Supplementary Information

Structural basis of cargo recognition by the myosin-X MyTH4-FERM domain

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Figure S1 Identification of the degradation loop of the human myosin-X MyTH4-FERM cassette.

(A) A TOF-MS spectrum of the fragment-1 (Frag-1) from the protein sample of the myosin-X MyTH4-FERM cassette. The inset shows the SDS-PAGE pattern of the sample. The protein sample of the recombinant human MyTH4-FERM cassette produces two major fragments (Frag-1 and Frag-2) during purification. A TOF-MS spectrum of Frag-2 showed a major peak corresponding to a molecular mass of 18,943.5 Da, which is closely related to the calculated value of 18,966 for a fragment of myosin-X residues Phe1893-Arg2058. The N-terminal analysis of Frag-2 suggested the N-terminal sequence of FRTGSV (data not shown), which is consistent with the proposed fragment. The peak at a m/Z value of 65,870.1 may correspond to trace amounts of the full-length protein of the construct and the peak at 46,864.7 to Frag-1. The observed m/Z value and our N-terminal analysis indicate that Frag-1 corresponds to residues 1486-1888 plus two extra residues (Gly-Pro) that were part of the HRV3C protease-recognition sequence (calculated 46,855 Da). Since Frag-2 corresponds to residues 1893-2058, only 4 residues are missing from our fragments.

(**B**) Binding assay of deletion mutants of the myosin-X MyTH4-FERM cassette. The deletion mutants Δ 47 (residues1845-1891), Δ 20 (1872-1891) and Δ 10 (1882-1891) are served for pull-down assays with GST-fused DCC P3 peptide (GST-DCC).

					α1x	α2x	η1x
			1490	1500	1510	1520	1530
hMyo-X	1486		TRI/SSATON	TDTKA <mark>PID</mark> T	PTQQ <mark>LI</mark> Q <mark>DIKEN</mark>	C <mark>ln<u>s</u>dvveq</mark> t	<u>YKRNPIL</u> RYTHH
bMyo-X			- <mark>RW</mark> SS <mark>AIQ</mark> N	/TDT <mark>KAPI</mark> D <mark>T</mark>	PTQQLI <mark>Q</mark> DIKEN	IC <mark>lns</mark> dv <mark>veq</mark> I	YKRNPILR <mark>HT</mark> H <mark>H</mark>
xMyo-X		TKLLNEA	M <mark>RW</mark> AN <mark>A I Q</mark> G	/IES <mark>KAPI</mark> ET	PTQQLI <mark>R</mark> DIKEN	S <mark>lns</mark> ea <mark>veq</mark> t	YKRNPILRY <mark>TQ</mark> H
		I	MyTH4	α1'M	α 1Μ		α2M
		1540	1550	1560	1570	1580	1590
hMyo-X	1539	PLHSPLLI	PLPYGD I <mark>NLI</mark>	NLLKDKGYTT	LQ <u>DEA</u> IK <mark>IFNSL</mark>	. <mark>QQ</mark> L <mark>E</mark> SMS <mark>DP1</mark>	PII <mark>O</mark> GILQT <mark>GH</mark> D
bMyo-X		PLHSPLLI	PLPYGD I <mark>NLI</mark>	NLL <mark>K</mark> DKGYTT	<mark>LQDEA</mark> IK <mark>IFNSL</mark>	. <mark>Q</mark> QL <mark>E</mark> SMS <mark>DP</mark> I	PIIQGILQT <mark>GH</mark> D
xMyo-X		<mark>PLHSPLLI</mark>	<mark>Plpygd</mark> v <mark>nli</mark>	NL <mark>Q</mark> KE <mark>kgytt</mark>	LQDEA <mark>VR</mark> IFNSL	. <mark>Q</mark> E I <mark>E</mark> TVQ <mark>DP</mark> V	<mark>PIIQGILQT</mark> CQ <mark>D</mark>
hSTAM1	1			MPLFA	TNP <u>FDQDVEKA</u> T	SEMNTAED <mark>WG</mark>	<u>IDICDKVGQ</u> S

