

Article

Title:

Translocation of the ABC transporter ABCD4 from the endoplasmic reticulum to lysosomes requires the escort protein LMBD1

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Table S1 List of oligonucleotide primers

Primer name	5'- sequence-3'
Fw-inv-ABCD4-HA	CAGACTACGCTGGTTACCCGTACGATGTACCAGACTACGCTTGACTC GAGTCTAGAGGGCCCGTTTAAAC
Rv-inv-ABCD4-HA	GTACATCGTACGGGTAACCAGCGTAGTCTGGTACATCGTACGGGTAT TCCACTTTGAT TCTCATCAGCTC
Fw-D1-TM1	CGGTTCCCTGTGGCTCCTGCGGCTGCTGTTC
Rv-D1-TM1	GTCTTTGTTGACGATGCAGCGGGCCAGCC
Fw-D1-TM2	AACAAAGACCCGCGGGCCTTTGGCTGGCAG
Rv-D1-TM2	GTAGAGGCGGTAGGCGTGGGCCACCAGACG
Fw-D1-TM3	CCGGACCAGTCTCTGACGGAGGACGTGG
Rv-D1-TM3	GCACTGGTAGGAAGTCACAGCCACGTCCAG
Fw-D1-TM4	GACCGTGGTGCTGCGGGCCTTCTCGCC
Rv-D1-TM4	GTAGAAAGCGATCTCCTCCGAGTTGGCCAC
Fw-D1-TM5	CATGTGGAGCTGGCCCTGCTACAGCGCTCC
Rv-D1-TM5	GGGGATTGCCACCATGAGCAGGCCCGAG
Fw-D1-TM6	CCCGCAGAGCGCACAGAAGCCTTCACTATTGCC
Rv-D1-TM6	GTAGCCAGCCAGCTCCGTCACCTCCTTGTACGAC
Fw-D4-invTM1	CTGCATCGTCAACAAAGACTTGGAAAGGGTTTAAAGACTC
Rv-D4-invTM1	CAGGAGCCACAGGAACCGCTGGAGAAATTG
Fw-D4-invTM2	CACGCCTACCGCCTCTACTTCCGGGGCC
Rv-D4-invTM2	GGCCCGCGGGTCTTTGTTTCCCAGGACC
Fw-D4-invTM3	GTGACTTCCTACCAGTGCTTCCAAAGCACAGGC
Rv-D4-invTM3	CGTCAGAGACTGGTCCGGTTATCGATGTC
Fw-D4-invTM4	GAGGAGATCGCTTTCTACAGAGCTGGGCATGTGG
Rv-D4-invTM4	GGCCCGCAGCACACCGGTCCCCAGGATG
Fw-D4-invTM5	CTCATGGTGGCAATCCCCATTTTCAGCGGG
Rv-D4-invTM5	GGGCCAGCTCCACATGCCAGCTCTGTAG
Fw-D4-invTM6	CGGAGCTGGCTGGCTACACGCACAGAATTG
Rv-D4-invTM6	CTTCTGTGCGCTCTGCGGGACTCAGGTCTC
Fw-LMBD1-Kpn	CTGGTACCATGGCGACTTCTGGCG
Rv-LMBD1-Xho	GCATCTCGAGTCAAGCAGAATAGACAGAGG
Fw-LMBD- Cla	GATGACCTCGAGTCTAGAGGGCC
Rv-LMBD- Cla	GATAGCAGAATAGACAGAGGGCTCATCATC
Fw-LMBD1-del1405G	ATGCTCCTGAAGATCAGTGTACTGTTACC
Rv-LMBD1-del1405G	TGCATCACATCTCTTTGGCACAGAAAGG
Fw-LMBD1-del848-851	GCATTTAGAATTCATTGAAAACAGCTGGTGG
Rv-LMBD1-del848-851	CTCTCTTCTTAAAGTGTTTCGTAACCTTTCTTC
Fw-mutLMBD1-GFP	ATCGATATGGTGAGCAAGGGCGAGGAGC
Rv-mutLMBD1-D469fs	AAAATACCCCAAGAAAGGCCAGTTACCAAAATAG
Rv-mutLMBD1-K281fs	AATGCCTCTCTTCTTAAAGTGTTTCGTAACCTTTCTTC
Fw-LMBD1-233AAAA	CAGCTGCGGAAAACACTGAAGACATTGAAG

Rv-LMBD1-233AAAA	CAGCAGCAGCGCTTCTAGTGCCTTTTATC
Fw-LMBD1-294AAAA	CTTGTGGCGCTCTGCGTCCCCTGAAGATC
Rv-LMBD1-294AAAA	CTGCTGCCGCCAGCTGTTTTCAATGAATTCTAAATGC
Fw-GFP- Cla	ATCGATATGGTGAGCAAGGGCGAGG
Rv-GFP- Cla	ATCGATTTACTTGTACAGCTCGTCCATGCC

Supplementary Figure Legends

Supplementary Figure 1 - Stable expression of LMBD1-GFP in CHO cells. PNS was prepared from CHO cells with or without the expression of LMBD1-GFP. LMBD1-GFP was detected by immunoblot analysis using an anti-GFP antibody.

