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Review article

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

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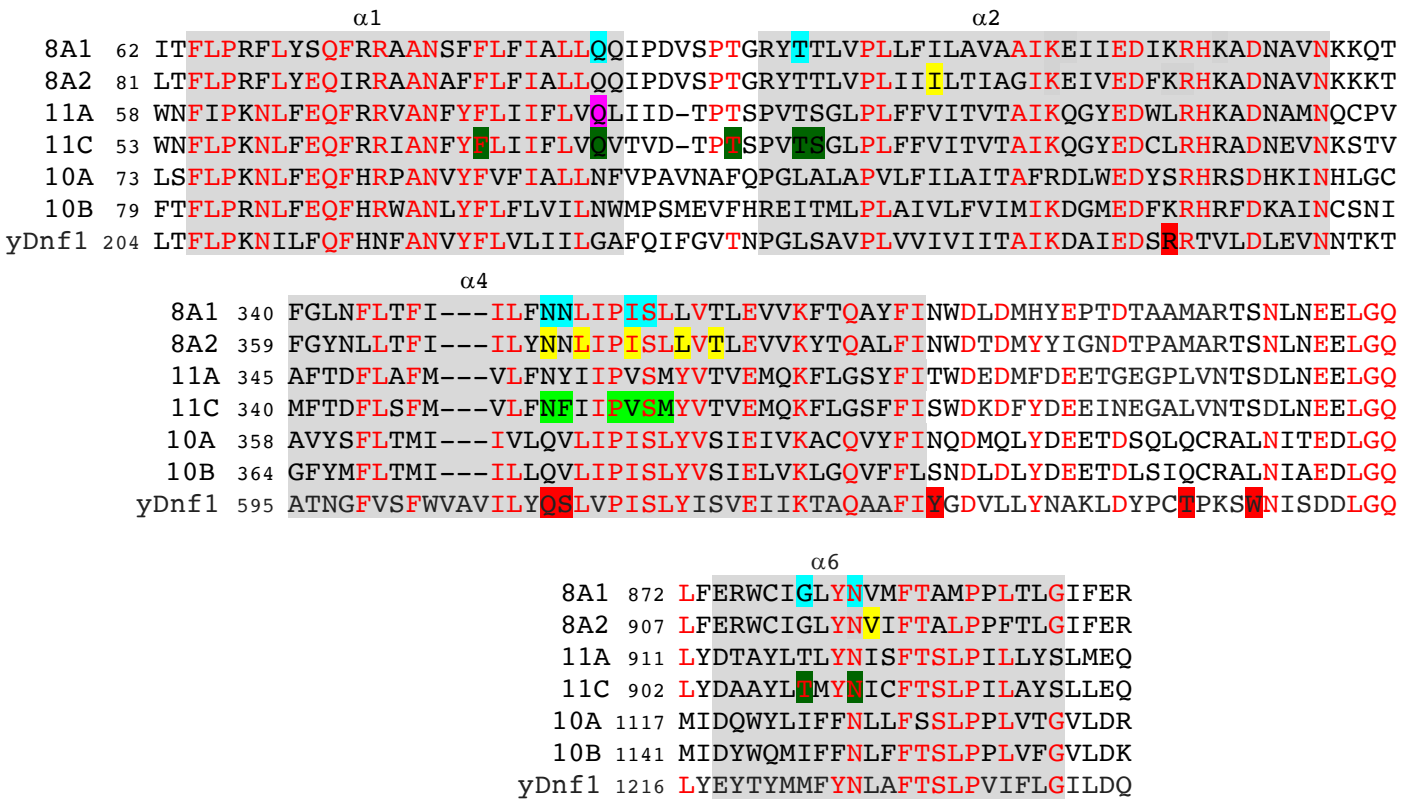
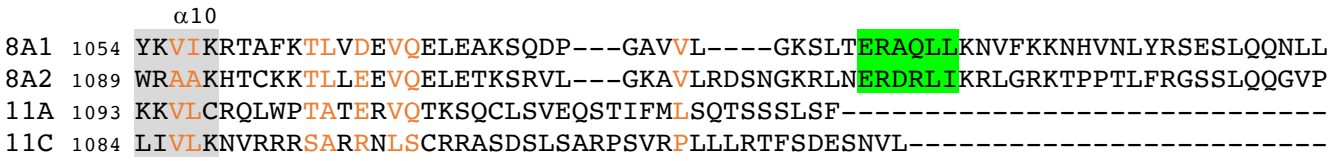
Supplementary Information

a



b



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 α1, α2, α4, and α6, 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[1]. The replacement of Q84 by Glu in ATP11A (highlighted in purple) concurs with the molecule flip of PtdSer and PtdCho[2]. The amino acids that coordinate the serine residue of PtdSer in ATP11C are highlighted in dark green[2]. The amino acid residues necessary for the flipping activity of ATP8A2[3] are highlighted in yellow, while the residues proposed to determine the substrate specificity in ATP8A1[4,5] 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[6] 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[7] is highlighted in green.

