**Review article** 

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## Regulation of phospholipid distribution in the lipid bilayer by flippases and scramblases

In the format provided by the authors and unedited

## **Supplementary Information**

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				α1					_		α	2					_
8A1	62	ITFL	PRFI	JYSQFRRAANS	SFFLFIA	ALL <mark>Q</mark> Q	IPDV	S <mark>PT</mark> GF	Y <mark>T</mark> TL	VPLLI	FILAN	AAIF	EII	DIK	RHKA	DNAV	NKKQT
8A2	81	LTFL	PRFI	YEQIRRAAN	AF <mark>FLFI</mark>	A <mark>LL</mark> QQ	IPDV	S <mark>PT</mark> GF	YTTL	VPLI	[ <mark>I</mark> LT]	AGI	(EIV	DFK	RHKA	DNAV	NKKKT
11A	58	WNFI	PKNI	JFEQFRRVAN	YFLII	FLV <mark>Q</mark> L	IID-'	r <mark>pt</mark> sf	VTSG	L <mark>PL</mark> FI	VITV	TAIF	QGY	DWL	RHKA	DNAM	NQCPV
11C	53	WNFL]	PKNI	FEQFRRIAN	Y <mark>F</mark> LII	FLV <mark>Q</mark> V	TVD-'	Г <mark>РТ</mark> SF	V <mark>TS</mark> G	L <mark>PL</mark> FI	VITV	TAIF	QGY	DCL	RHRA	DNEV	NKSTV
10A	73	LSFL	PKNI	FEQFHRPAN	VYFVFI	ALLNF	VPAV	NAFQF	GLAL	A <mark>P</mark> VLI	FILAI	TAFF	RDLW	DYSI	RHRS	DHKI	NHLGC
10B	79	FTFL	PRNI	JFEQFHRWAN	LYFLFL	JILNW	MPSM	EVFHF	EITM	L <mark>PL</mark> A]	[VLF\	/IM <mark>I</mark>	DGM	DFKI	RHRF	DKAI	NCSNI
yDnf1	204	LTFL]	PKN1	LFQFHNFAN	/YFLVL	II <mark>L</mark> GA	FQIF	GVTNE	GLSA	VPLVV	/IVI]	TAI	DAI	EDS <mark>R</mark> I	<b>R</b> TVL	DLEV	NTKT
$\alpha 4$																	
		8A1	340	FGLN <mark>FL</mark> TFI-	ILF	NNLIP	ISLL'	<b>V</b> TLEV	V <mark>K</mark> FT	QAYF1	NWDI	DMH3	(EPTI	TAAN	4ART:	S <mark>N</mark> LN	EELGQ
		8A2	359	FGYNL <mark>LTF</mark> I-	ILY <mark>I</mark>	NNLIP	ISL <mark>L</mark>	V <mark>T</mark> LEV	V <mark>K</mark> YT	QALF]	[NWD]	י <mark>DMY</mark> ?	IGN	TPAN	1ART	S <mark>N</mark> LN	EELGQ
		11A	345	AFTD <mark>FLAF</mark> M-	VLFI	NYI <mark>IP</mark>	V <mark>SMY</mark>	<b>√</b> TVEM	Q <mark>K</mark> FL	GSY <mark>F</mark> I	TWDE	DMFI	DEET(	GEGPI	LVNT	SDLN	EELGQ
		11C	340	MFTD <mark>FLSF</mark> M-	VLF	NFIIP	V <mark>S</mark> MY	<b>V</b> TVEM	Q <mark>K</mark> FL	GSF <mark>F</mark> I	SWDF	(DFYI	DEEIN	IEGAI	LVNT	SDLN	EELGQ
		10A	358	AVYS <mark>FL</mark> TMI-	<mark>I</mark> VL	QVLIP	ISLY	VSIEI	VKAC	QVYF1	I NQ <mark>D</mark> M	IQL <mark>Y</mark> I	)EETI	SQL	)CRA	L <mark>N</mark> IT!	EDLGQ
		10B	364	GFYM <mark>FL</mark> TMI-	ILL	QVLIP	ISLY	<b>V</b> SIEI	V <mark>K</mark> LG	QVF <mark>F</mark> I	SNDI	DLYI	)EETI	LSI	)CRA	L <mark>N</mark> IA	EDLGQ
	Σ	yDnf1	595	ATNGFVSFW	/AVILY	2SLVP	ISLY	ISVEI	I <mark>K</mark> TA	QAAF1	[ <mark>Y</mark> GD\	/LL <mark>Y</mark> N	JAKL	)YPC	PKS	NIS!	DD <mark>LGQ</mark>
α6																	
						8A1	872 L	FERWO	∶⊥ <mark>G</mark> LY		I'AMPI	2LTL(	JIFEI	R			
						8A2	907 L	F ERWO	JGL <mark>Y</mark>	NVIF.		?F"TL(	JIFEI	R			
						11A	911 L	YDTAY	LTLY	NISF	TSLP.	ILLY:	SLME(	2			
						11C	902 L	YDAAY	L <mark>I</mark> MY	NICF	TSLP.	LAY:	SLLE(	2			
						10A 1	117 M	IDQW	LIFF	NLLF	SSLPI	PLVT(	JVLDI	R			
					_	10B 1	141 M	IDYWÇ	QMIFF	NLFF	TSLP1	PLVF(	JVLD	K			
					УĽ	<b>nt</b> 1 1	216 L	YEYTY	(MMF' <mark>Y</mark>	NLAF"	<b>TSLP</b>	/IFL(	GILD	$\tilde{\Sigma}$			
b																	
0-1			α10				<i></i>										
841	10	54 YK		'''AFKTLVDEV	() ET EAT	SODP	Gi	$\Delta V V T_{-}$	G'	KSLT	RAOT	. KNV	/ F'KKN	JHVNT	YRS	ESLO	DNT T

