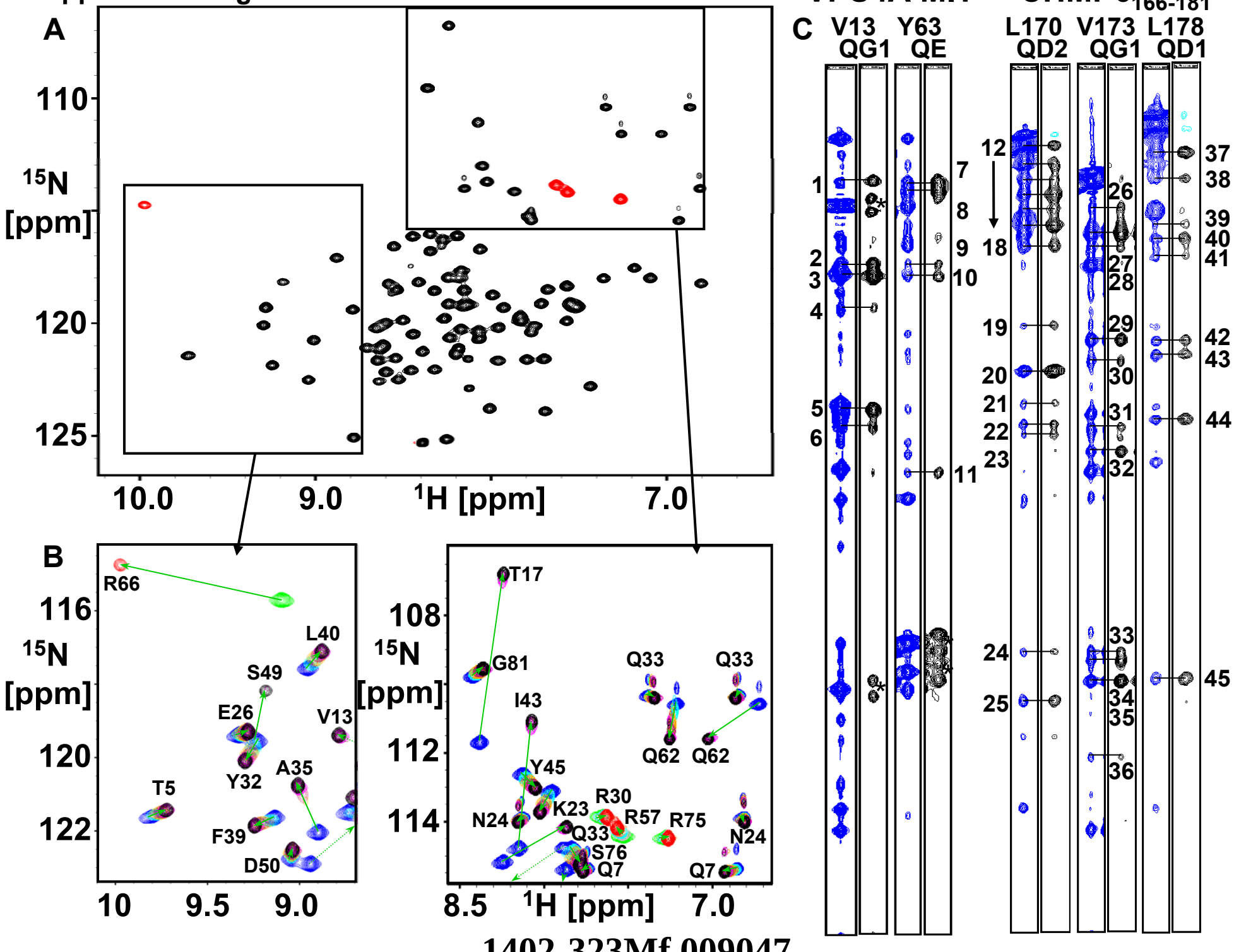
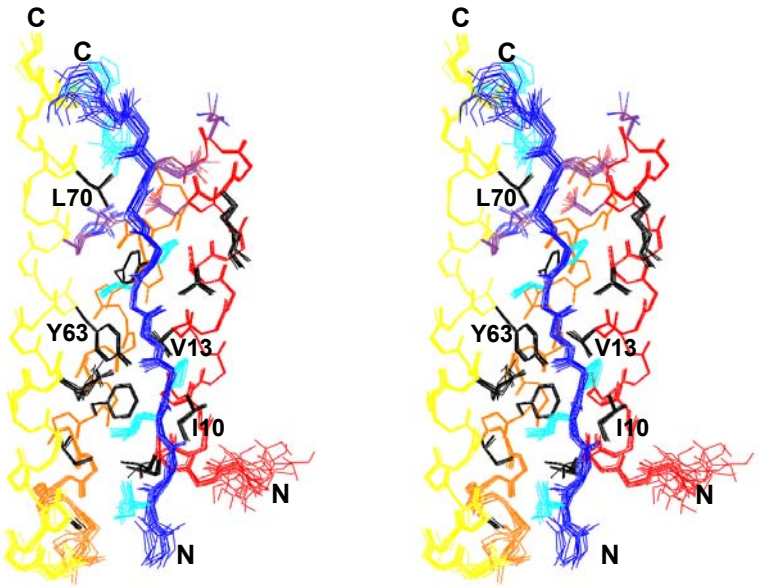
Supplemental Figure 1