		o	ι3M		α4M		α6M		
		1600	1610	<u>1</u> 620	1630	<u>1</u> 640	1650		
hMyo-X	1599	L <mark>R</mark> PLRDEL	<u>YCQLIKQT</u> NK <mark>v</mark>	<mark>/P</mark> H <mark>P</mark> G <mark>Svgnl</mark>	YS <mark>WQ</mark> I <mark>L</mark> T <mark>C</mark> L <mark>S</mark>	<u>CT</u> FLPS <mark>R</mark> GIL	<mark>KYLK</mark> FHLK	<mark>r i re</mark>	
bMyo-X		<mark>l</mark> rp <mark>lr</mark> d <mark>e</mark> l	YCQLIKQTN <mark>k</mark> v	<mark>/P</mark> H <mark>P</mark> g <mark>Svgnl</mark>	CS <mark>WQ</mark> ILT <mark>C</mark> LS	CTFLPS <mark>R</mark> GIL	.K <mark>ylkfhl</mark> r	<mark>R</mark> I <mark>RE</mark>	
xMyo-X		<mark>l</mark> ks <mark>lr</mark> nev	YCQLIKQTNH	<mark>/P</mark> e <mark>pns</mark> pgnl	RH <mark>wq</mark> l <mark>l</mark> s <mark>cms</mark>	CTFLP <mark>gr</mark> d1l	.R <mark>YLKFHL</mark> K	RV <mark>RE</mark>	
hSTAM1	40	RT <u>GPKDCL</u>	.RSIMRRVNHKD	PHVAMQALT	<u>llg<mark>a</mark>cv<mark>sn</mark>cg</u>	K I <mark>F</mark> hlevcsf	DFASEVSN	VLNK	

			α 7Μ		α8M	FERM β1A	β2Α
		1660	<u>1</u> 670	1680	1690	1700	1710
hMyo-X	1659	<u>Q</u> FPGT <u>e</u> mek	(Y <mark>A</mark> L <mark>F</mark> TYES <mark>L</mark> K	K <mark>tkcref</mark> vp	<mark>PS<mark>RD<mark>EI</mark>E<mark>AL</mark>IH</mark>R-</mark>	––––QE <mark>MT</mark> st <mark>v</mark> yc	<mark>hg<u>ggsc</u>k</mark>
bMyo-X		Q <mark>FPGTE</mark> M <mark>e</mark> k	(Y <mark>A</mark> L <mark>F</mark> IYES <mark>LK</mark>	KTKCRE <mark>FVP</mark>	<mark>PS</mark> RD <mark>ET</mark> E <mark>AL</mark> TH <mark>R</mark> -	––––QE <mark>mt</mark> st <mark>v</mark> hc	<mark>hgggsc</mark> k
хМуо-Х		S <mark>FPGTE</mark> V <mark>E</mark> G)F <mark>a</mark> h <mark>f</mark> vvda <mark>lk</mark>	KTKCREMVP	<mark>?S</mark> QE <mark>EI</mark> S <mark>AL</mark> LT <mark>R</mark> -	––––SH <mark>MT</mark> ts <mark>v</mark> yc	<mark>HGGGSC</mark> Q
hSTAM1/h	Rad	<u>GH<mark>P</mark>KVCEKL</u>	.K <mark>a</mark> lmvewtde	<u>FK</u> ND <u>PQLSL</u>	<u>ISAM<mark>I</mark>KN<mark>L</mark>KEQ</u>	/ MPKP <u>invr<mark>v</mark>t</u> t	M-DA <u>ele</u>

