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**Supplementary information**

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**The tertiary structure of the human Xkr8–  
Basigin complex that scrambles  
phospholipids at plasma membranes**

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In the format provided by the  
authors and unedited

**Supplementary Table 1. Primers for mutagenesis of human BSG, and human and mouse Xkr8**

**hBSG mutants N152Q and N186Q**

	forward primer	reverse primer
	TCCGCTAGCGGGCCAACTCCTAAAAAACCGCCACCATG	TCTCGAATTCGTCGATCACTTGTC
	complementary mutagenizing primers	
N152Q	<u>CTCATGCAAGGCTCCGAGAGCAGGTTCTTC</u>	<u>GGAGCCTTGATGAGGGCCTTGTCCTCAGAG</u>
N186Q	<u>CGGTGCCAAGGCACCAGCTCCAAGGGC</u>	<u>GGTGCCTTGGCACCGTACTGGCCGGG</u>

**hBSG mutants A207C and P211C**

	forward primer	reverse primer
	GTGTCGTGAGGAATTCGCCACCATGGCGGCTGCGCTGTTTC	ATGGGTACATGAATTCGGAAGAGTTCCTCTGGCGGAC
	complementary mutagenizing primers	
A207C	<u>CACCTGTGCGCCCTCTGGCCCTTCCTGG</u>	<u>GAGGGCGCACAGGTGGCTGCGCACGC</u>
P211C	<u>CTCTGGTGCTTCCTGGGCATCGTGGCTGAGG</u>	<u>CAGGAAGCACAGAGGGCGGCCAGGTG</u>

**hBSG mutant E230C**

	forward primer	reverse primer
	CTAGACTGCCGGATCCGCCACCATGGCGGCTG	ATGGGTACATGAATTCGGAAGAGTTCC
	complementary mutagenizing primers	
E230A	<u>ATCTACGCGAAGCGCCGGAAGCCC</u>	<u>GCGCTTCGCGTAGATGAAGATGATGGTGACCAGC</u>

**mXkr8 mutants**

	forward primer	reverse primer
	GTGAGGAATTGGATCCATCATGC	ACAGATTCTCGAATTCGAGGACTCC
	complementary mutagenizing primers	
D12A	<u>GCCTTAGCCG</u> TGGTCGTAGGCCTGGTG	<u>GACCACGGCTAAGGC</u> CACATGGTGGTGC
D26A	<u>CTGCTGGCTCTGGT</u> CGCTGACCTGTGG	<u>GACCAGAGCCAGCAGG</u> AAAGACAAGATACTCAC
D30A	<u>GTCGCTGCCCTGTGGG</u> CCGTTGTCCAG	<u>CCACAGGGCAGCGACC</u> AGATCCAGCAGG
R42A	<u>CCTTGGCGCTTATCT</u> GTGGGCCGCGCTG	<u>AGATAAGCGCCAAGG</u> AGCACGTACTGGAC
W45A	<u>TTATCTGGCGGCCG</u> CGCTGGTACTGG	<u>GCGGCCGCCAGATAA</u> CGGCCAAGGAGCACG
R98A	<u>CTGTATGCGTGTTT</u> GCACGGAATGCATCAAGG	<u>CAAACACGCATACAG</u> GTAGCCGAGCTGCAG
D129A	<u>TCCCTGGCCATCAGC</u> ATGCTGAAGCTTTTCGAG	<u>GCTGATGGCCAGGG</u> AGAGAAAGTCTGCGTAG
K134A	<u>ATGCTGGCGCTTTT</u> TCGAGAGCTTCCTGGAGG	<u>GAAAAGCGCCAGCA</u> TGCTGATGTCCAGGGAG
E137A	<u>CTTTTCGCGAGCTT</u> CCTGGAGGCGACG	<u>GAAGCTCGCGAAA</u> AGCTTCAGCATGCTGATG
E141A	<u>TCCTGGCGGCGAC</u> GCCACAGCTC	<u>CGTCGCCGCCAGGA</u> AGCTCTCGAAAAGCTTC
Q155A	<u>TGTATTGGCGAATG</u> GCCAGGCGGAATACTACC	<u>CCATTGCGCAATA</u> CAATTGCCAGCACCAGTGTG
D180A	<u>CTGCTGGCTTACC</u> ATCGGTCTCTGCGTACC	<u>ATGGTAAGCCAGC</u> AGTGCCCACGAGATG
R183A	<u>TACCATGCGTCTC</u> TGCGTACCTGTCTTCCC	<u>CAGAGACGCATGG</u> TAATCCAGCAGTGCCCAC
W310A	<u>GGCACCGCGCTG</u> CCCAGTGGGATCTCATTG	<u>GGGCAGCGCGGTG</u> CCGTGTGTCAACCAG