1402-323Mf 009047

Supplemental Figure 2



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Supplemental Table 1. Measured Dissociation Constants for VPS4 MIT Domain-CHMP Protein Interactions

ESCRT-III Construct	VPS4A MIT K_D (μM)	VPS4B MIT K_D (μM)	VPS4A MIT L64D K_D (μM)	VPS4A MIT V13D K_D (μM)
$CHMP6_{1-201}$	38±7[a]	785(6)[c]		
$CHMP6_{1-181}$	23.2±0.2[a]		93.6(7)[c]	
$CHMP6_{150-181}$	8±1[b]			
$CHMP6_{155-181}$	7.0±0.1[b]			
$CHMP6_{160-181}$	6.6±0.1[b]			
$CHMP6_{165-181}$	5.8±0.8[a]		26.1±0.8[b]	>1,000[b]
$CHMP6_{170-181}$	8.0±0.1[b]			
$CHMP6_{175-181}$	>1,000[b]			
$CHMP6_{165-181,L170D}$	>1,000[b]			
$CHMP6_{165-181,V173D}$	>1,000[b]			
$CHMP6_{165-181,L178D}$	44.4(2)[c]			
$CHMP1B_{180-196}$	20±1[a]		>1,000[a]	23.2±0.7[b]
$CHMP4A_{1-222}$	384±28[b]	600(6)[c]		
$CHMP4A_{188-203}$	265±7[b]		893(7)[c]	
$CHMP4B_{1-224}$	204±3[b]	507(3)[c]		
$CHMP4B_{187-202}$	120±15[b]		360(3)[c]	
$CHMP4C_{1-233}$	810±30[b]			
$CHMP4C_{181-196}$	82±11[b]		248(2)[c]	
$CHMP5_{1-219}$	180(1)[c]	421(2)[c]		

[a]Dissociation constant is the mean of three or more independent measurements and the error is the standard deviation in the measurement.
[b]Dissociation constant is the mean of two independent measurements and the error is the range in the two measurements.
[c]Dissociation constant and error were estimated from a statistical fit of a single binding isotherm derived from triplicate measurements at 10 different VPS4A MIT protein concentrations. The number in parentheses represents the error in the last reported digit.

Supplemental Table 2. Alignment of CHMP6 MIM2 Sequences from 14 Eukaryotic Model Organisms

