Supporting Information

McCullough et al. 10.1073/pnas.0801567105

А

В



Fig. S1. ALIX_{Bro1} in complex with the C-terminal CHMP4A helix. (A) Ribbon diagram showing the complex between ALIX_{Bro1} and the C-terminal helix from CHMP4A (purple). This figure is oriented so that the CHMP4A helix in Fig. 1 *Lower* is rotated \approx 90° toward the viewer. (*B*) CHMP4A is represented in sticks against a solid ALIX surface, with the Fo-Fc peptide omit map contoured at 2 × rmsd and displayed over the peptide. To generate the peptide omit map, the peptide was deleted from the model, random shifts (0.3 Å in *x*, *y*, *x*) were applied throughout and the model rerefined without the peptide in REFMAC. The orientation is the same as in Fig. 3.



Fig. S2. Stereoviews of ALIX_{Bro1}-CHMP4B and ALIX_{Bro1}-CHMP4C interfaces. (A) ALIX_{Bro1}-CHMP4B. (B) ALIX_{Bro1}-CHMP4C. Views and designations are the same as in Fig. 3A, except that CHMP4B is shown in green and CHMP4C is shown in orange.



Fig. S3. Comparative analyses of the ALIX_{Bro1}-CHMP4B interface. (A) Overlay of ALIX_{Bro1}-bound CHMP4A-C helices illustrating how hydrophobic residues at the N-terminal ends of the helices can make nearly equivalent contacts despite displacement of the CHMP4B helix (green). (B) Expanded view of the CHMP4B C terminus, highlighting the unique ALIX_{Bro1} contacts made by the C-terminal Met-224 residue.

