

Fig. S1. Structural determination of ATP11C-CDC50A complex

(A) Construction of TP11C and CDC50A used in crystallization. See Methods for details. (B) Purification of ATP11C-CDC50A complex. Lane 1: solubilized cell lysate, lane 2: pass-through of Flag resin, lane 3: wash fraction, lane 4: elution by Flag peptide (subjected to ATPase assay), lane 5: TEV proteinase- and endoglycosidase-treated sample, lane 6: pass-through fraction of Ni-NTA and amylose resin, lane 7: concentrated peak fractions by size-exclusion chromatography (arrowhead in the left panel). Arrowheads on the right indicate as follows, a: HSP70, b: EndoHf, c: cleaved eGFP, d: TEV proteinase. The elution profile of ATP11C-CDC50A complex by size-exclusion column chromatography is shown on the left. (C) Representative X-ray diffraction obtained from a plate-like crystal shown in **D**. Diffraction spots better than 3.6Å were obtained along the c^* -axis, whereas these are limited to around 4~6Å in directions along the a^* - and b^* axes, thus strongly anisotropic. (D,E) Three-dimensional crystals obtained from the samples purified from Expi293 cells, showing thin, but large plate-like crystals (D). In contrast, small crystals were obtained from CDC50A-KO cells (E). (F) Data acquisition strategy. We employed normal type LithoLoops for helical scan data acquisition from large single crystals. However, because crystals showed strong anisotropy, we also collected data sets by irradiating the X-ray beam from the direction perpendicular to the c-axis using 90° bent type LithoLoops. For the small but well diffracting crystals obtained from CDC50A-KO cells, data from each individual crystal were collected for 10°. All of these crystals showed identical unit cell size and symmetry regardless of crystal morphologies and expression cell types, as seen in the histograms of unit cell dimensions (G). (H) The result of correlation coefficient (CC) based clustering of data sets by KAMO. $[1 - CC(i, j)]^{1/2}$ was used as a distance between two data sets i and j, where CC(i, j) denotes the CC of intensity (52). The vertical axis represents 1,588 data sets, whereas the horizontal axis represents the distances between clusters. All diffraction data were finally merged into a single data set, and used for the molecular replacement. (I) F/σ is plotted for the three principle directions of the crystal using Diffraction Anisotropy Server. $R_{\text{work}}/R_{\text{free}}$ (J) and completeness (K) are plotted for the analyzed resolution shells. Dotted grey lines indicate the highest resolution shell (3.9Å) for the present structural analysis.



Fig. S2. Crystal packing.

(A-C) An asymmetric unit contains four protomers (A, B, C and D) shown in blue, green, yellow and red, respectively. Their symmetry-related molecules are shown in light colors. Yellow boxes indicate unit cells viewed from different directions in A-C as indicated in the figures. (D) Molecules in the asymmetric unit are displayed according to their temperature factors. Colors gradually change from blue (16) to red (283). (E,F) Comparison of the molecular conformation of four protomers. Arrowheads indicate loop structures, the conformations of which are variable among the four protomers. (G) $2F_{o}$ - F_{c} electron density maps of the four protomers at the same contour level of 1.5σ . (H) Close-up view of a crystal contact site at the interface of CDC50A subunit of protomer A (blue) and protomer B (green) achieved by a GlcNAc attached to Asn294^{CDC50A}. Grey mesh represents $2F_{o}$ - F_{c} map with 1.5σ contour level.



Fig. S3. Electron density maps

Surface represents $2F_0$ - F_c electron density maps of the indicated regions with 1.5σ contour level. Color code as in Fig. 1.