8A1	1054	YKVIKRTAFKTLVDEVQELEAKSQDPGAVVLGKSLT <mark>ERAQLL</mark> KNVFKKNHVNLYRSESLQQNLL
8A2	1089	WRAAKHTCKKTLLEEVQELETKSRVLGKAVLRDSNGKRLN <mark>ERDRLI</mark> KRLGRKTPPTLFRGSSLQQGVP
11A	1093	KKVLCRQLWPTATERVQTKSQCLSVEQSTIFMLSQTSSSLSF
11C	1084	I.TVLKNVRRRSARRNI.SCRRASDSI.SARPSVRPI.LI.RTFSDESNVI

Supplementary information Fig. 1. Alignment of P4-ATPases that work as a flippase in the plasma membranes and endosomes. (a) The amino acid sequences of the regions spanning the transmembrane helices  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 4$ , and  $\alpha 6$ , of human ATP8A1 (UniProt: Q9Y2Q0), 8A2 (Q9NTI2), 11A (P98196), 11C (Q8NB49), 10A (O60312), 10B (O94823), and yeast Dnf1 (P32660) are aligned to obtain the maximum homology. The residues that are identical in 5 or more members are red. The conserved "PVSM" motif that seems to work as a 'gate' is highlighted in green in ATP11C<sup>1</sup>. The replacement of Q84 by Glu in ATP11A (highlighted in purple) concurs with the molecule flip of PtdSer and PtdCho<sup>2</sup>. The amino acids that coordinate the serine residue of PtdSer in ATP11C are highlighted in dark green<sup>2</sup>. The amino acid residues necessary for the flipping activity of ATP8A2<sup>3</sup> are highlighted in yellow, while the residues proposed to determine the substrate specificity in ATP8A1<sup>4,5</sup> are in light blue. The residues coordinated in the entry site (Q610 and S611) or exit site (R264, Y633, T648, and W-652) of the yeast Dnf1<sup>6</sup> are highlighted in red. (b) The amino acid

sequences of the C-terminal region of the human members are aligned, and the homologous residues are in orange. The acidic di-leucine motif ([DE]XXXL[LI]) for endosome sorting<sup>7</sup> is highlighted in green.