DNAS

	β1			α3		β2	
H. sapiens P. troglodytes P. pygmaeus E. caballus C. familiaris B. taurus G. gallus O. anathinus X. taevis X. tropicalis D. perio S. purpuratus T. castaneum P. monodon A. meliflera N. vectensis D. melanogaster D. pseudoobscura B. malayi A. aegypti A. aegypti C. briggase C. briggase A. gambiae C. elegans	1 MATFISVQLKKT MATFISVQLKKT MAFF-ISVQLKKT 	20 SEVDLAKPLVKFIQOTYPSC SEVDLAKPLVKFIQOTYPSC SEVDLAKPLVKFIQOTYPSC SEVDLAKPLVKFIQOTYPSC SEVDLAKPLVKFIQOTYPSC SEVDLAKPLVKFIQOTYPSC SEVDLAKPLVKFIQOTYPSC SEVDLAKPLVKFIQOTYPSC SEVDLAKPLVKFIQOTYPSC SEVDLVKFLKFIQOTYPSC SEVDLVKFLKFIQOTYPSC SEVDLVKFLKFIQOTYPSC SEVDLVKFLKFIQOTYPSC SEVDLVKPLKFIQOTYPSC SEVDLVKPLKFINTSEI SEVDLVKPLKFINTSEI SEVDIVKPLKNLISSTYNG SEVDIVKPLKNLISSTYNG SEVDIVKPLKNLISSTYNG SEVDIVKPLKNLISSTYNG SEVDIVKPLKNISSINNYKNI NEVDLVKPLKTIVGSVINYKNI	40 40 G = EEQAQYCRAAEELSK G = EEQAQYCRAAEELSK - DAQAEHCRAAEELSK - DAQAEHCRAAEELSK - DOQTSYCRAVEELKK - EEQAEYLRAVDELNK - EEQAEYLRAVDELNK - EEQAEYLRAVDELNK - EEQAEYLRAVDELNK - EEQAEYLRAVDELNK - EUCHTSPANDELSK VLKEN-SDAISDLNK SEEEKAKYGEAVMEFSK SSEEVKAKYLEANVEFSK SSEEVKAKYLEANVEFSK SSEEVKAKYLEANVEFSK SSEEVKAKYLEANVEFSK SSEEVKAKYLEANVEFSK SSEEVKAKYLEANVEFSK SSEEVKAKYLEANVEFSK SSEEVKAKYLEANVEFSK SSEEVKAKYLEANVEFSK SSEEVKAKYLEANVEFSK SSEVKAKYLEAN	60 60 1 LIRRAAVGRPLDKHEGALETLLRY LIRRAAVGRPLDKHEGALETLLRY LIRRAAVGRPLDKHEGALETLLRY LIRRAALGRPLDKHEGALETLLRY LIRRAALGRPLDKHEGALETLLRY LIRRSALGRPLDKHEGALETLLRY LIRRSALGRPLDKHEGALETLLRY LIRRSALGRPLDKHEGALETLLRY LIRRSALGRPLDKHEGALETLLRY LIRKSAVGRPLDKHETSLETVMRY LIRKSAVGRPLDKHETSLETVMRY LIRKSAVGRPLDKHETSLETVMRY LIRKSAVGRPLDKHETSLETVMRY LIRKSAVGRPLDKHETSLETVMRY LIRNAAVMCFEVSESLEI IVGY LIRNAAVMCFEVSESLEI IVGY LIRNAAVMRFEVSESLEI IVGY LIRNAAVMRFEVSESLEI IVGY LIRNAAVMRFEVSESLEI VVAY MQNNAAVKACNQPLDKHESALEPULRY QINTAI WKFEKYEASLEI LVYY LIRNAAVMRFEVSESLEI LVYY LIRNAAVMCPDCHESLEI LVYY LIRNAAVMCPDCHESLEI LVYY LIRNAAVMCPDCHESLEI LVYY LIRNAAVMCPDCHESLEI LVYYY LIRNAAVMCPDCHESLEI LVYYY LIRNAAVMCPDCHESLEI LVYYY LIRNAAVMCPDCHESLEI LVYYY LIRNAAVMCPDCHESLEI LVYYY	80 DQICSIEPKPPFSENQ-I DQICSIEPKPPFSENQ-I DQICSIEPKPPFSENQ-I DQICSIEPKPPFSENQ-I DQICSIEPKPPFSENQ-I DQICSIEPKPPFSENQ-I DQICSIEPKPPFSENQ-I DQICSIEPKPPFSENQ-I DQICSIEPKPPFSENQ-I DQICSIEPKPPFSENQ-I DQICSIEPKPPFSENQ-I DQICSIEPKPPFSENQ-I DQICALEKPPFSENQ-I DQICALEKPFPFSENQ-I DQICALEKIPPSEN-I DQICALEKIPHAI DQICALEKIPHAI DQICALEKISVSE	100 LITTWKDAPDKGSLFGGS CLITTWKDAPDKGSLFGGS CLITTWKDAPDKGSLFGGS CLITTWKDAPDKGSLFGGS