**hXkr8 mutants, R214G, R280E, R284E, and D295K**

	forward primer	reverse primer
	AGTTAATTAAGGATCCGCCACCATGCCCTGGTCTAG	CCTTGCTCACGAATTCTCCTTTCACAGGGCTGGCG
	complementary mutagenizing primers	
R214G	<u>GTGGCCCGGAGTGCTGGCCGTGGCC</u>	<u>AGCACTCCGGGCCACAGCAGCAGC</u>
R280E	<u>AGGGCGAGACAAGAGGCAGAGCCATCATC</u>	<u>CTCTTGTCTCGCCCTCGGCCACGTTG</u>
R284E	<u>AGAGGCCGAAGCCATCATCCACTTCGCTTTTC</u>	<u>GATGGCTTCGCTCTTGTCCGGCCCTC</u>
D295K	<u>CTGAGCAAGAGCATCCTGCTGGTGGCTAC</u>	<u>GATGCTCTTGCTCAGCAGAAAAGCGAAGTGG</u>

**hXkr8 mutants, T302C and T305C**

	forward primer	reverse primer
	GTGAGGAATTGGATCCGCCACCATGCCCTGGTCTAG	ACAGATTCTCGAATTCTCCTTTCACAGGGCTGGCG
	complementary mutagenizing primers	
T302C	<u>GTGGCTTGCTGGGTCACCCACTCTAGCTGG</u>	<u>GACCCAGCAAGCCACCAGCAGGATGCTGTC</u>
T305C	<u>TGGGTC TGCCACTCTAGCTGGCTGCCTAGC</u>	<u>AGAGTGGCAGACCCAGGTAGCCACCAGCAG</u>

\*Mutated residues are underlined. The first 15 nucleotides are complementary to each other between the complementary mutagenizing primers. Each mutant was prepared by PCR. Using hBSG, mXkr8 or hXkr8 cDNA as a template, PCR was carried out with the forward primer and the second mutagenizing primer for the 5' part, and with the first mutagenizing primer and the reverse primer for 3' part. The resultant PCR products for the 5' and 3' parts and linearized vector were fused by In-Fusion HD Cloning Kit (Takara).

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-----10-----20-----α1-----30-----40-----50-----α2-----60-----70-----80-----
human  MPWSSRGALLRDLVGLVLTAAFLLDLGTDLWAAVQYALGGRYLWAAIVLALLGLASVALQLFSWLWLRADPAGLHGSQP----
mouse  MPLSVHHHVALDVVVGVSILSFLDLVADLWAVVQYVLLGRYLWAAIVLVLGQASVLLQLFSWLWLTADPTELHHSQL----
Chicken MAARGPARFGPWOWLLAAAGAAAADVADAGADVWVAVGYARAGQPGCAALGLLWAAGSAARQACSWLWFRSDPSTLRPEL----
Fugu   MRVMGQATISNYSWIDFVFSVIGVFTFFVLDWGAADVWVATEFYSRGDFEFWFGILLVSLMVLSVSVLVQMFSWFWLKYDRELDPVCRQSGGG