Organism	NCBI Accession Number	MIM2 Sequence
Homo Sapiens	NP_078867	.EQIELPEVPSEPLPEK.
Pongo pygmaeus	Q5R861	.EQIELPEVPSEPLPEK.
Macaca mulatta	XP_001110906	.EQIELPEVPSEPLPEK.
Mus musculus	NP_001078967	.EQMELPEVPSEPLPDR.
Rattus norvegicus	NP_001099326	.EQIELPEVPSEPLPDT.
Xenopus laevis	Q6NU11	.EDLELPEAPSEPLPDT.
Danio rerio	Q503V0	.ADLELPEVPGEELPEV.
Drosophila melanogaster	NP_726213	.KGAQLPDVPTEDLPIP.
Caenorhabditis elegans	NP_490762	.GTVQLPEAPSHELPEA.
Anopheles gambiae	EAA11586	.ISTRLPDVPDEELVLE.
Arabidopsis thaliana	NP_196488	.IVEDMPEVPTTELMPE.
Arabidopsis thaliana	NP_568980	.EKLDLPDVPTKTPVAS.
Leishmania infantum JPCM5	XP_001469630	.KLPEMPAVPSQKLPAQ.
Saccharomyces cerevisiae	NP_013794	.STEGLPSLPQGEQTEQ.
Schizosaccharomyces pombe	NP_596691	.GVDVLPSVPLKNAIPS.

CHMP6 protein sequences from 14 model organisms were identified using BLAST searches, and sequence alignments were created in Clustal W (http://align.genome.jp) by aligning them with *Homo sapiens* CHMP6 residues 166-181. The percent occurrence of amino acid residues at each position of the aligned CHMP6 MIM2 sequences is shown in Figure 2A.

Supplemental Table 3. Expression Vectors

1A. Bacterial Expression Vectors

Plasmid Name	Source	Reference	Internal ID	Backbone	Cloning Site	Epitope Tags
pGEX2T	Amersham		WISP01-69			N.GST
pET16B	Novagen		WISP01-71			N.6XHIS
pAED4	Matsudaira Lab		WISP07-61			N.10XHIS
pET16B-VPS4A$_{1-84}$	AF11215	A[8]	WISP05-43	pET16B	NdeI/BAMHI	N.6XHIS
pET16B-VPS4A$_{1-84,L64D}$	AF11215	B[8]	WISP07-120	pET16B	NdeI/BAMHI	N.6XHIS
pGEX2T-VPS4A$_{1-84}$	AF11215	B[8]	WISP05-60	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-VPS4A$_{1-84L64D}$	AF11215	B[8]	WISP07-117	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-VPS4A$_{1-84,V13D}$[1]	AF11215		WISP08-49	pGEX2T	NdeI/BAMHI	N.GST
pET16B-VPS4B$_{1-86}$	AF038960	B[8]	WISP04-153	pET16B	NdeI/BAMHI	N.6XHIS
pGEX2T-CHMP6$_{1-201}$[2]	BC010108		WISP07-133	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-CHMP6$_{1-181}$[2]	BC010109		WISP08-50	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-CHMP6$_{150-181}$[2]	BC010110		WISP08-51	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-CHMP6$_{155-181}$[2]	BC010111		WISP08-52	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-CHMP6$_{160-181}$[2]	BC010112		WISP08-53	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-CHMP6$_{165-181}$[2]	BC010113		WISP08-54	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-CHMP6$_{170-181}$[2]	BC010114		WISP08-55	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-CHMP6-$_{175-181}$[2]	BC010115		WISP08-56	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-CHMP6$_{165-181,L170D}$[2]	BC010117		WISP08-58	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-CHMP6$_{165-181,V173D}$[2]	BC010118		WISP08-59	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-CHMP6$_{165-181,L178D}$[2]	BC010118		WISP08-73	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-CHMP1B$_{180-196}$	AF306520	B[8]	WISP06-22	pGEX2T	NdeI/BAMHI	N.GST
pAED4-(HIS)$_{10}$-TRPΔLE-CHMP1A$_{180-196}$	NM_002768	B[8]	WISP06-62	pAED4	NdeI/BAMHI	N.10XHIS
pAED4-(HIS)$_{10}$-TRPΔLE-CHMP6$_{166-181}$[3]	BC010116		WISP08-57	pAED4	NdeI/BAMHI	N.10XHIS
pGEX2T-CHMP4A	BC010893	D[8]	WISP06-197	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-CHMP4A$_{188-203}$[7]	BC010893		WISP08-74	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-CHMP4B	AL050349	D[8]	WISP07-126	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-CHMP4B$_{187-202}$[7]	AL050349		WISP08-75	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-CHMP4C	BC002463	F[8]	WISP06-200	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-CHMP4C$_{181-196}$[7]	BC002463		WISP08-76	pGEX2T	NdeI/BAMHI	N.GST
pGEX2T-CHMP5[7]	BC007457		WISP07-144	pGEX2T	NdeI/BAMHI	N.GST