Supplementary Figure 2 - Interaction between ABCD4 and LMBD1. ABCD4-HA constructs were transfected to CHO cells with or without the expression of LMBD1-GFP. Cell homogenates from the cells were treated with 1% digitonin and subjected to immunoprecipitation using an anti-GFP antibody. Co-precipitate proteins were analyzed by SDS-PAGE followed by immunoblotting using an anti-HA antibody.

Supplementary Figure 3 - Interaction between ABCD4-HA and ABCD4-His. ABCD4-His constructs were transfected to HuH7 cells with or without the expression of ABCD4-HA. Cell homogenates from the cells were treated with 1% digitonin and subjected to immunoprecipitation using an anti-HA antibody. Co-precipitated proteins were analyzed by SDS-PAGE followed by immunoblotting using an anti-His antibody.

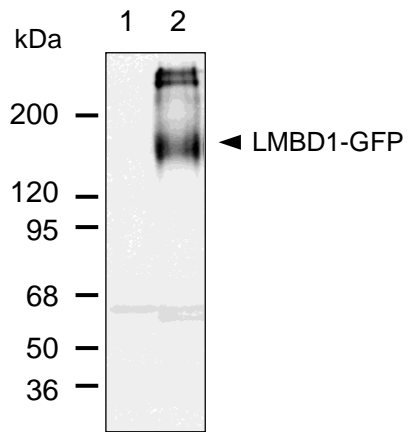
Supplementary Figure 4 - Amino acid sequence alignment of ABCD4, ABCD1 and CmABCB1. Each of the shaded sequences was exchanged between ABCD4 and ABCD1.

Supplementary Figure 5 - Subcellular localization of LMBD1(K281fs)-GFP in HEK293 cells. The distribution of LMBD1(K281fs)-GFP was compared with that of ER or lysosomes labeled with anti-KDEL and anti-LAMP1, respectively. Bar, 20 μ m.

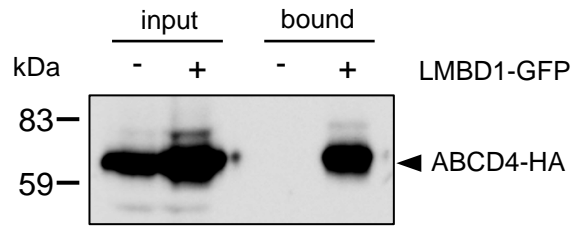
Supplementary Figure 6 - Subcellular localization of ABCD4-HA in HEK293 cells

expressing mutant LMBD1-GFP. The distribution of ABCD4-HA and LMBD1(295WTKF/AAAA)-GFP was compared with that of lysosomes stained with anti-LAMP1. Bar, 20 μ m.

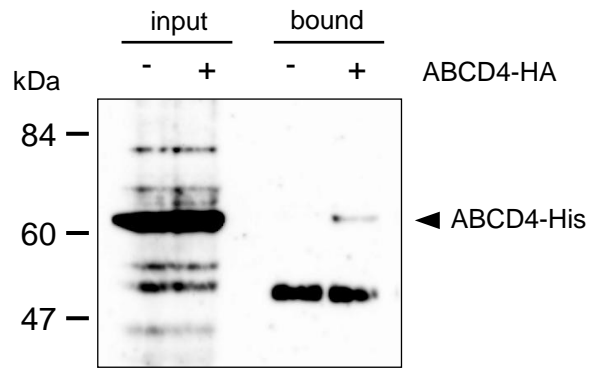
Supplementary Figure 7 - Construction of *LMBRD1* knockout cells. (A) Sequence analysis of HEK293 cells injected with PX459-lmbrd1 targeting *LMBRD1* gene. The PCR products were analyzed by direct sequencing to identify mutations in *LMBRD1*. (B) PNS was prepared from wild type and *LMBRD1* knockout cells. LMBD1 was detected by immunoblot analysis using an anti-LMBD1 antibody (left) and an anti-Actin antibody (right). (C) Detection of endogenous ABCD4 in HEK293 cells. PNS (1.5 mg) from the wild type or *LMBRD1* knockout cells was concentrated by Na_2CO_3 treatment. PNS (10 μ g) of CHO cells expressing ABCD4 was used as the positive control. Endogenous ABCD4 was detected by immunoblot analysis using an anti-ABCD4 antibody. The asterisk indicates a non-specific signal.