-----α3a-----90-----100-----α3b-----110-----120-----α4a-----130-----140-----α4b-----160
human  -----PRRCLALLHLLQLGGLYLYKCVQELRQGLLVWQEEPESEFDLAYADFLALDISMLRLEFSTFLATAPQLTLVLAIMLQSGRAE
mouse  -----SRPFLALLHLLQLGGLYLYKCLHGMHQGLSMCYQEMPSECDLAYADFLSLDISMLKLEFSTFLATPQLTLVLAIVLQNGQAE
Chicken -----SRRPLAALHLLQLGGLYLYKCLRALRLGWRLCREEAT-EEERDHMAFLTHDISMLRLEFSTFLANAPQLAVLLYSVLRTNKAE
Fugu   TTVLFGDRVQLSWLLHVLQLGLFLCKHISAIRQGFRVWWRQQ---EGSEYAVYLTHTDLSMLRLITFSSAPQLTLMVHVMMLCTNRRAR

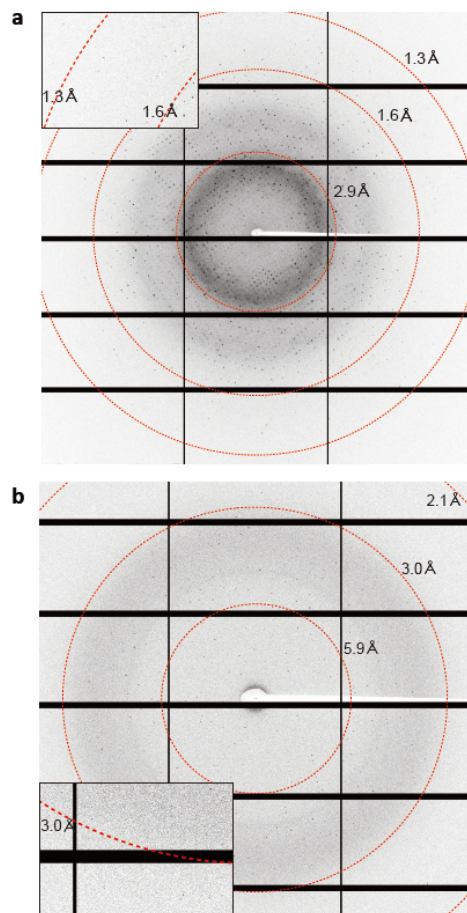
-----170-----α5-----180-----190-----200-----210-----α6-----220-----230-----α7-----240-----
Human  YYQWVGICTSFLGISWALLDYHFRALNTCLPSKPLLGLGSSVIYFLWNLLLWPRVLAVALFSAFSPSYVALHFLGLWLVLLWVWLQ
mouse  YYQWFGISSFLGISWALLDYHRSLENTCLPSKPRLGRSSAIYFLWNLLLWPRICAIALFSAVFPYYVALHFFSLWLVLLFWIWLQ
Chicken PSQGMGICTALLCVTWLLDYHQSLNLSFLQDKYEMGWSSVIYFLWNLLCPRIALALFALLWPYGVAVHFFPLVWLAMFIWVSLQ
Fugu   TVQ-----SWMVVDYHRSLEAFLPDKAKQGWSSLIYFLWNFLLIAPRVAALALFASVVGGFVAVHFLLLWCVFVMWAWLQ

250-----260-----α8-----270-----280-----290-----α9-----300-----310-----320-----α10-----330-----
Human  GTDFMPDPSSEWLYRVTVATILYFSWFNVAEGRTRGRAI IHFAFLLSMSILLVAT-WVTHSSWLPSGIPQLWLVPVCGCFFLGLLAL
mouse  GTNFMPDSKGEWLYRVTMALILYFSWFNVSGGRTRGRAVIHLIFIFSSVLLVTTWSWTHGTWLPVSGISLLMWVTIGGACFFLGLLAL
Chicken GTDFMESPGPEQLYRAMVAVILYFSWFNVAPGRTLYPSIIYHSFILLSTLLTLA-WLWGHPPPEEQRPYLIPVLCALPCYLGLLLL
Fugu   GTEFMDSVCGEGLYRATVGI IWYFSWFNVAEGQTRGSI IYHSFITDGGILLLT-WWCYRDPVQTEPYGLALLVTLFLFSYLLGLLFF