Supplemental Table 3. Expression Vectors (continued)

1B. Mammalian Expression Vectors

Plasmid Name	Source	Reference	Internal ID	Backbone	Cloning Site	Epitope Tags
pEGFP-C1	Clontech		WISP98-114			N.EGFP
pcDNA3.1(-)A-myc-HIS	Invitrogen		WISP02-257			C.myc.6XHIS
pcDNA3.1(-)B-myc-HIS	Invitrogen		WISP03-153			C.myc.6XHIS
pDsRed-C1	Clontech		WISP02-24			N.DsRed
pEGFP-VPS4A	AF11215	C[8]	WISP01-111	pEGFP	EcoRI/BAMHI	N.EGFP
pEGFP-VPS4A$_{L64D}$	AF11215	B[8]	WISP 07-99	pEGFP-C1	EcoRI/BAMHI	N.EGFP
pEGFP-VPS4A$_{V13D}$[4]	AF11215		WISP 08-60	pEGFP-C1	EcoRI/BAMHI	N.EGFP
pEGFP-VPS4A$_{L64D,V13D}$[4]	AF11215		WISP 08-61	pEGFP-C1	EcoRI/BAMHI	N.EGFP
pEGFP-VPS4A$_{K173Q}$	AF11215	C[8]	WISP 01-112	pEGFP-C1	EcoRI/BAMHI	N.EGFP
pEGFP-VPS4AK$_{173Q,L64D}$	AF11215	B[8]	WISP 07-101	pEGFP-C1	EcoRI/BAMHI	N.EGFP
pEGFP-VPS4A$_{K173Q,V13D}$[4]	AF11215		WISP 08-62	pEGFP-C1	EcoRI/BAMHI	N.EGFP
pEGFP-VPS4A$_{K173Q,L64D,V13D}$[4]	AF11215		WISP 08-63	pEGFP-C1	EcoRI/BAMHI	N.EGFP
pDsRed-VPS4B	AF038960	D[8]	WISP02-25	pDsRed-C1	EcoRI/BAMHI	N.DsRed
pcDNA3.1-VPS4B$_{WT}$[6]	AF038960		WISP 08-72	pcDNA3.1/*myc*-His(-)A	EcoRI/BAMHI	C.myc.6XHIS
pcDNA3.1-VPS4B$_{siRes685}$[6]	AF038960		WISP 08-64	pcDNA3.1/*myc*-His(-)A	EcoRI/BAMHI	C.myc.6XHIS
pcDNA3.1-VPS4BK$_{180Q,siRes685}$[6]	AF038960		WISP 08-65	pcDNA3.1/*myc*-His(-)A	EcoRI/BAMHI	C.myc.6XHIS
pcDNA3.1-VPS4$_{L66D,siRes685}$[6]	AF038960		WISP 08-66	pcDNA3.1/*myc*-His(-)A	EcoRI/BAMHI	C.myc.6XHIS
pcDNA3.1-VPS4B$_{A15D,siRes685}$[6]	AF038960		WISP 08-67	pcDNA3.1/*myc*-His(-)A	EcoRI/BAMHI	C.myc.6XHIS
pcDNA3.1-VPS4B$_{A15D,L66D,siRes685}$[6]	AF038960		WISP 08-68	pcDNA3.1/*myc*-His(-)A	EcoRI/BAMHI	C.myc.6XHIS
pcDNA3.1-CHMP6$_{WT}$	BC010108	E[8]	WISP 04-152	pcDNA3.1/*myc*-His(-)B	EcoRI/BAMHI	C.myc.6XHIS
pcDNA3.1-CHMP6$_{siRes}$[5]	BC010108		WISP 08-69	pcDNA3.1/*myc*-His(-)B	EcoRI/BAMHI	C.myc.6XHIS
pcDNA3.1-CHMP6$_{L170D,siRes}$[5]	BC010108		WISP 08-70	pcDNA3.1/*myc*-His(-)B	EcoRI/BAMHI	C.myc.6XHIS
pcDNA3.1-CHMP6$_{V173D,siRes}$[5]	BC010108		WISP 08-71	pcDNA3.1/*myc*-His(-)B	EcoRI/BAMHI	C.myc.6XHIS