		AN7 A-domain
human ATP11C	1	PVSETEAYIAORFCDNRIV
human ATP11A	1	MDCSLVRTLVHRYCAGE-ENWVDSRTIYVGHREPPPGAEAYIPORYPDNRIV
human ATP8A1	1	MPTMRRTVSEIRSRAEGYEKTDDVSEKTSLAD-OEEVRTIFINOPOLTKFCNNHVS
human ATP8A2	15	LPRRSRIRSSVGPVRSSLGYKKAEDEMSRATSVGD-QLEAPARTIYLNQPHLNKFRDNQIS
veast Drs2	141	AVTNNELDDNYLDSRNKFNIKILFNRYILRKNVGDAEGNGEPRVIHINDSLANSSFGYSDNHIS
Consensus		-:: *-: ::*::
		M1 M2
human ATP11C	47	SSKYTLWNFLPKNLFEQFRRIANFYFLIIFLVQVTVD-TPTSPVTSGLPLFFVITVTAIKQGYEDWLRHP
human ATP11A	52	SSKYTFWNFIPKNLFEQFRRVANFYFLIIFLVQLIID-TPTSPVTSGLPLFFVITVTAIKQGYEDWLRHK
human ATP8Al	56	TAKYNIITFLPRFLYSQFRRAANSFFLFIALLQQIPDVSPTGRYTTLVPLLFILAVAAIKEIIEDIKRHK
human ATP8A2	75	TAKYSVLTFLPRFLYEQIRRAANAFFLFIALLQQIPDVSPTGRYTTLVPLIIILTIAGIKEIVEDFKRHK
yeast Drs2	205	TTKYNFATFLPKFLFQEFSKYANLFFLCTSAIQQVPHVSPTNRYTTIGTLLVVLIVSAMKECIEDIKRAN
Consensus	11	::***:*: *:-:: : ** :** :* - :**- *: -*:-:: ::-:*: ** * -
		M2 A-domain
human_ATP11C	116	ADNEVNKSTVYIIENAKRVRKESEKIKVGDVVEVQADETFPCDLILLSSCTTDGTCYVTTASLDGESN
human_ATP11A	121	ADNAMNQCPVHFIQHGKLVRKQSRKLRVGDIVMVKEDETFPCDLIFLSSNRGDGTCHVTTASLDGES
human_ATP8A1	126	ADNAVNKKQTQVLRNGAWEIVHWEKVAVGEIVKVTNGEHLPADLISLSSSEPQAMCYIETSNLDGETN
human_ATP8A2	145	ADNAVNKKKTIVLRNGMWHTIMWKEVAVGDIVKVVNGQYLPADVVLLSSSEPQAMCYVETANLDGET
yeast_Drs2	275	SDKELNNSTAEIFSEAHDDFVEKRWIDIRVGDIIRVKSEEPIPADTIILSSSEPEGLCYIETANLDGET
Consensus	48	:*::*:: : -: **::: * : :*-* : *** :- *:: *:-***:-
		A-domain 🗈
human_ATP11C	184	CKTHYAVRDTIALCTAESIDTLRAAIECEQPQPDLYKFVGRINIYSNSLEAVARSLGPENLLLKGATLKN
human_ATP11A	189	HKTHYAVQDTKGFHTEEDIGGLHATIECEQPQPDLYKFVGRINVYSDLNDPVVRPLGSENLLLRGATLKN
human_ATP8A1	194	LKIRQGLPATSDIKDVDSLMRISGRIECESPNRHLYDFVGNIRLDGHGTVPLGADQILLRGAQLRN
human_ATP8A2	213	LKIRQGLSHTADMQTREVLMKLSGTIECEGPNRHLYDFTGNLNLDGKSLVALGPDQILLRGTQLRN
yeast_Drs2	345	LKIKQSRVETAKFIDVKTLKNMNGKVVSEQPNSSLYTYEGTMTLNDRQIPLSPDQMILRGATLRN
Consensus		* : - * : - : : - : -* *: ** : * : :*:::*:*:*:*
		A-domain M3
human_ATP11C	254	TEKIYGVAVYTGMETKMALNYQGKSQKRSAVEKSINAFLIVYLFILLTKAAVCTTLKYVWQSTPYNDEPW
human_ATP11A	259	TEKIFGVAIYTGMETKMALNYQSKSQKRSAVEKSMNAFLIVYLCILISKALINTVLKYMWQSEPFRDEPW
human_ATP8A1	260	TQWVHGIVVYTGHDTKLMQNSTSPPLKLSNVERITNVQILILFCILIAMSLVCSVGSAIWNRR-HSGKDW
human ATDOA2		TORRECTION TO DEVICE THE ADDRESS OF
numan_AIF0A2	279	IQWVFGIVVIIGDDIKLHQNSIKAPLKKSNVEKVINVQILVLFGILLVHALVSSAGALIWNKS-NGEKNW
yeast_Drs2	410	TAWIFGLVIFGHETKLLRNATATPIKRTAVEKIINRQIIALFTVLIVLILISSIGNVIMSTA-DAKHLS
yeast_Drs2 Consensus	279 410 110	TAWIFGLVIFGHETKLEMONSTRAFERKSNVERVINVOILVEGIEVEGIEVMALVSSAGALIWNRS-HGERNW TAWIFGLVIFTGHETKLERNATATPIKRTAVEKIINRQIIALFTVLIVLILISSIGNVIMSTA-DAKHLS * :-*:-::** :**: * - * : **: * :: : :*: : :
yeast_Drs2 Consensus	279 410 110	TAWIFGLVITGHDTKLMONSTRAFLKKSNVEKTINQILVLFGILLVMALVSSAGALIWNKS-HOEKNW TAWIFGLVIFTGHETKLLRNATATPIKRTAVEKIINRQIIALFTVLIVLILISSIGNVIMSTA-DAKHLS * :-*:-::** :**: * - * : **: * :: : :*: : @ @ #4 P-domain
yeast_Drs2 Consensus human_ATP11C	279 410 110 324	TAWIFGIVVIGHDIKLMONSIKAPLKKSNVEKVINVQILVLFGILVMALVSSAGALIWNKS-HGERNW TAWIFGLVIFGHETKLLRNATATPIKRTAVEKIINRQIIALFTVLIVLILISSIGNVIMSTA-DAKHLS * :-*:-::** :**: * - * : **: * :: : :*: : : P-domain YNOKTOKERETLKVLKMFTDFLSFMVLFNFIIPVSMYVTVEMQKFLGSFFISWDKDFYDEEINEGALVNT