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                                                                                    α1
16C (327) IRKLINNGSYIAAFPPHEGAYKSSQPIKTHGPQNNRHLLYERWARWGMWYKHQPLDLIRLYFGEKIGLYF
16E (237) IERLLNSNTYSSAYPLHDGQYWKP--SEPPNPTNERYTLHQNWARFSYFYKEQPLDLIKNYYGEKIGIYF
16F (232) INRLVNSGIYKAAFPLHDCKFRRQ--SEDPSCPNERYLLYREWAHPRSIYKKQPLDLIRKYYGEKIGIYF
16K (158) LRRLLTSGIVIQVFPLHDSEALKK--------------LEDTWYT-RFALKYQPIDSIRGYFGETIALYF

16C (397) AWLGWYTGMLIPAAIVGLCVFFYGLFTMNNSQVSQEICKAT---EVFMCPLCDKNCSLQRLNDSCIYAKV
16E (305) VFLGFYTEMLFFAAVVGLACFIYGLLSMEHNTSSTEICDPEIGGQMIMCPLCDQVCDYWRLNSTCLASKF
16F (300) AWLGYYTQMLLLAAVVGVACFLYGYLNQDNCTWSKEVCHPDIGGKIIMCPQCDRLCPFWKLNITCESSKK
16K (213) GFLEYFTFALIPMAVIGLPYYLFVWE--------------------------------------------
                               α2
16C (464) TYLFDNGGTVFFAIFMAIWATVFLEFWKRRRSILTYTWDLIEWEEEEETLRPQFEAKYYKMEIVNPITGK
16E (375) SHLFDNESTVFFAIFMGIWVTLFLEFWKQRQARLEYEWDLVDFEEEQQQLQLREFEAMCKHRKLNAVTKE
16F (370) LCIFDSFGTLVFAVFMGVWVTLFLEFWKRRQAELEYEWDTVELQQEEQA--RPEYEARCTHVVINEITQE
16K (239) ----DYDKYVIFASFNLIWSTVILELWKRGCANMTYRWGTLLMKRKFEE-PRPGFHGV---LGINSITGK
                                         α3
16C (534) PE--PHQPSSDKVTRLLVSVSGIFFMISLVITAVFGVVVYRLVVMEQFASFK--------WNFIKQYWQF
16E (446) MEP-YMPLYTRIPWYFLSGATVTL-WMSLVVTSMVAVIVYRLSVFATFASFMESDAS-LKQVKSFLTPQI
16F (438) EERIPFTAWGKCIRITLCASAVFF-WILLIIASVIGIIVYRLSVFIVFSAKLPKNINGTDPIQKYLTPQT
16K (301) EEPLY---PSYKRQLRIYLVSLPFVCLCLYFSLYVMMIYFDMEVWALGLHENS------------SEWTS
                               α4                                   α5
16C (594) ATSAAAVCINFIIIMLLNLAYEKIAYLLTNLEYPRTESEWENSFALKMFLFQFVNLNSSIFYIAFFLGRF
16E (513) TTSLTGSCLNFIVILILNFFYEKIS-AITKMEIPRTYQEYESSLTLKMFLFQFVNFYSSCFYVAFFKGKF
16F (507) ATSITASIISFIIIMILNTIYEKVAIMITNFELPRTQTDYENSLTMKMFLFQFVNYYSSCFYIAFFKGKF
16K (357) VLLYVPSIIYAIVIEIMNRLYRYAAEFLTSWENHRLESAYQNHLILKVLVFNFLNCFASLFYIAFVLKD-
                                                               α6
16C (664) VGHPGKYNKLFDRWRLEECHPSGCLIDLCLQMGVIMFLKQIWNNFMELGYPLIQNWWSRHKIKRGIH---
16E (583) VGYPGKYTYLFNEWRSEECDPGGCLIELTTQLTIIMTGKQIFGNIKEAIYPLALNWWRRRKARTNSE---
16F (577) VGYPGDPVYWLGKYRNEECDPGGCLLELTTQLTIIMGGKAIWNNIQEVLLPWIMNLIGRFHRVSGSE---
16K (426) ------------------------MKLLRQSLATLLITSQILNQIMESFLPYWLQRKHGVRVKRKVQALK
                                                 α7                       α8
16C (731) ---DASIPQWENDWNLQPMNLHGLMDEYLEMVLQFGFTTIFVAAFPLAPLLALLNNIIEIRLDAYKFVTQ
16E (650) ----KLYSRWEQDHDLESFGPLGLFYEYLETVTQFGFVTLFVASFPLAPLLALINNIVEIRVDAWKLTTQ
16F (644) ----KITPRWEQDYHLQPMGKLGLFYEYLEMIIQFGFVTLFVASFPLAPLLALVNNILEIRVDAWKLTTQ
16K (472) ADIDATLYEQVILEKEMGTY-LGTFDDYLELFLQFGYVSLFSCVYPLAAAFAVLNNFTEVNSDALKMCRV
                                     α9
16C (798) WRRPLPARATDIGIWLGILEGIGILAVITNAFVIAITSDYIPRFVYEYKYGPCANHVEPSENCLKGYVNN
16E (716) YRRTVASKAHSIGVWQDILYGMAVLSVATNAFIVAFTSDIIPRLVYYYAYST------NATQPMTGYVNN
16F (710) FRRLVPEKAQDIGAWQPIMQGIAILAVVTNAMIIAFTSDMIPRLVYYWSFSVPP-YGDHTSYTMEGYINN
16K (541) FKRPFSEPSANIGVWQLAFETMSVISVVTNCALIGMSPQV------------------------------
                                                                              α10
16C (868) SLSFFDLSEL-----GMGKSGY-----CRYRDYRGPPWSSKPYEFTLQYWHILAARLAFIIVFEHLVFGI
16E (780) SLSVFLIADFPNHTAPSEKRDF---ITCRYRDYRYPPDDENKYFHNMQFWHVLAAKMTFIIVMEHVVFLV
16F (779) TLSIFKVADFKNKSKGNPYSDLGNHTTCRYRDFRYPPGHPQEYKHNIYYWHVIAAKLAFIIVMEHVIYSV
16K (581) -------------------------------------------NAVFPESKADLILIVVAVEHALLAL