[1]*VPS4A Bacterial Expression Constructs* The VPS4A$_{1-84,V13D}$ (WISP 08-49) MIT domain expression construct was mutated via the QuikChange method (Stratagene) using the wild-type VPS4A$_{1-84}$ (WISP 05-60) MIT domain pGEX2T (Amersham) plasmid with an engineered TEV protease cleavage site after the N terminal GST tag as the template.

[2]*GST-Tagged CHMP6 Bacterial Expression Constructs* The GST-CHMP6$_{1-201}$ construct was amplified from the ATCC template (MGC-19477), digested, and ligated into the (5')NdeI/(3')BamHI sites of the aforementioned modified pGEX-2T plasmid. Truncated GST-tagged CHMP6 constructs (GST-CHMP6$_{1-181}$, WISP 08-50; GST-CHMP6$_{150-181}$, WISP 08-51; GST-CHMP6$_{155-181}$, WISP 08-52; GST-CHMP6$_{160-181}$, WISP 08-53; GST-CHMP6$_{165-181}$, WISP 08-54; GST-CHMP6$_{170-181}$, WISP 08-55; GST-CHMP6$_{175-181}$, WISP 08-56) were amplified from the GST-CHMP6$_{1-201}$ template, digested, and ligated into the modified pGEX-2T plasmid by (5')NdeI/(3')BamHI sites. The GST-CHMP6$_{165-181,L170D}$ (WISP 08-58), GST-CHMP6$_{165-181,V173D}$, and GST-CHMP6$_{165-181,V173D}$ point mutants were created by the QuikChange method using GST-CHMP6$_{165-181}$ as the template.

[3]*TRPΔLE CHMP6 Bacterial Expression Constructs* The (His)$_{10}$-TrpΔLE-CHMP6$_{165-181}$ (WISP 08-57) expression construct was inserted by the QuikChange method using the (His)$_{10}$-TrpΔLE-CHMP1A$_{180-196}$ (WISP 06-62) template plasmid.

[4]*VPS4A Mammalian Expression Constructs* The mammalian expression constructs: pEGFP-VPS4A, pEGFP-VPS4A$_{L64D}$, pEGFP-VPS4A$_{K173Q}$, and pEGFP-VPS4A$_{L64D,K173Q}$ were previously described (Garrus *et al*., 2001; 2003; Stuchell-Brereton *et al*., 2007). Additional pEGFP-VPS4A mutants: pEGFP-VPS4A$_{V13D}$ (WISP 08-60), pEGFP-VPS4A$_{L64D,V13D}$ (WISP 08-61), pEGFP-VPS4A$_{K173Q,V13D}$ (WISP 08-62), and pEGFP-VPS4A$_{K173Q,L64D,V13D}$ (WISP 08-63) were mutated via QuikChange using the templates pEGFP-VPS4A (WISP 01-111), pEGFP-VPS4A$_{L64D}$ (WISP 07-99), pEGFP-VPS4A$_{K173Q}$ (WISP 01-112), and pEGFP-VPS4A$_{L64D,K173Q}$ (WISP 07-101), respectively.

[5]*siRNA Resistant CHMP6 Mammalian Expression Constructs* Wild-type siRNA resistant myc-HIS tagged CHMP6 (pcDNA3.1-CHMP6$_{WT,siRes}$, WISP 08-69) was mutated by the QuikChange method using pcDNA3.1-CHMP6$_{WT}$ (WISP 04-152) as the template. siRNA resistant CHMP6 point mutants (pcDNA3.1-CHMP6$_{L170D,siRes}$, WISP 08-70) and pcDNA3.1-CHMP6$_{V173D,siRes}$, WISP 08-71) were mutated by QuikChange using pcDNA3.1-CHMP6$_{WT,siRes}$ (WISP 08-69) as the template.