α1

16C	(327)	IRKLINNGSYIAAFPPHEGAYKSSQPIKTHGPQNNRHLLYERWARWGMWYKHQPLDLIRLYFGEKIGLYF
16E	(237)	IERLLNSNTYSSAYPLHDGQYW <mark>K</mark> PSEPP <mark>NP</mark> TNERYTLHQNWARFSYFYKEQPLDLIKNYYGEKIGIYF
16F	(232)	INRLVNSGIYKAAFPLHDCKFRRQSEDPSCPNERYLLYREWAHPRSIYKKQPLDLIRKYYGEKIGIYF
16K	(158)	LRRLLTSGIVIQVFPLHDSEALKKLEDTWYT-RFALKYQPIDSIRGYFGETIALYF
	· · ·	
16C	(397)	AWL <mark>G</mark> WYTGMLIPAAIVGLCVFFYGLFTMNNSQVSQEICKATEVFMCPLCDKNCSLQRLNDSCIYAKV
16E	(305)	VFLGFYTEMLFFAAVVGLACFIYGLLSMEHNTSSTEICDPEIGGQMIMCPLCDQVCDYWRLNSTCLASKF
16F	(300)	AWLGYYTQMLLLAAVVGVACFLYGYLNQDNCTWSKEVCHPDIGGKIIMCPQCDRLCPFWKLNITCESSKK
16K	(213)	GFLEYFTFALIPMAVIGLPYYLFVWE
	、 <i>,</i>	α2
16C	(464)	TYLFDNGGTVFFAIFMAIWATVFLEFWKRRRSILTYTWDLIEWEEEEETLRPQFEAKYYKMEIVNPITGK
16E	(375)	SHLFDNESTVFFAIFMGIWVTLFLEFWKQRQARLEYEWDLVDFEEEQQQLQLREFEAMCKHRKLNAVTKE
16F	(370)	LCIFDSFGTLVFAVFMGVWVTLFLEFWKRROAELEYEWDTVELOOEEOARPEYEARCTHVVINEITOE
16K	(239)	DYDKYVIFASFNLIWSTVILELWKRGCANMTYRWGTLLMKRKFEE-PRPGFHGVLGINSITGK
	· · ·	α3
16C	(534)	PEPHOPSSDKVTRLLVSVSGIFFMISLVITAVFGVVVYRLVVMEOFASFKWNFIKOYWOF
16E	(446)	MEP-YMPLYTRIPWYFLSGATVTL-WMSLVVTSMVAVIVYRLSVFATFASFMESDAS-LKOVKSFLTPOI
16F	(438)	EERTPFTAWGKCTRTTLCASAVFF-WILLTASVIGTTVYRLSVFTVFSAKLPKNINGTDPTOKYLTPOT
16K	(301)	EEPLYPSYKROLRTYLVSLPFVCLCLYFSLYVMMTYEDMEVWALGLHENSSEWTS
1010	(001)	
16C	(594)	ATSAAAVCINETIIMI.NI.AYEKIAYI.UTNI.EYPRTESEWENSFALKMELEOFVNI.NSSIEYIAFELGRE
16E	(513)	TTSLTGSCLNFTVILILNFFYEKIS-AITKMEIPRTYOEYESSITLKMFLFOFVNFYSSCFYVAFFKGKF
16F	(510)	ATSTTASTISETTIMILNTIYEKVAIMTTNEELPRTOTDYENSLTMKMELEOFVNY YSSCEVIAEEKGKE
16K	(357)	VILYVPSTIYATVIETMNRLYRYAAEFLTSWENHRLESAYONHLILKVLVFNFLNCFASLFYLAFVLKD-
1010	(337)	
16C	(664)	VGHPGKYNKI.FDRWRI.EECHPSGCI.TDI.CI.OMGVIMFI.KOIWNNFMEI.GYPI.TONWWSRHKIKRGIH
16E	(583)	VGYPGKYTYLENEWRSEECDPGGCLIELTTOLTIMTGKOIFGNIKEAIYPLALNWWRRKARTNSE
16F	(500)	VGYPGDPVYWLGKYRNEECDPGGCLLELTTOLTITMGGKAIWNNTOEVLLPWIMNLIGREHRVSGSE
16K	(377)	MKLLROSLATLITTSOILNOIMESFLPYWLORKHGVRVKRKVOALK
1010	(120)	
16C	(731)	DASTPOWENDWNLOPMNI.HCI.MDEVI.EMVLOFCETTTEVAAEPI.API.LAI.LNNTTETRI.DAVKEVTO
16E	(650)	KLYSRWEODHDLESEGPLGLEVEWLETVTOEGEVTLEVASEPLAPLIALINNIVEIRVDAWKLTT
16F	(644)	KTTPRWEODYHLOPMCKLCLEYEVLEMTTOFCEVTLEVASEPLAPLLALVNNTLETRVDAWKLTTO
16K	(472)	ADIDATI.YEOVILEKEMGTY_LGTEDDYLELELOFGYVSLESCVYPLAAAFAVLNNETEVNSDALKMCRV
TOIL	(4/2)	
160	(798)	WPRDLDARATDTCTWLCTLFCTCTLAUTTNAFUTATTCDVTDRFUVFVKWCDCANHUFDSFNCLKCVUNN
16E	(716)	
16F	(710)	FRELUERKANDIG WODTHIORAVID VALIVALITATIODITINI VITATOTICATOTICA VIDUALIDI VIDUALITATICA VIDUALIDI VIDUALIDI VALIVALITATICA VIDUALIDI VIDUALIDI VIDUALIDI VALIVALITATICA VIDUALIDI VALIVALIVALITATICA VIDUALIDI VALIVALITATICA VIDUALIDI VALIVALIVALIVALIVALITATICA VIDUALIDI VALIVALIVALIVALIVALIVALIVALIVALIVALIVALI
16K	(710)	FKRDFSFDSANICVWOLAFFTMSVISVVTNCALICMSDOV
TOK	(341)	
160	(868)	GI SEEDI SEICMCKSCVCDVDDVDCDDWSSKDVEETI OVWHII AADI AEI IVEEHI VECI
16F	(000)	SISTIDISEDGROKSGICKIKDIKGI WSSKITETIQIWHILAAKLAFIIVIBHUVFGI
16F	(700)	
161	(775)	
TOK	(301)	
1	60 (0)	
1	100 (94 6F (94	20) ΚΕΙΙΔΙΜΙΤΟΥΓΙΟΝΠΟΛΙΑΝΟΛΙΟΥΥΠΟΝΟΥΥΠΟΝΟΥΥΠΟΝΟΥΥΠΟΝΟΥΥΠΟΝΥ
1	6F (0)	10) KEELCAVIDUACKDAKCKIODEKALAOKIIILUUTUUTUUMANMUALVEDALEVAUAMITEEMVAATPAKAI 10) KEELCAVIDUACKDAKCKIODEKALAOKIIIUUTUUTUUMANMUALVEDALEVAUAMITEEMVAATPAKA
1	1617 (0'	UE 1 YEAT DE REDERTONKT VELECTENT KOUUMAT AMENI KEEDWEGGAEAVA
-	TOV (0	^ \ VI TEVLYTE NVE VIITÄLIVEVUTEL EDERPYÄÄÄLIVE A LEN PVEELIEDOVEVAI