yeast_Drs2 Consensus human_ATP11C human_ATP11A	279 410 110 324 329	TAWIFGLVITGHDIKLMONSTRAFLKKSNVEKVINVQILVLFGILVMALVSSAGALIWNKS-HGERNW TAWIFGLVITGHETKLLRNATATPIKRTAVEKIINRQIIALFTVLIVLILISSIGNVIMSTA-DAKHLS * :-*:-::** :**: * - *: **: * :: ::*: : : P-domain YNQKTQKERETLKVLKMFTDFLSFMVLFNTIPVSMYVTVEMQKFLGSFFISWDKDFYDEEINEGALVNT YNQKTESERQRNLFLKAFTDFLAFMVLFNTIPVSMYVTVEMQKFLGSFFISWDKDFYDEEINEGALVNT YNQKTESERQRNLFLKAFTDFLAFMVLFNTIPVSMYVTVEMQKFLGSFFISWDKDFYDEEINEGALVNT
yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1	279 410 110 324 329 329	TAWIFGIVITGHDIKLMONSTAPLKKSNVEKTINQILVLFGILVMALVSSAGALIWNKS-HOEKNW TAWIFGLVIFGHETKLLRNATATPIKRTAVEKIINRQIIALFTVLIVLILISSIGNVIMSTA-DAKHLS * :-*:-::** :**: * - *: **: * :: ::*: :: P-domain YNQKTQKERETLKVLKMFTDFLSFMVLFNFITPVSM/VTVEMQKFLGSFFISWDKDFYDEEINEGALVNT YNQKTESERQRNLFLKAFTDFLAFMVLFNYITPVSM/VTVEMQKFLGSYFITWDEDMFDEETGEGPLVNT YLNLNYGGASNFGLNFLTFILLFNNLTPISL_VTLEVVKFTQAYFINWDLDMHYEPTDTAAMART
human_ATF0A2 yeast_Drs2 Consensus human_ATF11C human_ATF11A human_ATF8A1 human_ATF8A2	279 410 110 324 329 329 348 470	TAWIFGIVITGHDIKLMONSTARELKKSNVEKTINQILVLFGILVMALVSSAGALIWNKS-HOEKNW TAWIFGLVITGHETKLLRNATATPIKRTAVEKIINRQIIALFTVLIVLILISSIGNVIMSTA-DAKHLS * :-*:-::** :**: * - *: **: * :: ::*: :: P-domain YNQKTQKERETLKVLKMFTDFLSFMVLFNTIPVSM/VTVEMQKFLGSFFISWDKDFYDEEINEGALVNT YNQKTESERQRNLFLKAFTDFLAFMVLFNTIPVSM/VTVEMQKFLGSYFITWDEDMFDEETGEGPLVNT YNQKTESERQRNLFLKAFTDFLAFMVLFNTIPVSM/VTVEMQKFLGSYFITWDEDMFDEETGEGPLVNT YLNLNYGGASNFGLNFLTFILLFNNLTPISL_VTLEVVKFTQAYFINWDLDMHYEPTDTAAMART YIKKMDTTSDNFGYNLLTFILLYNNLTPISL_VTLEVVKYTQALFINWDTDMYYIGNDTPAMART
yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2	279 410 110 324 329 329 348 479	TAWIFGLVITGHDIKLMONSTARELKKSNVEKVINVQILVLFGILVMALVSSAGALIWNKS-HGERNW TAWIFGLVITGHETKLLRNATATPIKRTAVEKIINRQIIALFTVLIVLILISSIGNVIMSTA-DAKHLS * :-*:-::** :**: * - *: **: * :: ::*: :: : P-domain YNQKTQKERETLKVLKMFTDFLSFMVLFNTIPVSMVVTVEMQKFLGSFFISWDKDFYDEEINEGALVNT YNQKTESERQRNLFLKAFTDFLAFMVLFNTIPVSMVVTVEMQKFLGSYFITWDEDMFDEETGEGPLVNT YNQKTESERQRNLFLKAFTDFLAFMVLFNTIPVSMVVTVEMQKFLGSYFITWDEDMFDEETGEGPLVNT YLNLNYGGASNFGLNFLTFILLFNNLTPISL_VTLEVVKFTQAYFINWDLDMHYEPTDTAAMART YIKKMDTTSDNFGYNLLTFILLYNNLTPISL_VTLEVVKYTQALFINWDTDMYYIGNDTPAMART YLVLEGTNKAGLFFKDFLTFWILFSNLVPISL_VTVELIKYYQAFMIGSDLDLYYEKTDTPTVVRT
yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus	279 410 110 324 329 329 348 479 137	IOWYGIVVIGHDIKHONSIAPLKKSNVEKVINVQILVEGILVALVSSAGALIWNKS-HOEKNW TAWIFGLVIFTGHETKLENNATATPIKKTAVEKVINNQILVEFGILEVHALVSSAGALIWNKS-HOEKNW TAWIFGLVIFTGHETKLENNATATPIKKTAVEKVINNQILALFVULVULVISSIGNVIMSTA-DAKHLS * :-*:-::** :**: * - * : **: * :: : :*: :: : P-domain YNQKTQKERETLKVLKMFTDFLSFNVLFNFIPVSMVVVEMQKFLGSFFISWDKDFYDEEINEGALVNT YNQKTESERQRNLFLKAFTDFLAFMVLFNYIIPVSMVVVEMQKFLGSYFITWDEDMFDEETGEGPLVNT YLNLNYGGASNFGLNFLTFIILFNNLIPISL_VTLEVVKFTQAYFINWDLDMHYEPTDTAAMART YIKKMDTTSDNFGYNLLTFIILYNNLIPISL_VTLEVVKYTQALFINWDTDMYYIGNDTPAMART YLYLEGTNKAGLFFKDFLTFWILFSNLVPISL * ::*:* * ::* * *:- * ::*:* * ::* * *:- * ::*:* * ::* *: * ::*:* * ::* *:*