CLITTWKDAPDKGSLFGGS CLITTWKDAPDKGSLFGGS CLITTWKDAPDKGSLFGGS CLITTWKDAPDKGSLFGGS CLITTWKDAPDKGSLFGGS CLITTWKDAPDKGSLFGGS QUPFKKDAPDKGS1FGGK QIPFKKKDAPDKGS1FGGS PISFKKKDAPDKGSLFGG VUPFKKKDAPDKGSLFGGS VUPFKKKDAPDKGS1FGGS VUPFKKKDAPDKGS1FGGS VUPFKKKDAPDKGS1FGGS VUPFKKKDAPDKGS1FGGS VUPFKKKDAPDKGS1FGGS VUPFKKKDAPDKGS1FGGS	120 120 VKLALASLGYEKSCVLF VKLALASLGYEKSCVLF VKLALASLGYEKSCVLF VKLALASLGYEKSCVLF VKLALASLGYEKSCVLF VKLALASLGYEKSCVLF VKLALASLGYEKSCVLF VKLALASLGYEKSCVLF VKLALASLGYEKSCVLF VKLALASLGYEKTCVLF VKLALASLGYEKTCVLF VKLALASLGYEKTCVLF VKLALASLGYEKTCVLF VKLALASLGYEKTCVLF VKLALASLGYEKTCVLF VKLALSSUPEKTCVLF
Brox HD-PTP	MTHW-FHRNPLKAT. MEAVPRMPMIWLDLKEA	APVSFNYYGVVTGPSASF GDFHFQPAVKKFVLKNYGE1	KICNDLRSSRARLLELFT NPEAYNEELKKLEI	DLSCNPEMMKNAADSYFSLLQGF LRQNAVRVPRDFEGCSVLRKYI	INSLDESTQESKLRY LGQLHYLQSRVPMGSGQEA	IQNFKWTDTLQGQ AVPVTWTEIFSG	VPSAQQDAVFELISMGF KSVAHEDIKYEQACILY
Rim20p Bro1p	MSELLAIPLKRT MKPY-LFDLKLKDT I I	LEVDFATEL-SKL-IDTTSH EKLDWKKGLSSYLKKSYGSS I I 20	Q-TASFFQSDILKVVD- SQ-WRTFYDEKATSELDH I 40	ARNNAI-APDISIDGLSALKE LIRNNAN-G-ELAPSSLSEQNLKY I I I 60	KYVIL-LQLEKKFPN-N KSFLEHLYFRLGSKGSR-L I 80	QIEFTWFQTLS-QKS KMDFTWYDAEYSSAQKG I I 100	RGTSQYSLQWEKLTIIY LKYTOHTLAFEKSCTLF I 120
	β1 	α1 	02 03	aa 		β2 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	β3 α5
H. sapiens P. troglodytes P. pygmaeus E. caballus G. familiaris B. taurus R. norvegicus G. gallus O. anathinus X. taevis X. tropicalis D. rerio S. purpuratus T. castaneum P. monodon A. mellifera N. vectensis D. melanogaster D. pseudoobscura B. malayi A. aegypti C. briggsae A. gambiae C. elegans Brox HD-PTP Rim20p Bro1p	140 NCAALASQIAAEQNLD- NCAALASQIAAEQNLD- NCAALASQIAAEQNLD- NCAALASQIAAEQNLD- NCAALASQIAAEQNLD- NCAALASQIAAEQNLD- NCAALASQIAAEQNLD- NCAALASQIAAEQNLD- NCAALASQIAAEQNLD- NIGALASQIAAEQNLD- NIGALASQIAAEQNLD- NIGALASQIASEQNLD- NIGALASQIASEQNLD- NIGALASQIASEQNLD- NIAALQSIASEQNLD- NIAALQSIASEQNLD- NIAALQSIASEQNLD- NIAALQSIASEQNLD- NIAALQSIASQUD- NIAALQSIAASQSIE- NIAALQSIAASQSIE- NIAALQSIAASQSD- NIAALQSIAASQSD- NIAALQSIAASQSD- NIAALQSIAASQCD- NIAALQSIAASQCD- NIGALSQIAAQQFH- NIAALQSIAASQCFH- NIAGIASQIAAQQFH- NIASLGAVASSQSD- NIGSLMSQIAAQQFH- NIASLGAVASSQSD- NIGSLMSQIAAQQFH- NIALQSIASSQIAAQQFH- NIASLGAVASSQSD- NIGSLMSQIAAQQFH- NIALQSIASSQIAAQQFH- NIALQSIASSQIAAQQFH- NIALQSIASSQIAAQQFH- NIALQSIASSQIAAQQFH- NIASLGAVASSQSD- NIGSLMSQIAAQQFH- NIACASSQIAAQQFH- NIACASSQIAAQQFH- NIACASSQIAASSQSD- NIGSLMSQIAAQQFH- NIACASSQIAASSQIA- NIACASSQIAASSQIA- NIGCMYSLLALNSND- NIAVIFULARENINE-	