Kawaguchi et al. Figure S1




Kawaguchi et al. Figure S2



Kawaguchi et al. Figure S3


*Ctr*ABCB1 MNAAHAQSFETANYSSVGENHRETLSSSI YETDPVSPDTAVSSEATAFQESFPSLKLKEKDPNRRRWRWFWARPSAAGEDPEAGDPKKAASGPESAY 100
*Hs*ABCD1 MPVL SRPRPWRGNTLKR TAVLL-----ALAA-----YGAHKVYPLVRQCLAPARGLQAPAGEPTQEASGV-----AAAKAGMNRVF----LQ 73
*Hs*ABCD4 MAVAGPAPGAGARP-----RDL LQF----LQ 22



*Ctr*ABCB1 TTGVTARR I FALAWSSSATMI V I GF I AS I LEGATLP AFA I V -FGRMFQVFTKSKSQ I EGETWKYSVGFVGI GVFEF I VAGSR T ALFG I ASERLARDLRVA 199
*Hs*ABCD1 RLLWLLRLLFPRVL CRETG L LALHSAAL VSR TFLSVYVARLDGRLARC I VRKDPRAFQWQLLQWLL I ALPATFVNSA I RYLEGQLAL SFRSRLVAHAYRL 173
*Hs*ABCD4 RFLQ I LKVLFPSSQNALMFL TLLCL T LLEQFV I YQVGL I PSQYYGVLGNKDL EGFKTL TFLAVML I VLNSTLKSFDQFTCNLL YVSWRKDL TEHLHRL 122

chimera 1


chimera 2



*Ctr*ABCB1 AFSNLVEQDVTYFDRRK-----AGELGGKLNNDVQV I QYSF SKL GAVL FNL AQCVVG I I VAF I FAPAL TGVL I ALSPLVVL AGAAQMI EMSGNTKRSS 292
*Hs*ABCD1 YFSQQTYYRVSNMDGRLRNPQQSL TEDVVAFAASVAHL YSNLTKPLLDVAVTSY TLLRAARSRGAGTAWPSA I AGLV VFL TANVLR AFSPKFGE LVAEEA 273
*Hs*ABCD4 YFRGRAYTLNVL RDD I DNP DQR I SQDVERFCRQLSSMASKL I I SPFTLVYYTYQCQSTGWLGP-----VS I FGVI I LGTVV NKTLMGP I VMKLVHQE 216


chimera 3

chimera 4



*Ctr*ABCB1 EAYASAGSVAEEVFSN I RTTKAFEAEYETQRYGSKLDPL YRLGRRRY I SDGLFFGLS-----MLV I FCVYALAL WGGQL I ARGSLNL----- 376
*Hs*ABCD1 RRGKELRYMHSRVVANSEE I AFYGGHEVELALL QRSYQDLASQ I NL I LERLWYVMLEQFLMKYVWSAGLLMVAVP I I TATGYSESDAEAVKKALEKK 373
*Hs*ABCD4 KLEGD FRFKHMQ I RVNAEPAAFYRAGHVEHMRDRRLQRLLQTQRELMSKELWLY I GI-NTFDYLGSI SYVV I A I P I FSGVY----- 298

chimera 5



*Ctr*ABCB1 -----GNLLTAFFSA I L GFMGVGQAAQVWPDVTRGLGAG-----GELFAMI DRV PQYRRPDPGAEVVTQPLVLK 440
*Hs*ABCD1 EEELVSE RTEAFT I ARNLL TAAADA I ER I MSSYKEVTELAGY TARVHEMFQVFEDVQRCHF KRPRELEDA GAGSGT I GRSGVR--VEGPLK I RGQVVDVE 471
*Hs*ABCD4 GDLSPAEL STL VSKNAFVC I YL I SCFTQL I DLSTLSDVAGYTHR I GQLRETL DMSLKSQDCE----- I LGE--SEW--GLDTPPGWPAEPAD 384

chimera 6

*Ctr*ABCB1 QG I VFENVHFRYPTRMNEVLRGI SLT I PNGKTVA I VGGSGAGKST I I QLLMRFYD I EPQGGLL LFDGTPAWN YDFHALRSQ I GLV SQEPVLFSGT I RD 540
*Hs*ABCD1 QG I I CEN I PI VTPSG--EVVVASLN I RVEEGMHLL I TGPNCGCKSSLFR I LGG---LWPTYGGVL YKPPPQ-----RMFY I PQRPYMSVGLSRD 555
*Hs*ABCD4 TAFLLERVS I SAPSS-DKPL I KDLSLK I SEGQSL I TGNTGTGKTSLLRVLGG---LWTRSGSVQMLTDF-----GPHGVLF LQKPFPTDGLTRE 472