human_ATP1C human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus	279 410 110 324 329 329 348 479 137 394	TAWIFGIVVIGHDIKHONSIKAPLAKASIVEKVINVQILVLPGILVHALVSSAGALIWNKS-HGERNU TAWIFGLVIFTGHETKLLRNATATPIKRTAVEKIINRQIIALFTVLIVLISISGNVIMSTA-DAKHLS * :-*:-::** :**: * - * : **: * :: ::*:
human_ATPOA2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP11C	279 410 110 324 329 348 479 137 394 394	TAWIFGIVVIGHDIKHONSIAPEAKKSIVEKVINVQILVLEVELUVALVSSAGALIWNKS-HGERNU TAWIFGLVIFTGHETKLLRNATATPIKRTAVEKIINRQIIALFTVLIVLISISGNVIMSTA-DAKHLS * :-*:-::**:**:* - *:*:*:::::::-
human_ATPOA2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1	279 410 110 324 329 329 348 479 137 394 399 394	TAWIFGIVVIGNDIKEMONSIAPEAKKSNVEKVINVQILVLEVELUVALVSSAGALIWNKS-HGERNW TAWIFGLVIFTGHETKLLRNATATPIKKTAVEKVINNQILVLEVELUSSIGNVIMSTA-DAKHLS * :-*:-::**:**:* - *:*:*:::::::-
human_ATP3A2 yeast_Drs2 Consensus human_ATP11C human_ATP1A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2	279 410 110 324 329 329 348 479 137 394 399 394 413	TAWIFGIVVIEGNDIKEMONSIKAPLAKASVEKVINVQILVLEVELUVALVSSAGALIWNKS-HGERNU TAWIFGLVIFTGHETKLLRNATATPIKKTAVEKVINNQILVLEVELVALVSSAGALIWNKS-HGERNU TAWIFGLVIFTGHETKLLRNATATPIKKTAVEKVINNQILVLEVELVU * :-*:-::**:**:*::::::::-
human_ATP3A2 yeast_Drs2 Consensus human_ATP11C human_ATP1A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP1A1 human_ATP8A2 yeast_Drs2	279 410 110 324 329 329 348 479 137 394 399 394 413 545	IOWYGIVVIGHUNSIKAPLAKASVEKVINUQILVLPULVISAGALIWAKS-HGERMU TAWIFGLVIFTGHETKLLRNATATPIKRTAVEKIINRQIIALFTVLIVLILISSIGNVIMSTA-DAKHLS *:-*:-::**:**:* - *: - YNQKTESERQRNLFLKAFTDFLSFMVLFNFI Pdomain YLNLNYGGASNFGLNFLTFILLFNNLTPISL VTLEVVKFTQAYFINWDLDMHYEPTDTAAMART YLVLEGTNKAGLFFKDFLTFWILFSNLVPISL VTVELVVKYTQALFINWDTDMYYIGNDTPAMART YLYLEGTNKAGLFFKDFLTFWILFSNLVPISL VTLEVVKYTQALFINWDTDMYYIGNDTPAMART YLYLEGTNKAGLFFKDFLTFWILFSNLVPISL VTVELVVYQAFMIGSDLDLYYEKTDTPTVVRT * :::::::::::::::::::::::::::::::
human_ATPOA2 yeast_Drs2 Consensus human_ATP11C human_ATP1A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP8A1 human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus	279 410 110 324 329 329 348 479 137 394 399 394 413 545 167	IOWYGIVYIGHDIKHONSIAREKKSIVEKYINVQILVLEYALVSSAGALIWKS-HOEKNW TAWIFGLVIFTGHETKLLRNATATPIKKTAVEKIINRQIIALFTVLIVLILISSIGNVIMSTA-DAKHLS *:-*:-::**:**:* - *:-*::::::*:::::::::::::::::::::::::::
human_ATPOA2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus	279 410 110 324 329 329 348 479 137 394 399 394 413 545 167	TOWYGIVVIGNOTKEMONSIAPEARKSNYERVINVQIEVEFGLEVMALVSSAGALIWNRS-HORAWL TAWIFGLVIFTGHETKLERNATATPIKRTAVEKIINRQIIALFULIVLILISSIGNVIMSTA-DAKHLS *:-*:-::**:**:* -:-:-* * -:-::*::*:::::::::::::::::::::::::::::