1NDEGLKIAAKHYQFASGNDEGLKIAAKHYQFASGNDEGLKIAAKHYQFASGNDEGLKIAAKHYQFASGNDEGLKIAAKHYQFASGNDEGLKIAAKHYQFASGNDEGLKIAAKHYQFASGNDEGLKIAAKHYQFASGNDEGLKIAAKHYQFASG	A C C C C C C C C C C C C C C C C C C C	180 SPTVDISPDTVGTLSLIMLAQAQE SPTVDISPDTVGTLSLIMLAQAQE SPTVDISPDTVGTLSLIMLAQAQE SPTVDISPDTVGTLSLIMLAQAQE SPTVDISPDTVGTLSLIMLAQAQE SPTVDISPDTVGTLSLIMLAQAQE SPTVDISPDTVGTLSLIMLAQAQE SPTVDISPDTVGTLSLIMLAQAQE SPTVDISPDTVGTLSLIMLAQAQE SPTVDISPDTVGTLSLIMLAQAQE SPTDISPDTVGTLSLIMLAQAQE SPTDISPTVGTLSLIMLAQAQE SPTDISPTVGTLSLIMLAQAQE SPTDISPTUGTLSLIMLAQAQE SPTDISPTUGTLSLIMLAQAQE SPTDISPTUGTLSLIMLAQAQE SPTDISPTUGTLSLIMLAQAQE SPTDISPTUGTLSLIMLAQAQE SPTDISPTUGTLSLIMLAQAQE SPTDISPTLISALSALMLAQAQE SPTPDISPTLISALSALMLAQAQE SPTPDLSUTLTVLQLMVAQAQE SPTPDLSUTLTVLQLMAQAQE SPTPDLMPDTLAALSALMLAQAQE SPTPDLMPDTLAALSALMLAQAQE SPTPDLMPDTLAALSALMLAQAQE SPTPDLMPDTLAALSALMLAQAQE SCGRDLESRLIEAYVLQCQAEAQE CVDMSRQILTLNVLMLGAQAQE SCGRDLESRLIEAYVLQCAAEAQE CVDLSSTNTFLAMLCAAQE C	200 VPFLKATRDK-MKDAI VPFLKATRDK-MKDAI VPFLKATRDK-MKDAI VPFLKATRDK-MKDAI VPFLKATRDK-MKDAI VPFLKATRDK-MKDAI VPFLKATRDK-MKDAI VPFLKATRDK-MKDAI VPFLKATRDK-MKDAI VPFLKATRDK-MKDAI VPFLKATRDK-MKDAI VPFLKATRDK-MKDAI VFVLKATSDK-MKDAI VFVLKATSDK-MKDAI VFVLKATSDK-MKDAI VFVLKATSDK-MKDAI VFVLKATSDK-MKDAI VFVLKATSDK-MKDAI VFVLKATSDK-MKDAI VFVLKATSDK-MKDGI I VVTVLKATSDK-MKDGI I VVTVKAIKS-MKDGI I VVTVKAIKS-MKDGI VITVKAIKS-MKDGI VTVKAIKS-MKDGI VFILKAIKDN-MNNII I VFILKAIKDN-MKDGI VFILK	220 AKLANQAADY FGDAFKQC(AKLANQAADY FGDAFKQC(AKLANQAADY FGDAFKQC(AKLANQAADY FGDAFKQC(AKLANQAADY FGDAFKQC(AKLANQAADY FGDAFKQC(AKLANQAADY FGDAFKQC(AKLANQAADY FGDAFKQC(AKLANQAADY GGDAFKQC(AKLANQAADY GGDAFKQC(AKLAQCQAEVYGDAKKQC(AKLAQCQEVYGDAKKQC(AKLAQCQEVYGDAKKQC(AKLAQCQEVYGDAKKQC(AKLAQCQEVYGDAKKQC(AKLAQQAEY YDAQKWM; XKLAQCQEVYDYKQAADHTLG AKLSQQI VDFYCAAINDF(AKLSQQI VDFYCAAINDF(AKLSQAINDFYCAAINDF(AKLSQAINDFYCAAINDF(AKLSQAINDFYCAANDF(AKLSQAINDFYCAANDF(AKLSQAINDFYCAANDF(AKLSQAINDFYCAANDF(AKLSQAINDFYCAANDF(AKLSQAINDFYCAANDF(AKLSQAINDFYCAANDFYCAANDF(AKLSQAINDFYCAANDF(AKLSQAINDFYCAANDF(AKLSQAINDFYCAANDFYCAANDF(AKLSQAINDFYCAANDF(AKLSQAINDFYCAANDFYCAANDF(AKLSQAINDFYCAANDFYCAANDF(AKLSQAINDFYCAANDF(AKLSQAINDFYCAANDFYCAANDFYCAANDF(AKLSQAINDFYCAAND	240 YKDTLPKEV YKDTLPKEV YKDTLPKEV YKDTLPKEV YKDTLPKEV YKDTLPKYFYPQEV YKDTLPKYFYPQEV YKDTLPKYFYPQEV YKDTLFKEV YKDTLFKEV YKDTLFKDW RESIKQLMBKDW RESIKQLMBKDW RESIKQLMBKEW RESIKQLMBKEW RDAVRGLPKDW SKDIVRGLMDKEW SKDIVRGLMDKDW
		α6		α7		α8	<u> </u>