-----340-----350-----α11-----360-----
human  RLVYYHWLHPSCCW---KDPDQVDGARSLLSP-EGYQLPQNRRMTHLAQKFFPKAKDEAASPVKG-----
mouse  RVIYYLWLHPSCSW----DPLVDGTLGLLSPHRPPKLIYNRRATLLAENFFAKAKARAVLTEEVQLNGVL-----
hicken RVTYYGWLHPNVRAQP-RSGGDEVDAEGRHDGVVEFRSISEPSELRSVSGPDLVNSRLRCLARSHFPLSALAEPRLLNGAAAV----
Fugu   RTVYYCCFHPTMRRPPARESSDLPDAEVTFRHFSIQDGAPSSPLLNRMAAHAARFYSERRAVKNLGGVDAATSSPP-----

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**Supplementary Fig. 1. Sequence alignment of Xkr8 orthologues.** Sequences of Xkr8 from humans (UniProt: Q9H6D3), mice (UniProt: Q8C0T0), chickens (Chick) (UniProt; Q49M60), and fugu (UniProt: H2TYQ9) were analyzed by the MUSCLE Program (EMBL-EBI). Numbers above the first line are the amino acid positions for hXkr8. Conserved residues are indicated in red. Eleven  $\alpha$ -helices are shadowed and numbered. Negatively and positively charged residues in the lipid layer are highlighted in green, while the hydrophobic amino acids forming the cleft for PtdCho are highlighted in yellow.



**Supplementary Fig. 2. X-ray diffraction analysis of the hBSG $\Delta$ -Fab complex.** The representative X-ray diffraction pattern of a crystal of Fab14 (**a**) and the lipidated hBSG $\Delta$ -Fab complex (**b**) in buffer containing 33% PEG400. The area of high resolution was enlarged in insets.

XKR8 -----MPWSSRGALLRDLVLGVLGTAFFLLDLGTDLWAAVQYALGGRYLWAAVLAALL  
 XK -----MKFPASVILASVFLFVAETTAALSLSSSTYRSGGDRMWAQALLLPS  
 XKR2 MDRVYEIPEEPNVDPVSSLEEDVIRGANPRFTFPFSLFSTFLYCGEAASALYMVRIYRKNSETYWMYTTSPF  
 XKR3 METVFEEMDEESTGGVSSSKEEIVLQRLHLSPFSLIFSTVLYCGEVAFGLYMFEIYRKANDTFWMSFTISFI  
 XKR4 MAAKSDGRLLK--85--ALCLRLRGREQRRYSLWDCWLILAAVAVYFADVGTDLVWLAVDYYLRGQRWVWFGTLFFV  
 XKR5 -----MCHARLLGLSALLQAAEQSARLYTVAYYFTTGRLLWGWLAL--8V  
 XKR6 MAAKSDGGGV--101--AARPEPPPPQVERPWLDCWLIVLALLVFFGAVGTDLWLALDYRKGQDYVYFGLTFFV  
 XKR7 MAAKSDGAAA--29--PGVVGAGGPGPRYELRDCCVLCCALLLVFFSDGATDLWLAASYLQNHQHTYFSTLLLV  
 XKR9 -----MKYTKQNFMMSSVLGIIIVTDLIVDIWVSVRFFHEGQYVFSALALSFM