Supplementary Fig. 2. TMEM16 Ca<sup>2+</sup>-dependent scramblase. The amino acid sequences of human TMEM16C (UniPlot: Q9BYT9), 16E (UniProt: Q75V66), 16F (Q4KMQ2), and 16K (Q9NW15) are aligned. The residues identical among the three or four members are shown in red. The transmembrane helices are shadowed and numbered. The number at the right is the amino acid number. The acidic residues involved in  $Ca^{2+}$  -binding are highlighted in yellow. In addition to the well-accepted five Glu/Asp residues (Glu-623, Glu-666, Glu-670, Glu-698, and Asp-702) in helices  $\alpha 6-8$  of human TMEM16F) that bind two Ca<sup>2+</sup> (<sup>8,9</sup>), Glu-394 in  $\alpha 2$  and Asp-858 in  $\alpha 10$ coordinate an additional  $Ca^{2+}$  (<sup>10,11</sup>). The domain responsible for scrambling phospholipids<sup>12,13</sup>, assigned in TMEM16F, is highlighted in light blue. The replacement of Phe-518 or Tyr-563 (highlighted in green) by Lys in TMEM16F concurs with the mutant constitutive-active<sup>14</sup>. The gain-of-function mutations of TMEM16C found in dystonia (N225S, Y279N, I308L, G400V, V463M, W490C, R494W, E510K, A657T, Y847C) (https://www.hgmd.cf.ac.uk/) and TMEM16E (R215G, C356R/G/Y, C360Y, S500F, T513I, and G518E) found in gnathodiaphyseal dysplasia<sup>15</sup> are highlighted in green. The mutations of N52S, F54S, R57W, R58W, D81G, V87I, D93E, L108R, G126V, I133F, H134R/Y, Y143C, E202K, R215G, G231V, K259N, N265S, P266L, L273F, G301V, C342Y, N366S, T368M, R404L, A432G, A464D, M470R, S506G, M543I, R547Q, T548I, S555I, Q564L, F578S, R642L, Y652C, W655C, Y673C, N701D, T714S, R758C, L781P, S796L, C804S, Y806C, Y819C, A830V, M833K, M839R, H841D, and I865L (https://www.hgmd.cf.ac.uk/) (highlighted in pink) in TMEM16E are loss of function mutations causing muscular dystrophy or myopathy<sup>15</sup>. Mutations of F171S, G229W, R263H, F337V, L510R, C513R, and D615O (https://www.hgmd.cf.ac.uk/) (highlighted in pink) in TMEM16K were found in human spinocerebellar ataxia<sup>16</sup>.