		α1Α β3Α			β4A	α	α2A	
		<u>1</u> 720	1730	1740	<u>1</u> 750	1760	<u>1</u> 770	
hMyo-X	1714	<u>ITI<mark>NSH</mark>TTAGEVV</u>	<mark>/EKLIRGL</mark> A <mark>M</mark> E	D <mark>srn<u>mfalfe</u>y</mark> n	IG- <mark>H</mark> V <mark>DKA</mark> I <mark>E</mark> S	RTV <mark>VADVL</mark> A	AK <mark>F</mark> EKL <mark>A</mark>	
bMyo-X		<mark>I</mark> TV <mark>NSHTTAGEVV</mark>	<mark>/EKLIRGL</mark> A <mark>M</mark> E	D <mark>srnmfalfe</mark> yn	IG- <mark>H</mark> V <mark>D</mark> K <mark>A</mark> I <mark>E</mark> S	RTI <mark>VADVLA</mark>	<mark>KFE</mark> K <mark>LA</mark>	
xMyo-X		<mark>I</mark> SI <mark>NSHTTAGEVV</mark>	<mark>/EKLIRGL</mark> S <mark>M</mark> D	N <mark>srnmfalfe</mark> hn	IK- <mark>h</mark> t <mark>draves</mark>	RVI <mark>VADVLA</mark>	<mark>KFE</mark> R <mark>LA</mark>	
hRad	18	<u>FAI</u> QPN <mark>TT</mark> GKQLF	DQVVKTVGLR	-EVWF <mark>fg<mark>l</mark>qyv</mark> d	OSKGY <u>stw</u> l <mark>k</mark> l	NK <mark>VTQ</mark> Q-D	OVKK	

2

Supplementary Figure S2 (continued)