16C (928) KSFIAYLIPDVPKGLHDRIRREKYLVQEMMYEAELEHLQQRRKSGQPVHHEWP------------
16E (847) KFLLAWMIPDVPKDVVERIKREKLMTIKILHDFELNKLKENLGINSNEFAKHVMIEENKAQLAKSTL
16F (849) KFFISYAIPDVSKRTKSKIQREKYLTQKLLHENHLKDMTKNMGVIAERMIEAVDNNLRPKSE-----
16K (606) KFILAFAIPDKPRHIQMKLARLEFESLEALKQQQMKLVTENLKEEPMESGKEKAT------------
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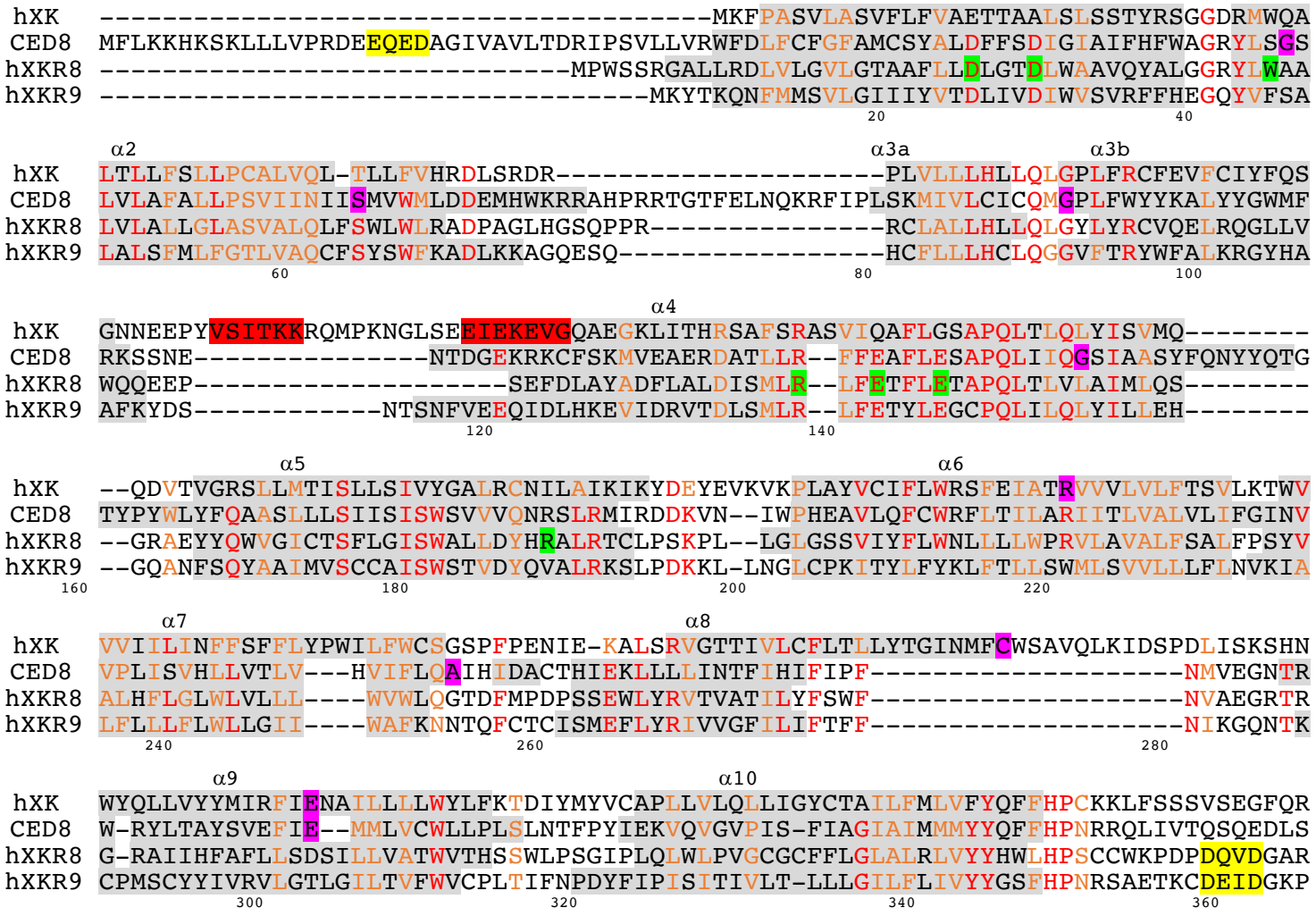
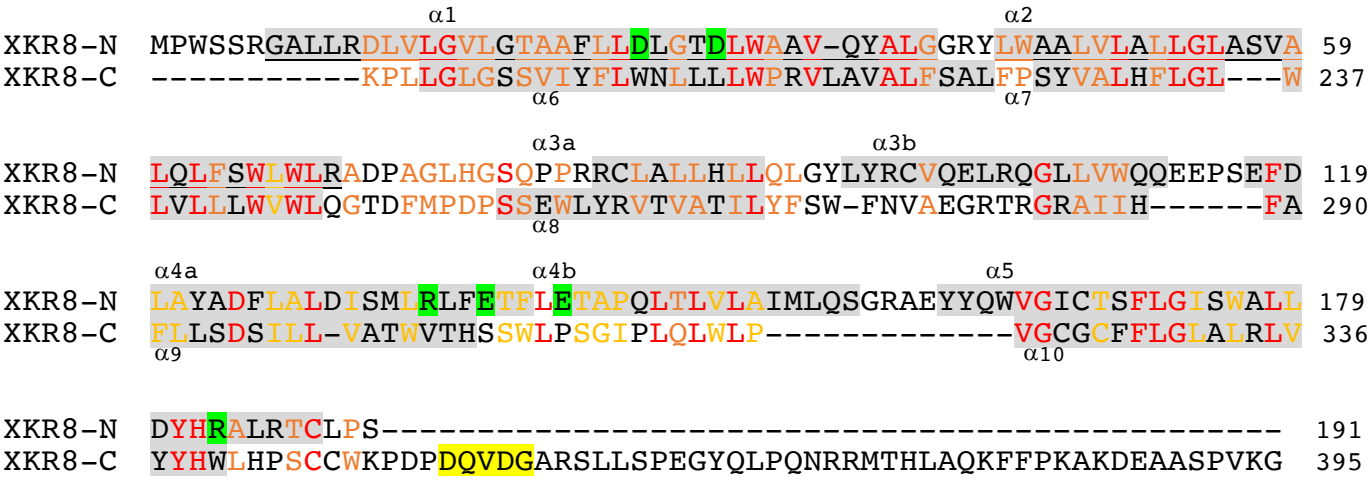
Supplementary Fig. 2. **TMEM16 Ca^{2+}-dependent scramblase.** The amino acid sequences of human TMEM16C (UniPlot: Q9BYT9), 16E (UniProt: Q75V66), 16F (Q4KMQ2), and 16K (Q9NW15) are aligned. The residues identical among the three or four members are shown in red. The transmembrane helices are shadowed 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 α6-8 of human TMEM16F) that bind two Ca^{2+} ([8,9]), Glu-394 in α2 and Asp-858 in α10 coordinate an additional Ca^{2+} ([10,11]). The domain responsible for scrambling phospholipids[12,13], 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[14]. 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[15] 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[15]. Mutations of F171S, G229W, R263H, F337V, L510R, C513R, and D615Q (https://www.hgmd.cf.ac.uk/) (highlighted in pink) in TMEM16K were found in human spinocerebellar ataxia[16].



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 α-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 β-hairpin structures are highlighted in yellow and red, respectively. The intramolecular six charged residues required for phospholipid scrambling and the Trp-45 gatekeeper[17] in human XKR8 are highlighted in green. The residues mutated in human patients

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



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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