human_ATPOA2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C	279 410 110 324 329 329 348 479 137 394 399 394 413 545 167 450	TWYFGIVYIGHDIKHMONSIARELKKSNVEKVINVQILVLFGILLVHALVSSAGALIWNKS-HGERMU TAWIFGLVIFTGHETKLLRNATATPIKRTAVEKIINRQIIALFGILVVHALVSSAGALIWNKS-HGERMU TAWIFGLVIFTGHETKLLRNATATPIKRTAVEKIINRQIIALFGILVIVIVILLISSIGNVIMSTA-DAKHLS * :-*:-::**:*:*:*:*::::::::::::::::::::
human_ATPOA2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A2 yeast_Drs2 Consensus human_ATP1C human_ATP11C human_ATP11C human_ATP11C	279 410 110 324 329 329 348 479 137 394 399 394 413 545 167 450 462	TWYFGIVYIGHDIKHMONSIAPEAKKSIVEKVINVQILVLEFULIVAAUSSAGALIWNKS-HGERMU TAWIFGLVIFTGHETKLLRNATATPIKKTAVEKIINRQIIALFTVLIVLILISSIGNVIMSTA-DAKHLS * :-*:-::** :**: * - * :**: * :: ::*: : * * :-*:-::** :**: * - * :**: * :: ::*: : * * :-*:-::**: :*: :: :: : * * :
human_ATPOA2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 yeast_Drs2 Consensus human_ATP11C human_ATP11C human_ATP11A human_ATP11A	279 410 110 324 329 329 348 479 137 394 399 394 413 545 167 450 462 456	TWYFGIVYIGHDIKHMONSIAPEAKKSIVEKVINVQILVLFGILLVHALVSSAGALIWNKS-HGERWL TAWIFGLVIFTGHETKLLRNATATPIKKTAVEKIINRQIIALFGILVVHALVSSAGALIWNKS-HGERWL TAWIFGLVIFTGHETKLLRNATATPIKKTAVEKIINRQIIALFGILVLIVLSSIGNVIMSTA-DAKHLS * :-*:-::**:*:*:*:*::::::::::::::::::::
human_ATPOA2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP11C human_ATP11C human_ATP11A human_ATP11A human_ATP1A1 human_ATP8A1 human_ATP8A2	279 410 110 324 329 348 479 137 394 399 394 413 545 167 450 462 456 476	TOWFGIVVIGNOTIGNOTRAFIAR SAFEKSIVEKVINVOILVLEGILLVAAUSSAGALIWKKS-HOEKNW TAWIFGLVIFTGHETKLLRNATATPIKRTAVEKIINRQIIALFGVLIVLIUISISGNVIMSTA-DAKHLS * :-*:-::**:***************************
human_ATPOA2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP1A1 human_ATP8A1 human_ATP8A2 yeast_Drs2	279 410 110 324 329 348 479 137 394 399 394 413 545 167 450 462 456 476 603	TOWFGIVVIGNOTIGNOTRAFIAREARSIVERVINVQILVLEFULUVALVSSAGALIWARS-HORAW TAWIFGLVIFTGHETKLLRNATATPIKRTAVEKIINRQIIALFTVLIVLILISSIGNVIMSTA-DAKHLS * :-*:-::**:**:**:*::::::::::::::::::::
human_ATPOA2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP11C human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus	279 410 110 324 329 348 479 137 394 399 394 413 545 167 450 462 456 476 603	TOWFGIVVIGNOTIGNOTRAPEARSAVERVINVOILVLETULIVALVSSAGALIWARS-HORAWA TAWIFGLVIFTGHETKLLRNATATPIKRTAVEKIINRQIIALFTVLIVLILISSIGNVIMSTA-DAKHLS * :-*:-::**:*:**:*:*:::::::::::::::::::
human_ATPOA2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP11A human_ATP1A1 human_ATP8A1 human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus	279 410 110 324 329 348 479 137 394 399 394 413 545 167 450 462 456 476 603	Idwirdivertight Idwirdivertight TAWIFGLVIFTGHETKLLRNATATPIKRTAVEKIINRQIIALFTVLIVLILISSIGNVIMSTA-DAKHLS * :-*:::::::::::::::::::::::::::::::::::