			β5A	\		α1B		
		178	80	1790	1800	1810) 18	20
hMyo-X	1773	<u>A</u> TSEVGDLP-	<mark>W</mark> KF <mark>YFKL</mark>	<mark>Yc</mark> fld-tdn	<mark>vpk</mark> ds <u>vef</u>	AFMFEQAHI	<u>AVIH</u> GHHF	PAP <u>EE<mark>NLQ</mark>V</u>
bMyo-X		A <mark>t</mark> sevgeqp-	<mark>W</mark> KF <mark>YFKL</mark>	<mark>Ycfld</mark> –tdn	<mark>vpk</mark> dsv <mark>ef</mark>	AFMFEQAHI	<mark>AVIH</mark> GHY <mark>F</mark>	PAPEENLQV
хМуо-Х		G <mark>T</mark> GDEEDDLO	3P <mark>W</mark> NL <mark>YFKL</mark>	<mark>YCFLD</mark> –VQS	<mark>VPK</mark> EGI <mark>EF</mark>	AFMFEQAHI	SLTS <mark>GH</mark> FF	PAPEETLQH
hRad	73	EN	IP <u>lqfk<mark>f</mark>ra</u>	<mark>k</mark> f <mark>f</mark> ped <u>vse</u>	<u>e</u> liq <u>eitq</u>	RLF <mark>F</mark> L <mark>Q</mark> VK	AILNDEIY	(C <mark>P<mark>PE</mark>TAVL</mark>
		α2B		α2'iB	α2"iB			
		_	1840	1850	18	60	870	1880
hMyo-X	1830	LAALRLQY	GDYTLHAA	IPP <u>LEE</u>	VYSLORLK	ARISQSTK	IF PCER	KRRTSFLEGT
bMyo-X		<mark>LAALRLQY</mark> LQ	0 <mark>GD</mark> YAPHAP	VPPLEE	<mark>v</mark> ysl <mark>qrlk</mark>	ARIS <mark>Q</mark> STK	SF <mark>T</mark> PGER <mark>LE</mark>	KR
xMyo-X		<mark>laalrlqy</mark> qf	I <mark>gd</mark> fskvtw	SLDT	<mark>v</mark> ypv <mark>qrlk</mark>	<mark>AKILQ</mark> ATK	ss <mark>t</mark> sght <mark>le</mark>	RR
hRad	125	L <mark>a</mark> syav <mark>q</mark> aky	(<mark>GD</mark> YNKEIH	KPGYLANDR	LL-P <mark>qr</mark> vl			
					α3	В		α4B
		1890	1900	1910	1920	193	30 1	940
hMyo-X	1889	LR <mark>I</mark> SFRTGS\	/VRQKVEE	ONLDMN I <mark>KE</mark>	<mark>e</mark> vss <mark>ar</mark> a <mark>s</mark>	I I D <mark>KW</mark> RKF	<mark>QG</mark> MN <mark>Q</mark> EQAN	IA <mark>KYMA</mark> LIK <mark>E</mark>
bMyo-X		LR <mark>R</mark> SFRT <mark>GS</mark>	AIR <mark>QK</mark> AEEE	<mark>qm</mark> vd <mark>mw</mark> vke	<mark>e</mark> vcs <mark>ar</mark> as	ILD <mark>KW</mark> KKF	<mark>ag</mark> ms <mark>q</mark> eqan	IA <mark>KYMA</mark> LIK <mark>E</mark>
xMyo-X		<mark>l</mark> k <mark>r</mark> gfkv <mark>gs</mark> n	IRK <mark>QK</mark> V <mark>EEE</mark>	<mark>qm</mark> me <mark>mw</mark> vke	<mark>e</mark> lsa <mark>ar</mark> ts	I AE <mark>kw</mark> srl	<mark>ag</mark> vs <mark>q</mark> hqan	IV <mark>kyma</mark> i vs <mark>e</mark>
hRad	159		<mark>E</mark>	<mark>Q</mark> ———H <mark>K</mark> L	T- <u>keqwee</u>	<u>r i qn<mark>w</mark>he</u> hi	R <mark>g</mark> ml <u>reds</u> n	IME <mark>YL</mark> KIAQD
		ĥ	31C	β2C	βЗС	ĥ	34C η1C (35C
			<u>1960</u>	1970	1980	199	0 2	000
hMyo-X	1950	<mark>wpgyg</mark> s <u>tlf</u> e	<mark>)VE</mark> C <mark>KE</mark> GGF	<mark>P</mark> Q <u>e<mark>lwlgv</mark>s</u>	<mark>a</mark> d <u>avsvyk</u>	<mark>RG</mark> EGR- <mark>PLI</mark>	V <mark>FQYEH</mark> IL	<u>.S<mark>FG</mark>APLA<mark>NT</mark></u>
bMyo-X		<mark>wpgyg</mark> s <mark>tlf</mark> e	<mark>VE</mark> C <mark>KEGGF</mark>	<mark>P</mark> QD <mark>lwlgvs</mark>	<mark>a</mark> da <mark>vsvyk</mark>	<mark>Rg</mark> egr- <mark>pli</mark>	EV <mark>FQYEHI</mark> L	.S <mark>FGAP</mark> LA <mark>NT</mark>
хМуо-Х		<mark>wpgyg</mark> p <mark>tlf</mark> [<mark>)VE</mark> Y <mark>KEGGF</mark>	<mark>p</mark> nd <mark>lwlgvs</mark>	<mark>a</mark> en <mark>vsvyk</mark>	<mark>Rg</mark> dak- <mark>pli</mark>	T <mark>FQYEHI</mark> I	F <mark>fgap</mark> qp <mark>nt</mark>
hRad	198	LEM <mark>y</mark> gv <u>ny<mark>f</mark>e</u>	<u>EIKN<mark>K</mark>KG—–</u>	-T <u>e<mark>lwlgv</mark>d</u>	<mark>a</mark> l <u>glni<mark>y</mark>e</u>	hddklt <mark>p</mark> k	IG <mark>F</mark> PWSE <mark>I</mark> F	<u>RNISFN</u> -DK <u>K</u>
		β6C	β7C		α1C			
		2010	2020	2	030	2040	2050	2058
hMyo-X	2010	<u>Y<mark>kivv</mark>d</u> E	RELLFET-	se <mark>v</mark> vdva	<mark>Kl<mark>m</mark>kayis</mark>	<u>mivkkr</u> ys	FTRSA <mark>s</mark> sq@	as <mark>s</mark> r
bMyo-X		Y <mark>ki</mark> v <mark>vd</mark> ——e	RELLFET-	se <mark>v</mark> vdva	KL <mark>Mk</mark> ayis	<mark>mivkkr</mark> ys [.]	FSRSV <mark>s</mark> sq@	as <mark>s</mark> r
xMyo-X		F <mark>ki</mark> t <mark>vd</mark> ——[RELFFET-	——TQ <mark>V</mark> GE I T	<mark>k</mark> i <mark>m</mark> rayin	MI VKKR <mark>cs</mark> v	/RSVT <mark>s</mark> qds	SQ <mark>s</mark> snwar
hRad	255	FV <mark>I</mark> KPIDKKA	APDFV <mark>F</mark> YAP	RLR I N <mark>K</mark> R I L	ALCMGNHE	LY MRRR KPI	DTIEVQQMM	AQAR