hXK	MKF <mark>PAS</mark> V	LASVFLFVAETTA	ALSISTYRSGGDRMWQA
CED8	MFLKKHKSKLLLVPRDE <mark>EQED</mark> AGIVAVLTDRIPSVLLVRWFDLFCF	<b>GFAMCSYALD</b> FFS	SDIGIAIFHFWAGR <mark>Y</mark> LS <mark>G</mark> S
hXKR8	MPWSSRGALLRDLVLG	<b>VLGTAAFLL<mark>D</mark>LGI</b>	DLWAAVOYALGGRYLWAA
hXKR9	MKYTKONFMMS	VLGIIIYVT <mark>D</mark> LIV	<b>DIWVSVRFFHEGQYVF</b> SA
		20	40
	α2	α3a	α3b
hXK	LTLLFSLLPCALVQL-TLLFVHRDLSRDR	PLVLLLHLI	<b>QLGPLFRCFEVFCIYFQS</b>
CED8	LVLAFALLPSVIINII <mark>S</mark> MVWMLDDEMHWKRRAHPRRTGTFELNQKR	FIPLSKMIVLCIC	COMGPLFWYYKALYYGWMF
hXKR8	LVLALLGLASVALQLFSWLWLRADPAGLHGSQPPR	RCLALLHLI	OLGYLYRCVQELRQGLLV
hXKR9	LALSFMLFGTLVAQCFSYSWFKADLKKAGQESQ	HCFLLLHCI	<b>OGGVFTRYWFALKRGYHA</b>
	60	80	100
	$\alpha 4$		
hXK	GNNEEPY <mark>VSITKK</mark> RQMPKNGLSE <mark>EIEKEVG</mark> QAEGKLITHRSAFSRA	SVIQA <mark>FL</mark> GS <mark>APQ</mark> I	TLQLYISVMQ
CED8	RKSSNENTDGEKRKCFSKMVEAERDATLLR-	-FFEAFLESAPQI	JIIQ <mark>G</mark> SIAASYFQNYYQTG
hXKR8	WQQEEPSEFDLAYADFLALDISML <mark>R</mark> -	–LF <mark>E</mark> TFL <mark>E</mark> TAPQI	TLVLAIMLQS
hXKR9	AFKYDSNTSNFVEEQIDLHKEVIDRVTDLSMLR-	-LFETYLEGCPQI	ILQLYILLEH
	120 14	0	
	α5	α6	
hXK	QDVTVGRSLLMTISLLSIVYGALRCNILAIKIKYDEYEVKVKPL	AY <mark>VCIFLW</mark> RSFEI	AT <mark>R</mark> VVVLVLFTSVLKTWV
CED8	TYPYWLYFQAASLLLSIISISWSVVVQNRSLRMIRDDKVNIWPH	EA <mark>V</mark> LQ <mark>FCW</mark> RFLTI	LARIITLVALVLIFGINV
hXKR8	GRAEYYQWVGICTSFLGISWALLDYH <mark>R</mark> ALRTCLPSKPLLGLG	SS <mark>V</mark> IY <mark>FLW</mark> NLLLI	WPRVLAVALFSALFPSYV
hXKR9	GQANFSQYAAIMVSCCAISWSTVDYQVALRKSLPDKKL-LNGLC	PKITYLFYKL <mark>F</mark> TI	LSWMLSVVLLLFLNVKIA
160	180 200		220
	α7 α8		
hXK	VVIILINFFSFFLYPWILFWCSGSPFPENIE-KALSRVGTTIVLCF	LTLLYTGINMF <mark>C</mark> W	VSAVQLKIDSPDLISKSHN
CED8	VPLISVHLLVTLVHVIFLQAIHIDACTHIEKLLLLINTFIHIF	IP <mark>F</mark>	NMVEGN <mark>T</mark> R
hXKR8	ALHFLGLWLVLLLWVWLQGTDFMPDPSSEWLYRVTVATILYF	SWF	NVAEGR <mark>T</mark> R
hXKR9	LFLLLFLWLLGIIWAFKNNTQFCTCISMEFLYRIVVGFILIF	TF <mark>F</mark>	NIKGQN <mark>T</mark> K
	240 260		280
	α9 α10		
hXK	WYQLLVYYMIRFIENAILLLLWYLFKTDIYMYVCAPLLVLQLLIGY	CTAILFMLVFYQF	FHPCKKLFSSSVSEGFQR
CED8	W-RYLTAYSVEFI <mark>E</mark> MMLVCWLLPLSLNTFPYIEKVQVGVPIS-F	LAGLALMMMYYQE	'F'HPNRRQL1VTQSQEDLS
hXKR8	G-RAIIHFAFLLSDSILLVATWVTHSSWLPSGIPLQLWLPVGCGCF	FLGLALRLVYYHW	VLHPSCCWKPDPDQVDGAR
hXKR9	CPMSCYYIVRVLGTLGILTVFWVCPLTIFNPDYFIPISITIVLT-L		<b>FHPNRSAETKCDEID</b> GKP
	500 520	540	560
1. 171			
		JI LNAEDLCSA	
	KO SLLSPEGIULPUNKKMTHLAUKFFPKAKDEAASPVKG		
пхк	\KY_V <u>L</u> KECK <mark>M</mark> KYFLME		