Fig. S4. (Continued on next page.)

PNAS PNAS

	26	0 2	280	300	320	340		359
H. sapiens	FPVLAAKHCIMQANAEYHC	SILAKQQKKFGEEIARLQ	HAAELIKTVASRY-DEY	VNVKDFSDKINRAL	AAAKKDNDFIYHDRVPDLKD	LDPIGK <mark>A</mark> TLVKSTP	VNVPISQKF	TDLFEKMV
P. troglodytes	FPVLAAKHCIMQANAEYHQ	SILAKQQKKFGEEIARLC	HAAELIKTVASRY-DEY	VNVKDFSDKINRAL	TAAKKDNDFIYHDRVPDLKD	LDPIGK <mark>ATL</mark> VKSTP	VNVPISQKF	TDLFEKMV
P. pygmaeus	FPVLAAKHCIMQANAEYHQ	SILAKQQKKFGEEIARLQ	HAAELIKTVASRY-DEY	VNVKDFSDKINRAL	TAAKKDNDFIYHDRVPDLKD	LDPIGK <mark>A</mark> TLVKSTP	VNVPISQKF	TDLFEKMV
E. caballus	FPILAAKHCIMQANAEYHQ	SILAKQQKKFGEEIARLQ	HAAELIKTVASRY-DEY	VNVKEFSDKISRAL	TAAKKDNDFIYHDRVPDLKD	LDPIGK <mark>A</mark> TLVKSTP	VNVPISQKF	TDLFEKMV
C. familiaris	FPVLAAKPCIMQAYAEYHQ	SILAKQQKKFGEEIARLQ	HAAELIKTVASRY-DEY	VNVKEFSDKISRAL	TAAKKDNDFIYHDRVPDLKD	LDPIGK <mark>A</mark> TLVKSTP	VNVPISQKF	TDLFEKMV
B. taurus	FPVLAAKHCIMQANAEYHQ	SILAKQQKKFGEEIARLQ	HAAELIKTVASRY-DEY	VNVKDFSDKISRAL	TAAKKDNDFIYHDRVPDLKD	LDPIGK <mark>A</mark> TLVKSTP	VSVPISQKF	TDLFENMV
R. norvegicus	FPTLAAKQCIMQANAEYHQ	SILAKQQKKFGEEIARLQ	HAAELIKNVASRY-DEY	VNVKDFSDKINRAL	AAAKKDNDFIYHDRVPDLKD	LDPIGK <mark>A</mark> TLVKPTP	VNVPISQKF	TDLFEKMV
M. musculus	FPTLAAKQCIMQANAEYHQ	SILAKQQKKFGEEIARLQ	HAAELIKNVASRY-DEY	VNVKDFSDKINRAL	TAAKKDNDFIYHDRVPDLKD	LDPIGK <mark>A</mark> TLVKPTP	VNVPVSQKF	TDLFEKMV
G. gallus	FPVLAAKHCIMQANAEYHQ	SILAKQQKKFGEEIGRLQ	HAADLVKTVASRY-DEY	INVKDLVDKINRAL	TAAKKDNDFIYHDRVPDLKD	LEPIGK <mark>A</mark> SLVKSTP	VAVPLSQKF	TDLFEKMV
O. anathinus	FPVLAAKHCIMQANAEYHQ	SILAKQQKKFGEEIGRLQ	HAAELIKTVTSRY-DEY	VNVKELSDKINRAL	TAAKKDNDFIYHDRVPDLKD	LDPIGK <mark>A</mark> S <mark>L</mark> VKSTP	VIVPLSQKF	SDLFDKMV
X. laevis	FPILAAKHCIMQAHAEYHQ	SVLAKQQKKFGEEIGRLQ	HASDLVKTVSSRY-DEY	VNVKDLADKINRAL	TAAKKDNDFIYHDRVPDLKD	LDPVGK <mark>A</mark> S <mark>L</mark> VKSTP	VNVPLSQKY	TDLFEKMV
X. tropicalis	FPILAAKHCIMQAHAAYHQ	SVLAKQQKKFGEEIGRLQ	HASDLVKTVSSRY-DEY	VNVKD- LADKINRAL	TAAKKDNDFIYHDRVPDLKD	LDPIGK <mark>A</mark> S <mark>L</mark> VKPTP	INVPLSQKY	TDLFEKMV
D. rerio	LPVLAAKHCMMQATAEMHQ	SALANQKKKFGEEIARLQ	HAQELVKTAASRY-DEY	VNVKDLSDKINRAL	TAAKKDNDFIYHDRVPEVKD	lehigk <mark>a</mark> s <mark>l</mark> vkata	VQTPLSQKF	TDLFEKMV
S. purpuratus	IPTVLGKQYYMHAISEYHQ	ALVCRASKSYGPEVGRLK	HASDLLSEVERKG-GQY	IDTKDFAARVSQEL	KAVKKDNDFIYHDIVPSVDQ	lsrpgt <mark>a</mark> q <mark>i</mark> aklvd	FSTPLSTES	NDLFASLV
I. castaneum	IPTIAGKQAALHAVAQFYQ	QSLVCRSNKQIGEEIARLE	HSVSLFQTAQQRS-GKA	SLFQELQHKAQRNL	AEVKKDNDFIYHERIPDIKS	LEPIGK <mark>A</mark> Q <mark>P</mark> AKVLP	IAQPMSQGF	KDLFSELV