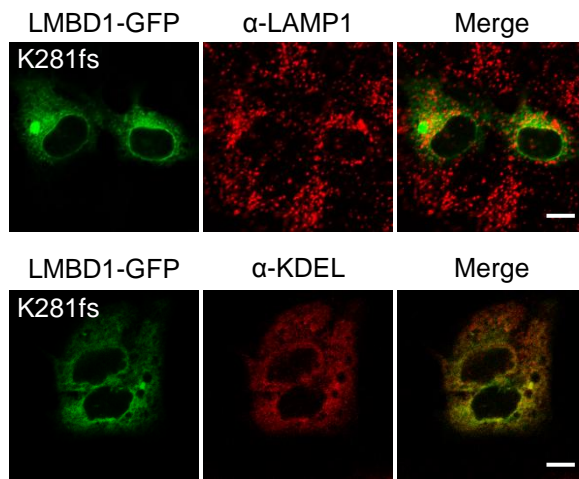
Walker A

*Ctr*ABCB1 N ILYGK-----RDATDEEV I QALREANAYSFVMALPDGLDTEVGERGLALSGGQKQRI A I ARA I LKHPTLLCLDESTSALDAESEALVQEALDRMMAS 633
*Hs*ABCD1 QV I YPDSVEDMQRKGYSEQDLEA I LDVVHLHH I LQREGGW--EAMCDWKDVL SGG EKQR I GMARMFYHRPKYALLDECTSAVS I DVEGK---- I FQA AKD 649
*Hs*ABCD4 QV I YPLKEVYPDSGSADDER I LRFL ELA GLSNLVARTEGLDQQVDWNNYDVLSPGEMQRLSFARLFYLQPKYAVLDEATSALTEEVESE----LYR I GQQ 568

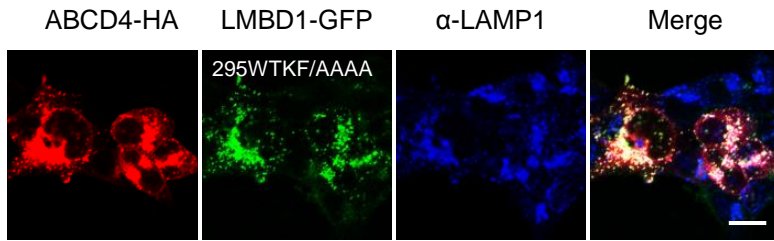
Walker B

*Ctr*ABCB1 DGVT SVV I AHRLSTVARADL I LVMQDGVVVEQGNHSELMALGPSGFYYQLVEKQLASGDMSAA----- 696
*Hs*ABCD1 AG I ALLS I THRPSLWKYHTHLLQFDGEG----GWKFEKLD SAA--RLSL TEEKRLEQLAG I PKMQRR LQELCQ I LGEAVAPAHVPAPSPQGPGLQGA 743
*Hs*ABCD4 LGMTF I SVGHRQSLEKFHSLV LKCGGG----RWELMR I KVE----- 606

Kawaguchi et al. Figure S4



Kawaguchi et al. Figure S5

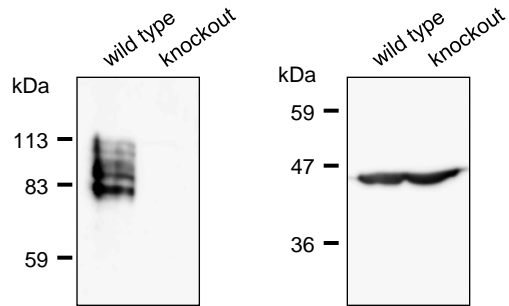


Kawaguchi et al. Figure S6

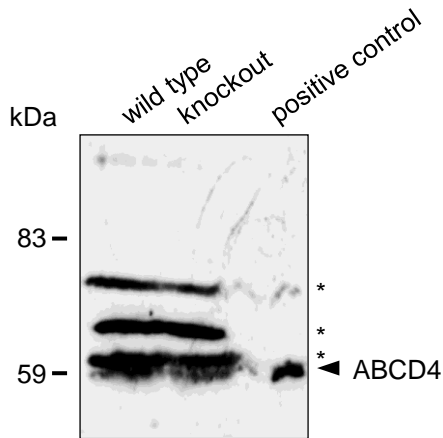
A

wild TGGATATATGTTTCGTAAATACCAAAGTCGGCGGGAAAGTGAAGTTGTCTC
knockout TGGATATATGTTTCGTAAAT-CC-----GCGGGAAAGATAAGGTGTCTC

B



C



Kawaguchi et al. Figure S7