XKR8 GLASVALQLFSWLWLRADPAGLHG---SQPPRRCLALLHLLQLGLYRCAVQELRQGLLVWVQQEEPS-----  
 XK LIPCALVQLTLL-LFVHRDLRSRDRP-----LVLHLLHLLQLGLFRFCFEVFCIYFQSGNNEEY-14-SEE  
 XKR2 MFSSIMVQLTLL-LFVHRDLAKDKP-----LSLFMHLLILGLPVIRCLEAMIKYLTWKKKEEQE-16-EVL  
 XKR3 IVGAILDQIILL-MPFNKDLRRNKA-----ALLFWHILLGLPIVRCLEHTIRNYHKWLKLNKQE-11-NTM  
 XKR4 VIGSLSVQVFSFRWFVHDFSTEDS-72-SFCIWLLQSLIHILQLGQIWRYPHTIYLGIKRSRQSGEND-----  
 XKR5 LPPGFLVQALSYLWFRADGHP-----GHCSLMLHLLQLGVWKRHWDAALTSLQKELEAPHR-----  
 XKR6 LPPSLLVQSLFRWFVQDYTGGL-41-RLSVWIQWVVIHLLQMGQVWRYIRTMYLGIQSRQRKEHQ-----  
 XKR7 LPPSLVVQLLSFRWFVVDYSEPA-53-RLCIWLLQTLVHLLQLGQVWRYLRALYLGLQSRWRGERL-----  
 XKR9 LGLTLVAQCFSYSWFKADLKA-----GQESQHCFLHLLHCLQGGVFTRYWFAKRGYHAAFKYDSN----TSN

XKR8 -----EFDLAYADFLALDISMLRLETFETAPQLTIVLAIMLQSGR-AEYQWVGICTSFLGISWALLDYH-R  
 XK IEKEVGOAEGKLIHRSASFRAVSIQAFVGSAPQLTQLYISVMQQD-VTVGRSILLMTISLLSIVYGALRCNIL  
 XKR2 IEWEVGHISIRTLAMHRNAYKRMFSIQAFVGSVPLTYQLYVSLISAE-VPLGRVVLMMVFSLSVSVTYGATLCNML  
 XKR3 LEREIAFSIRDNFMQOKAFKYSVSIQAFVGSVPLIQLQMYISLTIRE-WPLNRALLMTFSLLSVTYGAIRCNIL  
 XKR4 ----RWRFYWKMVYEVADVSMHLLATFESAPQLVQLCTIIVQTHS-LQALQGFATAASLSVSLAWALASYQ-K  
 XKR5 -----GWLQEQEADLSARLLEALQTGPHLLIQLTYVFLASDF-TDIVPGVSTLFSWSSLSWALVSYT-R  
 XKR6 ----RRFYWAMYEVADVNMRLLETFFESAPQLVQLYIMLQKNS-AETLPCVSSVTSLMSLAWVLASYH-K  
 XKR7 ----RRHFYWQMLFESADVSMRLLETFFESAPQLVQLSLLVHRGGAPDLLPALSTASLSVSLAWTLASYQ-K  
 XKR9 FVEEQIDLHKEVIDRVTDLSMRLLETFFEGCPQLIQLYITLLEHQ-ANFSQYAAIMVSCCAISWSTVDYQ-V

XKR8 ALRTRCLPSKPLGL-GSSVIYFLWNLLLVPRVLAVALFSALFPPSYVALHFGGLWLVLLVWVWVWQ---GTDFM  
 XK AIKIKYDEYEVKVKPLAYVCIFLWRSFEIATRVVVLVLFSTVSKTWWVVIILINFFSFLYPWILFWCSGSPFP  
 XKR2 AIQIKYDDYKIRLGPLVLCITITWRTLEITSRLLILVLFSATLKLKAVPFLVNLIELIILFEPWIKFWRSGAQP  
 XKR3 AIQISNDTTIKLPPIEFVCMVWRFLEVISRVVLAFFIASLKLKSLPVLIIYFVSLAPWLEFWKSGAHL  
 XKR4 ALRDRSRDDKPKISY-MAVLIQFCWHFPTIARVITFALFASVFQLYFGIFVVLHWCIMTFWIVHC----ETDFC  
 XKR5 FMGFMPGHLAMPW-AALFCQQLWRMGMLGTRVLSLVLFYKAYHFVWVAVAGAHWLVMTFVWVAQ---QSDII  
 XKR6 LLRDRSRDDKKSMSY-RGAVIQVFWRLFTISSRVISFALFASIFQLYFGIFVVLHWCAMAFWIIHG---GTDFC  
 XKR7 VLRDRSRDDKRPLSY-KGAVAQVLWHLFSIAARGLAFALFASVYKLYFGIFVVAHWCVMTFVVIQ---ETDFC  
 XKR9 ALRKSPLDKKLLNGLCPKITLYLFYKFLTLLSWMLS VVLLLFLNVKIALFLFLFLWLLGIWAFKN---NTQFC