P. monodon	IPRISGKQAGYAGLAEYHQ	SRVCNSDKSVGEEIARLN	KGLEMFKAAQARS-GDL	TMFADNLGRAKNCL	DEAVKDNDFIYHERVPDVKS	LSAIGK <mark>A</mark> A <mark>L</mark> AKPTP	LPEKFSATF	TDLFETLV
A. mellifera	VPLIAGKQAGYRAMTEFY	SLVCKNNKSIGEEIARLE	RSVELFKAAQQRS-NKP	HLFQDYANRAQRNL'	TEVKKDNDFIYHERIPDIKS	LEPVGK <mark>A</mark> C <mark>V</mark> AKLIS	MPEIFSSNF	KDLFNDLL
N. vectensis	TAVLGAKQKYFKAISQYHQ	ATVAMEKGKYGENVARLK	LANQLVGEAAKES-MGM	FDTKTWVDRIKRGF	VEAEKDNNLIYHDMIPSVDT	LAVIGR <mark>A</mark> S <mark>L</mark> AKSLP	LSKPASINF	IDMFTKLV
D. melanogaster	IPTIAGKQAGFHALTQLYQ	OSLVCRAAKKIGEEIARLR	NAIDLFKAAQTRS-GNE	TYLDEYFSRAKRNL'	TESTKDNEFIYNEIIPELST	LTSPGK <mark>A</mark> Q <mark>L</mark> AKPLP	IAVPLAENF	KDIFSSLV
D. pseudoobscura	IPTVAGKQAGFHALTQLYQ	SLVCRAAKKIGEEIARLR	NAIDLFKAAQSRG-GNE	TYLDEYFSRAKRHL	AESTKDNEFIYNEMIPELST	LASPGK <mark>A</mark> Q <mark>L</mark> AKSLG	IASPMASNF	RDIFTNLV
B. malayi	TNTVKGKALGLSALAQYHM	AVDNADSKNIGEQLSRLI	ESQSLMQQAISYMPHET	FNIQYAAIEKAYT	SAKKDNDFIYHERIADFRSL	PHLPKA <mark>A</mark> V <mark>A</mark> KIVPI	TFPITPRF	KDMFSSLV
A. aegypti	ISTVAGKQAGFHALTMFYF	ISLVSKSNKAIGEEISRLQ	KSVELFKIAQSRS-GKP	NFLEEYASKAQRNL	IEAKKDNDFIYNEMIPDINS	LPGPGK <mark>A</mark> Q <mark>L</mark> AKYLP	IASPMSQNF	RDLFADLV
C. briggsae	SSIVNGKTIAYQALAQFHQ	SEVNGESRQIGEQLSRLA	ESLKLFETAQKYLPKDI	TGIWDIYPTVSKAH	TAAKKDNDFIYHERVADFRS	LPALQK <mark>A</mark> V <mark>L</mark> AKPTP	VPNPITPNF	RDMFSSLV
A. gambiae	IPQVAGRQAAMHALTMLYF	ISKVSKANKAFGEEISRLÇ	KAVELFKTAQSRS-SNP	TLFQEYANRAQKSL	AEAKKDNDFIYNEVIPDVNN	LPSPGK <mark>A</mark> Q <mark>L</mark> AKVLP	LATPLSEDF	HDLFGALV
C. elegans	SAIVSGKNLAYQALAQYHQ	SEVCGEARQIGEQLSRLA	ESLKLFDTAQKYLPRDI	TGIWDIYPSVSKAH	AAAKKDNDFIYHEKVSDFRT	LPTLPK <mark>A</mark> V <mark>L</mark> AKPTP:	MQTPMTPSF	RDMFAVLV
Brox	RKYLHLKMCFYTAYAYCYF	IGETLLASDKCGEAIRSLQ	EAEKLYAKAEALC	KEYGETKGP	GPTVKPSGHLFFRKLG	-N <mark>-</mark> -LVKNTL	EKCQRENGF	IYFQ-KT
HD-PTP	KKLVQMKIYYFAAVAHLHM	IGKQAEEQQKFGERVAYFQ	SALDKLNEAIKLAKGQP	DTVQDALRFTMDVIGGKY	NSAKKDNDFIYHEAVPALDT	LQPVKG <mark>A</mark> P <mark>L</mark> VKPLP	VNPTDPAVTG	PDIFAKLV
Rim20p	INHLKAKKAYFSAVTYYRI	ALSFNEKKQFGNVVKALQ	MGLQFINESTLSSQAKF	KTVVESSL	KEAQRDNEFIYLQEVP	SE <mark>L</mark> PSIKPAL	MVKPSSSATLLPS	SIKKDETLFKDLI
Bro1p	KTTVTCKLHFYKSLSAYYH	IGLHLEEENRVGEAIAFLD	FSMQQLISSLPFKTWLV	EFIDFDGFKETLEKKQI	KELIKDNDFIYHESVPAVVQ	VDSIKA <mark>L</mark> D <mark>A</mark> IKSPT	WEKILEPYMQDVA	NKCDSLYRGII
	1 1	1 I	I I	1	1 1	I I	1	1 1
	240	260	280	300	320	340		360 367
	- AXAXAXAXAXAXAX	AXAXAXXXXXXXXXXXX			YX-	\ \		
	~0		210	011			a12 a13	
	u.g		10	u11			u12 013	u 14