human_ATPOA2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP1A2 yeast_Drs2 Consensus human_ATP8A1 human_ATP8A1 human_ATP1C human_ATP1A1 human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP8A2	279 410 110 324 329 329 348 479 137 394 399 394 413 545 167 450 462 456 476 603 503	TAWIFGLVITIGHDIKLENDWSTKAPELKSDVEKVINVQILVEEGULUVELUVELUVSSAGALIWKS-RGEKNW TAWIFGLVITIGHETKLENDWSTKAPELKSDVEKVINVQILVEEGULUVELUVELUSSIGNVIMSTA-DAKHLS * :-*:-::**:**:**:** P-domain YNQKTQKERETLKVLKMFTDFLSFMVLFNFII YNQKTESERQRNLFLKAFTDFLAFMVLFNYII YNQKTESERQRNLFLKAFTDFLAFMVLFNYII YNQKTESERQRNLFLKAFTDFLAFMVLFNYII YNQKTGSDNFGLNFLTFIILFNNLIPISL VTLEVVKFTQAYFINWDLDMHYPETDTAAMART YLKKMDTSDNFGUNLTFIILFNNLIPISL VTLEVVKYTQALFINWDDDMYYIGNDTPAMART YLYLEGTNKAGLFFKDFLTFWILFSNLVPISL VTVELIKYYQAFMIGSDLDLYYEKTDTPTVVRT * P-domain SDLNEELGQVDYVFTDKTGTLTENSMEFIECCIDGHKYGVT SDLNEELGQVEYIFTDKTGTLTENNMEFKECCIEGHVVPHVICNGQVLPESSGIDMIDS PSU
human_ATPOA2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP8A1 human_ATP8A1 human_ATP8A1 human_ATP8A1 human_ATP8A2 yeast_Drs2	279 410 110 324 329 329 348 479 137 394 413 545 167 450 462 456 476 603 503 516	TAWFGIVYTGHDIRLENDNSTRAPERRSMVERVINVQIEVERGIEVAALVSSAGALIWERSS-RGENIW TAWIFGLVIFTGHETKLLRNATATPIRRTAVEKIINRQIIALFTVLIVLILISSIGNVIMSTA-DAKHLS * :=*:=::**:**:* - *::::::::::::::::::::
human_ATPOA2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP11C human_ATP1A1 human_ATP8A1 human_ATP1A2 yeast_Drs2 Consensus human_ATP1A1 human_ATP8A1 human_ATP11C human_ATP11C human_ATP11A human_ATP1A1	279 410 110 324 329 329 348 479 137 394 394 413 545 167 450 462 456 476 603 503 516 513	TAWIFGLVIFTGHETKLLRNATATPIKTAVEKVINVQILVIFGILVIAGUSAGALIWIKS-HGENW TAWIFGLVIFTGHETKLLRNATATPIKTAVEKVINVQILVIFGILVILVILISIGNVIMSTA-DAKHLS * :-*:-::**:**:**: P-domain YNQKTQKERETLKVLKMFTDFLSFMVLFNYI IPVSMVVVEMQKFLGSFFISWDKDFYDEEINEGALVNT YNQKTGKERETLKVLKMFTDFLSFMVLFNYI IPVSMVVVEMQKFLGSFFISWDKDFYDEEINEGALVNT YNQKTGKERETLKVLKMFTDFLSFMVLFNYI IPVSMVVVEMQKFLGSYFITWDDLDMFYDETGEGPLVNT YLNLNYGGASNFGLNFLTFIILFNNLIPISLVVLEVVKYTQAYFINWDLDMHYEPTDTAAMART YLKKMDTSDNFGYNLLTFILLFNNLIPISLVVLEVVKYTQALFINWDTDMYYIGNDTPAMART YLVLEGTNKAGLFFKDFLTFWILFSNLVPISLVVLEVVKYTQALFINWDTDMYYIGNDTPAMART YLVLEGTNKAGLFFKDFLTFWILFSNLVPISLVVELIKYYQAFMIGSDLDLYYEKTDTFVVRT *
human_ATPOA2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP11C human_ATP1A1 human_ATP1A1 human_ATP8A1 human_ATP1A2 yeast_Drs2 Consensus human_ATP1A1 human_ATP1A1 human_ATP1A1 human_ATP1A1 human_ATP1A1 human_ATP8A1 human_ATP8A1 human_ATP8A2	279 410 110 324 329 329 348 479 137 394 394 413 545 167 450 462 456 476 603 503 516 513 533	TAWIFGLVIFTGHETKLLRNATATPIKRTAVEKVINVQITVEFGILUVEFGILUSSAGALIWARS-HGEANU TAWIFGLVIFTGHETKLLRNATATPIKRTAVEKVINVQITVEMQKFIGSIGNVINSTA-DAKHLS * :-*::::::::::::::::::::::::::::::::::
human_ATPOA2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP8A2 yeast_Drs2 Consensus human_ATP11C human_ATP11A human_ATP8A1 human_ATP1A2 yeast_Drs2 Consensus human_ATP11C human_ATP1A1 human_ATP8A1 human_ATP1A2 yeast_Drs2 Consensus human_ATP11C human_ATP1A2 yeast_Drs2 Consensus	279 410 110 324 329 329 348 479 137 394 394 413 545 167 450 462 456 476 603 516 513 516 513 533 660	TAWIFGLVIFTGHETKLLRNATATPIKRTAVEKVINVQITVEFGLLVALVSJAGALVWARJ-HGEANV TAWIFGLVIFTGHETKLLRNATATPIKRTAVEKVINVQITVEFGLLVFGLLVSJAGALVWARJ-DAKHLS * :-*::::*:*:*:*:*:*:*:*:::::::::::::::