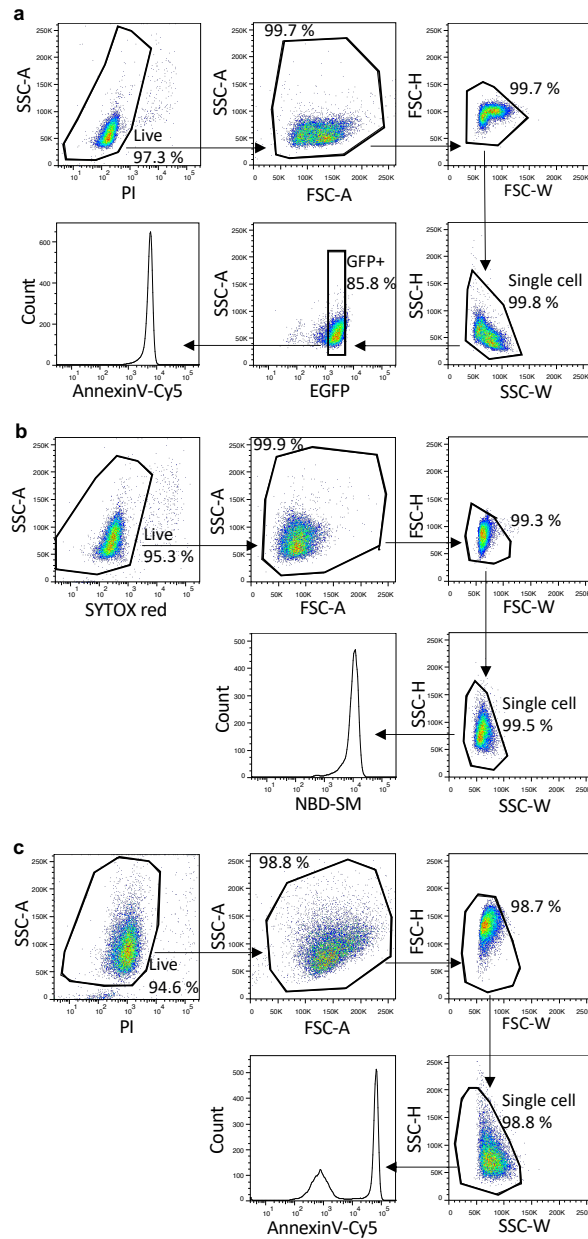
XKR8 -----PDPSSSEWLYRIVVATILYFSWFNVA-----EGRTRGRAIHFALSDSI-LLVATWVT  
 XK ENIEKALSRVGTIVLCFLTLYTGINMFCWSAVQLKIDSPDLISKSHNWWYQLLVYYMIRFIENA-ILLLWYL  
 XKR2 NNIKFNFSRVGTIVLVVISVTLIYAGINFSCWSALQLRLADRDLVDKQNWGHMGLHYSVRLVENV-IMVLFKF  
 XKR3 GNKENNSNMVGTIVLMLFLITLYAAINFSCWSAVKQLSDDKIIDGRQRWGHRIHYSFQFLENV-IMLVFRF  
 XKR4 -----ITKWEIIVFDVVGIIYIFSWFNK-----EGRTRCLFIYFFVILENT-ALSLWYL  
 XKR5 -----DSTCHWRLFNLVGAVALCYLFW-----DSPSRNRMVTFYVMILENI-ILLLLAT-  
 XKR6 -----MSKWEIILFNVVGIVYIFCWFNVK-----EGRTRYRMFAYYTIVLTENA-ALTFWYF  
 XKR7 -----MSKWEIIVFNVVGIIYIFCWFNVK-----EGRSRRRMTLYHCVLLENA-ALTGFWYS  
 XKR9 -----TCISMEFLYRIVVGFILIFTFNFK-----GQNTKCPMSCYIIVRVLGTLGLTVFWVC