Fig. 54 (Continued). Sequence alignments and secondary structures of Bro1 domains. Secondary structures of the Bro1 domain of ALIX (*Upper*) and Bro1p (*Lower*) (PDB entry 1zb1) are shown together with aligned primary sequences of ALIX_{Bro1} domains from 25 representative metazoan species (top sequence block), Bro1 domains from the human Brox and HD-PTP proteins (middle sequence block), and Bro1 domains from the yeast Rim20p and Bro1p proteins (bottom sequence block). ALIX_{Bro1} and Bro1p were aligned by least squares overlap of the two structures, and other sequence alignments were performed by using the ClustalW server http://npsa-pbil.ibcp.fr/NPSA/npsa_clustalw.html [Combet C, Blanchet C, Geourjon C, Deléage (2000) NPS@: Network protein sequence analysis. *Trends Biochem Sci* 25:147–150]. Residues highlighted in yellow make contacts with CHMP4A in the ALIXBro1-CHMP4A₂₀₅₋₂₂₂ structure and residues italicized in bolck ALIX binding and HIV-1 budding when mutated to Asp.

IAS PNAS



Fig. S5. Selectivity of the CHMP4A and CHMP1B recognition helices for $ALIX_{Bro1-V}$ and Vps4A MIT. Biosensor isotherms showing $ALIX_{Bro1-V}$ and VPS4A MIT domain binding to immobilized GST-CHMP4A₂₀₅₋₂₂₂ and GST-CHMP1B₁₈₀₋₁₉₆. Estimated dissociation constants for $ALIX_{Bro1-V}$ interactions were: GST-CHMP4A₂₀₅₋₂₂₂, $A \pm 6 \mu$ M, GST-CHMP1B₁₈₀₋₁₉₆, > 1 mM. Estimated dissociation constants for VPS4A MIT domain interactions were: GST-CHMP4A₂₀₅₋₂₂₂, > 1 mM, GST-CHMP1B₁₈₀₋₁₉₆, $18.5 \pm 0.6 \mu$ M (dissociation constant and error were estimated from a statistical fit of a single binding isotherm derived from duplicate measurements at six different Vps4A MIT domain concentrations over a range of 0–140 μ M). Binding to a control GST surface was negligible in all cases (data not shown).

Table S1. Data collection and refinement statistics for ALIX_{Bro1}-CHMP4 complexes

PNAS PNAS

Statistic	CHMP4A	CHMP4B	CHMP4C	
Data collection				
Space group	C2	C2	C2	
Cell dimensions				
a, b, c, Å	120.7, 62.7, 76.1	120.5, 62.6, 76.1	120.9, 62.4, 76.4	
b, deg.	122.1	121.5	121.6	
Resolution, Å	50.0–2.15 (2.23–2.15)	50.0–2.1 (2.18–2.10)	50.0-2.02 (2.09-2.02)	
R _{sym}	0.063 (0.334)	0.067 (0.413)	0.066 (0.596)	
<i>Ι/σ(I)</i>	19 (1.8)	14 (1.7)	9 (1.5)	
Completeness, %	94.9 (75.1)	92.3 (67.6)	95.5 (81.0)	
Redundancy	9.5 (3.8)	7.2 (4.6)	6.6 (4.4)	
Refinement				
Resolution, Å	50.0–2.15 (2.21–2.15)	50.0–2.10 (2.154–2.10)	50.0-2.02 (2.07-2.02)	
Reflections, no.	25,024	26,458	30,381	
R _{work}	0.214 (0.305)	0.228 (0.326)	0.243 (0.333)	
R _{free}	0.284 (0.344)	0.290 (0.386)	0.291 (0.411)	
Number of atoms				
Protein	2911	2953	2905	
Ligand/ion	6	6	6	
Water	127	90	56	
(B)-factors				
Protein	33.2	35.3	34.0	
Ligand/ion	82.5	78.9	85.8	
Water	49.9	55.0	51.3	
rmsd				
Bond lengths, Å	0.019	0.017	0.016	
Bond angles, deg.	1.710	1.653	1.542	
Ramachandran plot, %				
Most favored	89.7	88.0	90.6	
Additionally allowed	9.7	10.8	8.6	
Generously allowed	0.3 (Q27)	1.2 (K207,Q27,S31,C40)	0.3 (K207)	
Disallowed	0.3 (Q88)	0.0	0.6 (Y29, E34)	

Diffraction data from one crystal were used to determine each structure. This includes the CHMP4A complex, for which data were collected in two separate sweeps from different beamlines. Values in parentheses refer to the high-resolution shell.