		R-domain c c
human ATP11C	572	RVQNHEIELTKVHVERNAMDGYRTLCVAFKEIAPDDYERINRQLIEAKMALQDREEKMEKVFDDIET
human ATP11A	585	RVIEGKVDQIRARVERNAVEGLRTLCVAYKRLIQEEYEGICKLLQAAKVALQDREKKLAEAYEQIEK
human ATP8A1	581	RLA-ETSKYKEITLKHLEQFATEGLRTLCFAVAEISESDFQEWRAVYQRASTSVQNRLLKLEESYELIEK
human ATP8A2	601	RLS-KDSKYMEETLCHLEYFATEGLRTLCVAYADLSENEYEEWLKVYQEASTILKDRAQRLEECYEIIEK
yeast Drs2	730	RLDDEANQYVEATMRHLEDYASEGLRTLCLAMRDISEGEYEEWNSIYNEAATTLDNRAEKLDEAANLIEK
Consensus	243	*: - : ::* * :* ****_* : ::: * :-:* :: : **-
		N-domain P-domain
human ATP11C	639	NMNLIGATAVEDKLODOAAETIEALHAAGLKVWVLTGDKMETAKSTCYACRLFOTNTELLELTTKTIEES
human ATP11A	652	DLTLLGATAVEDRLOEKAADTIEALOKAGIKVWVLTGDKMETAAATCYACKLFRRNTOLLELTTKRIEEQ
human ATP8A1	650	NLOLLGATAIEDKLODOVPETIETLMKADIKIWILTGDKOETAINIGHSCKLLKKNMGMIVINEGSLDGT
human ATP8A2	670	NLLLLGATAIEDRLOAGVPETIATLLKAEIKIWVLTGDKOETAINIGYSCRLVSONMALILLKEDSLDAT
veast Drs2	800	NLILIGATAIEDKLODGVPETIHTLOEAGIKIWVLTGDROETAINIGMSCRLLSEDMNLLIINEETRDDT
Consensus	271	·· *·***··**·** ·* * ·* * ***** ***
	2/2	P-domain
human ATP11C	709	ERKEDRIHELLIEVEKKLIHEFPKSTR-SEKKAWTEHOEVGLIIDGSTISLIINSSODSSSNNYKST
human ATP11A	722	SLHDVLFFLSKTVLRHSGSLTRDNLSGLSADMODYGLTLDGAALSLTMKPRFDGSSGNYRFL
human ATPSA1	720	PTISPHCTTISPALREENDEALTIDGKTLEVALTEGVROY
human ATPSA2	740	PDIGNLIGKENDVALIDGHTLKVALSEEVRES
VADAT Dre?	870	FNI I FETNNI NEHOI STHDMATI AI VIDGESI GENI
Yeast_DIS2	070	
Consensus		
human ATDIIC	775	FUCTOR COMMON OF A COMMUNITY CONTRACT STORE AND A COMMUNITY CONTRACT OF A COMM
human_AIPIIC	704	FLQICHACIAVLCCRHAFLQAAQIVRHVANLAGJFIILJIGDGANDVSHILEJHVGIGIKGAEGRQAARN
human_AIPIIA	761	FLEICKSCSAVLCCRUCHLOUCEURUE WOUWDUTLAIGDGANDVSMICTAUUCUCISCUECIONNE
human_AIPSAI	761	FLDLALSCKAVICCRVSFLQKSEVVEMVK-KQVKVVILAIGDGANDVSMIQIAHVGVGISGNEGLQAANS
numan_AIP8A2	781	FLDLALSCKAVICCRVSPLQKSEIVDVVK-KKVKAIILAIGDGANDVGMIQIAHVGVGISGNEGMQAINN
yeast_Drs2	914	LLIVAKLCKAVICCKV5PLQKALVVKMVK-RK555LLLAIGDGANDV5MIQAAHVGVGI5GMEGMQAAR5
Consensus	324	
100110		MS MS
human_ATPIIC	845	SDISVERERHLERELLAHGHLYYVRIAHLVQIFFYRNLCFILPQFLYQFFCGFSQOPLYDAAYLIMYNIC
human_ATPIIA	854	SDYAIPKFKHLKKMLLVHGHFYYIRISELVQYFFYKNVCFIFPQFLYQFFCGFSQQTLYDTAYLTLYNIS
human_ATP8A1	830	SDYSIAQFKYLKNLLMIHGAWNYNRVSKCILYCFYKNIVLYIIEIWFAFVNGFSGQILFERWCIGLYNVM
human_ATP8A2	850	SDYAIAQFSYLEKLLVHGAWSYNRVTKCILYCFYKNVVLYIIELWFAFVNGFSGQILFERWCIGLYNVI
yeast_Drs2	983	ADIAVGQFKFLKKLLLVHGSWSYQRISVAILYSFYKNTALYMTQFWYVFANAFSGQSIMESWTMSFYNLF
Consensus	373	:* :: :**:::*: ** * *:: : * **** : : :: : * -** * : : : :
		MS Q. M7
human_ATP11C	915	FTSLPILAYSLLEQHINIDTLTSDPRLYMKISGNAMLQLGPFLYWTFLAAFEGTVFFFGTYFLFQTASLE
human_ATP11A	924	FTSLPILLYSLMEQHVGIDVLKRDPTLYRDVAKNALLRWRVFIYWTLLGLFDALVFFFGAYFVFENTTVT
human_ATP8A1	900	FTAMPPLTLGIFERSCRKENMLKYPELYKTSQNALDFNTKVFWVHCLNGLFHSVILFWFPLKALQYGTAF
human_ATP8A2	920	FTALPPFTLGIFERSCTQESMLRFPQLYRITQNGEGFNTKVFWGHCINALVHSLILFWFPMKALEHDTVL
yeast_Drs2	1053	FTVWPPFVIGVFDQFVSSRLLERYPQLYKLGQKGQFFSVYIFWGWIINGFFHSAIVFIGTILIYRYGFAL
Consensus	413	** * : -:::: : * ** : * : :-*
		M8 M9
human_ATP11C	985	E-NGKVYGNWTFGTIVFTVLVFTVTLKLALDTRFWTWINHFVIWGSLAFYVFFSFFWGGIIWPFLKQQR-
human_ATP11A	994	S-NGQIFGNWTFGTLVFTVMVFTVTLKLALDTHYWTWINHFVIWGSLLFYVVFSLLWGGVIWPFLNYQR-
human_ATP8A1	970	G-NGKTSDYLLLGNFVYTFVVITVCLKAGLETSYWTWFSHIAIWGSIALWVVFFGIYSS-LWPAIPMAPD
human_ATP8A2	990	T-SGHATDYLFVGNIVYTYVVVTVCLKAGLETTAWTKFSHLAVWGSMLTWLVFFGIYST-IWPTIPIAPD
yeast_Drs2	1123	NMHGELADHWSWGVTVYTTSVIIVLGKAALVINQWTKFTLIAIPGSLLFWLIFFPIYAS-IFPHANISRE
Consensus		* * *:* *- * * -* * ** :- :-: **: ::-* ::- ::*
		M10 C-terminal inhibitory domain
human_ATP11C	1053	MYFVFAQMLSSVSTWLAIILLIFISLFPEILLIVLKNVRRRSARRNLSCRRASDSLSARP
human_ATP11A	1062	MYYVFIQMLSSGPAWLAIVLLVTISLLPDVLKKVLCRQLWPTATERVQTKSQCLSVEQ
human_ATP8A1	1038	MSGEAAMLFSSGVFWMGLLFIPVASLLLDVVYKVIKRTAFKTLVDEVQELEAKSQDPGAVVLGKSL
human_ATP8A2	1058	MRGQATMVLSSAHFWLGLFLVPTACLIEDVAWRAAKHTCKKTLLEEVQELETKSRVLGKAVLRDSNGKRL
yeast_Drs2	1192	YYGVVKHTYGSGVFWLTLIVLPIFALVRDFLWKYYKRMYEPETYHVIQEMQKYNISDSRP
Consensus		-* *: :: -*- : :
human_ATP11C	1113	SVRPLLLRTFSDESNVL
human_ATP11A	1120	STIFMLSQTSSSLSF
human_ATP8A1	1104	TERAQLLKNVFKKNHVNLYRSESLQQNLLHGYAFSDDENGIVSQSEVIRAYDTTKQRPDEW
human_ATP8A2	1128	NERDRLIKRLGRKTPPTLFRGSSLQQGVPHGYAFSDEEHGAVSDEEVIRAYDTTKKKSRKK
yeast_Drs2	1251	-HVQQFQNAIRKVRQVQRMKKQRGFAFSQAEEGGQEKIVRMYDTTQKRGKYGELQDASANP
Consensus		: -
yeast_Drs2	1312	FNDNNGLGSNDFESAEPFIENPFADGNQNSNRFSSSRDDISFDI