XKR8 HSSWLPSPGIPLQLWLPVCGCGFFLGLALRLVYHWHLPSCCWKPDQVDGARSLLSPEGYQLPQ--25--END  
 XK FKTDIYMYVCAPLLVLQLLIGYCTAILFMLVYFQFHFPCCKLFSSSVSEGFQRWLRFCWACRQ--42--END  
 XKR2 FGVKVLNLYCHSLIALQLIIAYLISIGFMLFFQYVHPLRSLFTHNVVDYLHCVCCHQHPRTVE--15--END  
 XKR3 FGGKTLNCCDSLIAVQLIISYLLATCFMLLFYQYLYPWSGKVLPGRTENQPEAPYYVNIKT--30--END  
 XKR4 YKAPQIADAFAPALCVVFSFSLTGVMFLMYAFVHPNGPRFGQSPSCACEDPAAAFPLPPDVA-108--END  
 XKR5 -DFLQASWTSLQTIAGVLSGFLIGSVSLIYVSLHLPKSTDIWQGLRKSCKIAGGDKTERRDS-339--END  
 XKR6 YRDPETDTSYAVPALCCVFI SFVAGIAMLLIYGVLHPTGPRAKILASSCAELLWGIPLPPDVE-114--END  
 XKR7 SRNF-STDFYSLIMVCVVASSFALGIFMFCVYVCLHLPNGPMLGPQAPGCFRKAPEPCGPPADA-111--END  
 XKR9 PLTIFNPD-YFIPISITIVLTLLGILFLIVYYSFHPNRSATKCEIDGKPVLRRECRMRYFLME-----

**Supplementary Fig. 3. Alignment of amino acid sequences of the human XKR family.** Amino acid sequences of XKR8 (Q9H6D3), XK (P51811), XKR2 (Q6PP77), XKR3 (Q5GH77), XKR4 (Q5GH76), XKR5 (Q6UX68), XKR6 (Q5GH73), XKR7 (Q5GH72), and XKR9 (Q5GH70) were aligned by introducing several gaps to obtain maximum homology. Amino acids that were identical among all members are shown in red, while those in the same categories (non-polar: G, A, V, I, L, P, F, W, and M);

uncharged polar: S, T, C, Y, Q, and N; charged polar: D, E, K, R, and H) are in orange. Eleven  $\alpha$ -helices are shadowed and numbered. Hydrophobic amino acids forming the cleft carrying PtdCho are highlighted in green, while the charged amino acids that lie in the putative phospholipid path are in yellow. A pair of amino acids in XK (R222 and E327), the mutation of which was identified as missense mutations in McLeod syndrome<sup>34,35</sup>, are highlighted in magenta. Numbers above the first line are the amino acid positions for human XKR8.





**Supplementary Fig. 4. Representative gating strategy for flow cytometric analysis. a**, Gating strategy for flow cytometric analysis of Annexin V staining of Ba/F3 transformants expressing the GFP-tagged hXkr8 (Fig. 4a,d, Fig. 5h, and Fig. 6a,b,e,f). **b**, Gating strategy for flow cytometric analysis of NBD-SM incorporation by Ba/F3 transformants (Fig. 4c,d, Fig. 6d, and Extended Data Fig. 8a,b). **c**, Gating strategy for flow cytometric analysis of Annexin V staining of W3 transformants (Extended Data Fig. 1c).