Supplementary Figure S2 (continued)

Figure S2 Sequence alignment and secondary structures of the myosin-X MyTH4-FERM cassette.

Human, bovine and xenopus MyTH4-FERM cassettes are aligned with conserved residues highlighted in yellow (hMyo-X, bMyo-X and xMyo-X, respectively). Secondary structures are indicated with double underlines (α -helices) and single bold underlines (β -strands). In the current map of hMyo-X, N-terminal residues (1486-1500), which include T1486 and R1487 believed to belong to the upstream PH2 domain, and 12 C-terminal residues (2047-2058) were not defined. These residues are highlighted in grey with white letters and wavy underlines. Compared with radixin, the insertion residues (1851-1891) of human myosin-X are highlighted in grey, with deleted residues (1872-1891) shown as white letters. Basic residues that form the basic patch on the MyTH4 domain of human myosin-X are highlighted in blue. Residues that participate in forming the interface between the MyTH4 and FERM domains are highlighted in green and cyan, respectively.

The MyTH4 domain is also aligned with the VHS domain of human STAM1 (hSTAM1), and the FERM domain is aligned with the human radixin FERM domain (hRad). In the crystal structure of the STAM1 VHS domain bound to ubiquitin (PDB ID 3LDZ), key residues that directly interact with ubiquitin are highlighted in red (critical) or pink (not essential), with other residues (grey) conserved in the VHS domains. In the crystal structure of the radixin FERM domain bound to IP₃ (PDB ID 1GC6), key residues that directly interact with IP₃ are highlighted in blue or green, and other basic residues that participate in the basic cleft between subdomains A and C are shown as blue letters.



Supplementary Figure S3 (continued)

Figure S3 Comparison between FERM domains of myosin-X and radixin.

(A) Individual superposition of subdomains A, B and C from the myosin-X (cyan) and radixin (magenta) FERM domains with r.m.s. deviations of 2.3 Å (75 residues, 13% identity), 2.1 Å (102 residues, 22% identity) and 1.4 Å (86 residues, 20% identity), respectively. The poorly conserved extra residues of subdomains A and B are indicated with a label "insertion".

(**B**) Stereo-view of superimposition of FERM domains of myosin-X (cyan) and radixin (magenta) with a relatively large r.m.s. deviation of 4.8 Å for 273 residues that exhibit 18% identity.

(**C**) A shift in subdomain A orientation and position relative to subdomain B is induced by contacts made by the β 5A and β 3A strands from subdomain A with the α 1B helix from subdomain B. In the myosin-X FERM domain (*left*, cyan), subdomain A has a longer β -sheet comprising the β 3A (Phe1742) and β 5A (Phe1786) strands that sits on the α 1B helix (Phe1806) of subdomain B, pushing one side of the β -sheet contacting the α 1C helix upward from subdomain B. Contacts involving aromatic rings are shown. In the radixin FERM domain (*right*, magenta), subdomain A has a shorter β -sheet comprising the β 3A (Phe45) and β 5A (Phe80) strands. This sheet and the flanking α 1A- β 3A loop (Phe44) make contact with the α 1B helix (Leu101 and Phe102) of subdomain B.