Fig. S4. Sequence alignment.

Primary sequences of indicated P4-ATPases isoforms were aligned using software MAFFT ver.7(35) and large gaps introduced in the non-conserved region were manually edited. Cytoplasmic domains (A, pink; P, green; N, light blue), secondary structures (ahelices, β -sheets and TM helices) and mutations introduced for the crystallized construct $(\Delta N7, \Delta C38)$ are indicated above the alignment. The degree of conservation among evaluated sequences is indicated below the alignment. Acidic, basic, hydrophilic or hydrophobic amino acids are indicated as red, blue, green and black characters, respectively. Gene and protein ID are as follows; human ATP11C (NCBI: XM 005262405.1), human ATP11A (Uniprot: P98196-1), human ATP8A1 (UniProt: Q9Y2Q0-2), bovine ATP8A2 (Genebank: GQ303567.3), and Saccharomyces cerevisiae Drs2 (UniProt: P39524-1).



Fig. S5. Cytoplasmic domains

(A-D) Comparison of the relative orientations of the cytoplasmic domains viewed along with the membrane plane. Cytoplasmic domains of ATP11C are shown in worm models with the same color code as in Fig. 1C (A). Atomic models of Drs2p in E2P activated form (B, grey), ATP8A1 E2P form (C, orange) and ATP8A1 E2-P_i form (D, yellow) are superimposed on the ATP11C structure according to their P domain structures to show relative orientations of A and N domains. (E-G) Azimuthal position of the A domain is

compared. A domain of Drs2p (E), ATP8A1 E2P state (F) and E2-P_i state (G), and these models are superimposed on the ATP11C structure (only A domain is highlighted in magenta, and others are shown in transparent colors) as in A-D. Dotted arrow indicates the different azimuthal positions between ATP11C E2P state and ATP8A1 E2-P_i state. Phosphate analog BeF_x (red spheres) and DGES/T motif (sticks) in ATP11C structure are highlighted in all figures. (H-K) Comparison of the TM helix arrangement in ribbon representation. Atomic models of ATP11C E2P state (color codes as in Fig. 1), Drs2p E2P state (light grey) and ATP8A1 E2P state (tan) are aligned according to their TM helices (H,I). ATP8A1 E2-P_i transition state (light green) is also compared with ATP11C E2P state (J,K) Only catalytic subunits are shown in the figure, viewed from the exoplasmic side (H,J) or perpendicular to the membrane plane with exoplasmic side up (I,K). Phosphoserine (sticks) and Pro94 (spheres) in ATP11C, and PtdSer occluded in ATP8A1 E2-P_i state (sticks) are indicated in the figure.



Fig. S6. Comparison of membrane crevices

(A-D) Surface representation of the atomic models of ATP11C E2P state (A), Drs2p E2P activated form (B), ATP8A1 E2P (C) and E2-P_i state (D). PtdSer and phosphoserine are indicated as spheres. Brown background indicates approximate location of the lipid bilayer. (E,F) Vertical sections of the TM region in ATP11C (E) and Drs2p (F) viewed from as in A-D. Phosphoserine (sticks) bound to the ATP11C and scale bar are shown for comparison.



Fig. S7. Phospholipid-dependence of ATPase activity for mutants.

ATPase activities of indicated mutants are plotted as a function of DOPS (left) or POPE (right) concentration. Mutants are categorized as follows; cytoplasmic gate (1st row), surface of the membrane cleft (2nd row), occlusion site (3rd row), TM3-4 loop at exoplasmic cavity and residues in TM5 and 6 (4th row), and CDC50A exoplasmic domain facing the cavity (5th row). ATPase activity for the wild-type enzyme is shown in all graphs as a control (black lines).