Table S2. Thirty-four different CHMP4 protein sequences from 19 different metazoan species grouped together with the human CHMP4 isoform with which they show the greatest pair-wise identity throughout the entire protein

		NCBI protein	to most homologous	C-terminal	
CHMP4 protein	Organism	accession no.	human CHMP4, %	sequence	
4A	Homo sapiens	Q9BY43	100	PKVDEDEEA L KQ L AE W VS	
4A	Pan troglodytes	XP_001169270	99(A)	PKVDEDEEA l kQ l ae w VS	
4A	Canis familiaris	XP_537387	91(A)	PEADEDEAA l kq l ae w vs	
4A	Bos taurus	AAI33472	91(A)	PKADEDEAE l KQ l AE W VS	
4A	Monodelphis domestica	XP_001380207	81(A)	ASKTDEEKE m KQ l VD w VS	
4B	Homo sapiens	NP_789782	100	KKKEEEDDD M KE L EN W AGSM	
4B	Mus musculus	NP_083638	99(B)	KKKEEEDDD M KE L EN W AGSM	
4B	Macaca mulatta	XP_001105255	99(B)	KKKEEEDDD M KE L EN W AGSM	
4B	familiaris	XP_542966	97 (B)	KKKEEEEDD M KE L EN W AGSM	
4B	Bos taurus	AAI23448	97 (B)	KKKEEEDED M KE L ET W AGTI	
4B	Rattus norvegicus	XP_001073409	97 (B)	KKKEEEDDD M KE L EN W AGSM	
4B	Monodelphis domestica	XP_001381361	96 (B)	KKKEEEDDD M KE L EN W AGSM	
4B	Ornithorhynchus anatinus	XP_001518785	94 (B)	KKKEEEDDD M KE L EN W AGSM	
4B	Equus caballus	XP_001499057	94 (B)	KKKEEEDDD M KE L EN W AGTI	
4B	Gallus gallus	NP_001006286	91 (B)	KKEEEEDDD M KE L EA W AGNM	
4B	Xenopus laevis	Q5XGW6	87 (B)	KKQEEDDDD m re l en w ata	
4B	Xenopus tropicalis	Q6GL11	87 (B)	KKQEEDDDD m re l en w ata	
4B	Danio rerio	Q7ZVC4	86(B)	KKEEEDEDD m KD l ea w aan	
4B	Nematostella vectensis	XP_001639344	67 (B)	AKKKTEDDD l ae l ea w as	
4B	Ornithodoros moubata	AAS59855	66 (B)	SKAVMEDPD M IE L AQ W AS	
4B	Anopheles gambiae	XP_315330	64 (B)	AVAEEDDPD M KE L MS W AN	
4B	Caenorhabditis elegans	AAA68771	61 (B)	PRAKEADKD L ED L ES W AN	
4B	Drosophila melanogaster	NP_610462	57 (B)	AVEDDDDPD M KQ L LS W SN	
4C	Homo sapiens	Q96CF2	100	QRAEEEDDD i kq l aa w at	
4C	Pan troglodytes	XP_528179	99(C)	RRAEEEDDD i kq l aa w at	
4C	Macaca mulatta	XP_001093735	98 (C)	RRAEEEDDD i kq l aa w at	
4C	Canis familiaris	XP_535115	88 (C)	SKRTEEVDD I KQ l aa w ap	
4C	Bos taurus	AAI13332	88 (C)	RRTEGEDDD I QH L AA W AT	
4C	Monodelphis domestica	XP_001367000	88 (C)	SRRKEDEDD i kq l aa w as	
4C	Equus caballus	XP_001489156	87 (C)	RRAEEDDDD I KK l SA w AT	
4C	Mus musculus	Q9D7F7	84(C)	SRRAEEDDD f KQ l aa w at	
4C	Rattus norvegicus	Q569C1	84(C)	SRRAEEDDD f KQ l aa w at	
4C	Gallus gallus	XP_418312	72 (C)	RRRVEDEDD M KQ l aa w as	
4C	Xenopus laevis	Q6GNN8	69 (C)	SKKVEDDDD m qm l aa w at	

Conserved hydrophobic, Leu, and Trp positions in the terminal recognition helix are in bold. For reference, pairwise identities between the three human CHMP4 isoforms are 62% (A vs. B), 52% (A vs. C), and 61% (B vs. C), whereas the nearest identity between a human CHMP4 protein and another human CHMP protein is 30% (CHMP4A vs. CHMP5). Note that our current nomenclature for CHMP4A (NCBI protein accession locus Q9BY43), CHMP4B (NP_789782), and CHMP4C (Q96CF2) matches the current NCBI database annotations (www.ncbi.nlm.nih.gov/sites/entrez) but that the designations of CHMP4A and CHMP4B are reversed from those used in several previous publications.