(**D**) A large shift in subdomain C orientation and position relative to subdomain B is induced by contacts involving the linker between subdomains A and B. In the myosin-X FERM domain (*left*, cyan), the β 5A- α 1B loop (Leu1792), and the β 5A (Cys1790) and β 3A (Met1741) strands push the C-terminal end of the α 1C helix (Met2039) upwards. In the radixin FERM domain (*right*, magenta), Phe84 (β 5A- α 1B loop) and Trp43 (α 1A- β 3A loop) make contact with Leu290 (α 1C). The rotation of subdomain C is pivoted on myosin-X Leu2032 (α 1C), which corresponds to radixin Leu283 (α 1C).



Figure S4 Structural comparison of the myosin-X MyTH4-FERM domain cassettes obtained in this study.

The crystal of the free form of the myosin-X MyTH4-FERM domain cassettes contain two crystallographically independent molecules. Two molecules A (magenta) and B (grey) of the free forms are superimposed on the DCC-bound form (cyan).



Supplementary Figure S5

Figure S5 CD spectrum of the DCC P3 peptide (1373-1477).

The spectrum obtained clearly suggested the absence of typical secondary structures such as the α -helix at a sample concentration of ~0.1 mg/ml (13 μ M). CD spectra were recorded using a JASCO J-720W spectropolarimeter at 20°C. Secondary structure estimations were calculated using the JASCO Secondary Structure Estimation software.



Figure S6 Basic residues of the MyTH4 domain and the FERM-bound DCC P3 helix.

The C-terminus of the DCC P3 helix has a peptide stretch (Gly-Ser-Ala-Phe-COOH) which is flexible in our crystal, probably due to the glycine residue, and the last two residues are absent in our structure. These residues occupy the space (orange gradation) with a radius of about 10 Å or 15 Å and could reach some basic residues of the MyTH4 domain. Thus, the C-terminal flexible peptide region could interfere with accommodation of the tubulin C-terminal acidic tail at the concave region formed by MyTH4 and FERM domains.



Figure S7 Structural comparison of our myosin-X MyTH4-FERM-DCC complex with the MyTH4-FERM-DCC fusion protein.

(A) Superimposition of the myosin-X MTH4-FERM cassettes of our complex (cyan) and the recently-reported fused protein (PDB ID 3PZD, yellow) with an r.m.s. deviation of 1.9 Å for 494 residues. The DCC peptide (orange) of our complex is shown with the fused DCC peptide (green) of the fusion protein. The observed reorientation of subdomain C is indicated by a pink arrow and a shift of the bound DCC peptide by a green arrow.

(B) Superimposition of subdomain C's of our complex (cyan) and the fusion protein (yellow) with an r.m.s. deviation of 0.8 Å for 89 residues.

(C) A close-up view of the fused DCC peptide (green) bound to subdomain C of the fusion protein (yellow) superimposed upon our complex (cyan) and the bound DCC peptide (orange). The hydrogen bond between Lys2031 and Ser1445 of the fusion protein is shown with a black solid line.



Figure S8 Structural comparison of our myosin-X MyTH4-FERM - DCC complex with the myosin-VIIa MyTH4-FERM-SH3 - Sans complex.

Stereo-view of superimposition of MTH4-FERM cassettes of myosin-X (cyan) and myosin-VIIa (PDB ID 3PVL, yellow) with an r.m.s. deviation of 6.2 Å for 432 residues. The myosin-X MyTH4-FERM cassette binds DCC P3 peptide (orange) and the myosin-VIIa MyTH4- FERM cassette is linked to the SH3 domain (pink) and binds Sans CEN peptide (a green stick model).