Supplementary Fig. 3. The conserved amino acid sequence of XKR scramblases. The amino acid sequences of the XKR scramblases [human XK (UniProt; P51811), XKR8 (Q9H6D3), XKR9 (Q5GH70), and C. elegans CED8 (O17386)] were aligned with  $\alpha$ -helices numbered. The amino acid residues identical in three or more members are shown in red, while those conserved in the same category (non-polar: Gly, Ala, Val, Ile, Leu, Pro, Phe, Trp, and Met; uncharged polar, Ser, Thr, Cys, Tyr, Asn, and Gln; charged polar, Asp, Glu, Lys, Arg, and His) are in orange. The caspase-recognition sites and  $\beta$ -hairpin structures are highlighted in yellow and red, respectively. The intramolecular six charged residues required for phospholipid scrambling and the Trp-45 gatekeeper<sup>17</sup> in human XKR8 are highlighted in green. The residues mutated in human patients

(R222G, C294R, and E327K) (<u>https://www.hgmd.cf.ac.uk/</u>)<sup>18</sup>, and the residues mutated in C. elegans (G76E, S94L, G139R, G200E, A309T, and E356K)<sup>19</sup> are highlighted in purple. The number at the bottom line is the amino acid position of human XKR9.

	α1		α2		
XKR8-N	MPWSSRGALLRDLVLGVLGT.	AAFLL <mark>D</mark> LGT <mark>DLWAAV-</mark> Q	Y <mark>ALG</mark> GRY <mark>LWAALVI</mark>	LALLGLASVA	59
XKR8-C	KPLLGLGSS	VIYFLWNLLL <mark>LWPRV</mark> LA	VALFSAL FPSYVAI	HFLGLW	237
		x3a	α3b		
XKR8-N	LQLFSWLWLRADPAGLHGSQ	PPRRCLALLHL <mark>L</mark> QLGYL	YRCVQELRQ <mark>G</mark> LLVV	VQQEEPSEFD	119
XKR8-C	LVLLLWVWLQGTDFMPDPS:	EWLYRVTVATILYFSW- <sup>X8</sup>	-FNVAEGRTR <mark>GRAI</mark>	[H <mark>F</mark> A	290
	α4a	α4b	α5		
XKR8-N	LAYADFLALDISMLRLFETF	L <mark>E</mark> TAPQLTLVLAIMLQS	GRAEYYQW <mark>VG</mark> ICTS	<b>FLGISWALL</b>	179
XKR8-C	FLLSDSILL-VATWVTHSSW	LPSGIPLQLWLP	<mark>VGCGC</mark> E	FLGLALRLV	336
XKR8-N	DYHRALRTCLPS				191
XKR8-C	YYHWLHPSCCWKPDPDQVDG	ARSLLSPEGYQLPQNRF	RMTHLAQKFFPKAKI	DEAASPVKG	395

Supplemental Fig. 4. **Two similar domains in human XKR8.** The amino acid sequences of the N-terminal (amino acids 1-191) and C-terminal (amino acids 192-395) domains of human XKR8 (UniProtKB; Q9H6D3) are aligned. The identical amino acids are red, while the conserved amino acids are orange. The caspase 3-recognition site is highlighted in yellow, while the charged amino acids essential for XKR8's scrambling activity are green.

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