[6]*siRNA Resistant VPS4B Constructs* VPS4B was sub-cloned from pDsRed-VPS4B (WISP 02-25) into pcDNA3.1/myc-HIS(-)A (Invitrogen) using (5') EcoRI / (3')BAMHI restriction sites to make pcDNA3.1-VPS4B$_{WT}$ (WISP 08-72). Wild-type siRNA resistant myc-HIS

Supplemental Table 3. Expression Vectors (continued)

tagged VPS4B (pcDNA3.1-VPS4B$_{siRes685}$, WISP 08-64) was cloned by the QuikChange method using pcDNA3.1-VPS4B$_{WT}$ (WISP 08-72) as a template. siRNA resistant VPS4B point mutants (pcDNA3.1-VPS4B$_{A15D,siRes685}$, WISP 08-67; pcDNA3.1-VPS4B$_{L66D,siRes685}$, WISP 08-66; and pcDNA3.1-VPS4B$_{K180Q,siRes685}$, WISP 08-65) were mutated by QuikChange using pcDNA3.1-VPS4B$_{siRes685}$ (WISP 08-72) as the template. The siRNA resistant VPS4BA15D,L66D double point mutant (pcDNA3.1-VPS4$_{BA15D,L66D-siRes685}$, WISP 08-68) was created via QuikChange using pcDNA3.1-VPS4B$_{L66D,siRes685}$ (WISP 08-66) as the template.

[7]*GST tagged CHMP4A, B, C and CHMP5 Bacterial Expression Constructs* GST tagged CHMP4A, CHMP4B, and CHMP4C were previously described (von Schwedler, 2003; McCullough, 2008). CHMP5 was amplified from a cDNA template (ATCC# MGC-12181), digested, and ligated into the (5')NdeI/(3')BamHI sites of the aforementioned modified pGEX-2T to make GST-CHMP5 (WISP 07-144). The regions of CHMP4A, CHMP4B, and CHMP4C corresponding to the CHMP6 MIM2 sequence were amplified from their respective full-length GST tagged constructs. PCR products were digested and ligated into pGEX-2T via (5')NdeI/(3')BamHI restriction sites to make GST-CHMP4A$_{188-203}$ (WISP 08-74), GST-CHMP4B$_{187-202}$ (WISP 08-75), GST-CHMP4C$_{181-196}$ (WISP 08-76).

[8]*References for previously cloned constructs:* A) Scott, A., Gaspar, J., Stuchell-Brereton, M.D., Alam, S.L., Skalicky, J.J., and Sundquist, W.I. (2005b). Structure and ESCRT-III protein interactions of the MIT domain of human VPS4A. Proc Natl Acad USA *102*, 13813-13818. B) Stuchell-Brereton, M.D., Skalicky, J.J., Kieffer, C., Karren, M.A., Ghaffarian, S., and Sundquist, W.I. (2007). ESCRT-III recognition by VPS4 ATPases. Nature *449*, 740-744. C) Garrus, J.E., von Schwedler, U.K., Pornillos, O.W., Morham, S.G., Zavitz, K.H., Wang, H.E., Wettstein, D.A., Stray, K.M., Cote, M., Rich, R.L.*, et al.* (2001). Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. Cell *107*, 55-65. D) von Schwedler, U.K., Stuchell, M., Muller, B., Ward, D.M., Chung, H.Y., Morita, E., Wang, H.E., Davis, T., He, G.P., Cimbora, D.M.*, et al.* (2003). The protein network of HIV budding. Cell *114*, 701-713. E) Langelier, C., von Schwedler, U.K., Fisher, R.D., De Domenico, I., White, P.L., Hill, C.P., Kaplan, J., Ward, D., and Sundquist, W.I. (2006). Human ESCRT-II complex and its role in human immunodeficiency virus type 1 release. J Virol *80*, 9465-9480. F) McCullough, J., Fisher, R., Whitby, F.G., Sundquist, W.I., and Hill, C.P. (2008). CHMP4-ALIX interactions in the human ESCRT pathway. Proceedings of the National Academy of Science, USA, in press.