Supplementary Materials

Molecular Biology of the Cell

Anderson *et al*.

Supplemental Information

Supplemental figure legends

Fig. S1. BORC subunits MEF2BNB and KXD1 are critically required for BORC function.

A. Cell lysates were extracted from the indicated cells and subjected to immunoblotting with antibodies against BORC unique subunits myrlysin, diaskedin, and lyspersin, and the BORC-BLOC1-shared subunit snapin. PDI serves as a loading control. Quantification of the indicated proteins was from 3 independent experiments and was normalized to WT. **B.** The indicated cells were transfected with the plasmids encoding ARL8b-GFP, fixed at 24 h post transfection, immunostained with the antibodies against GFP and LAMP1, and imaged with confocal microscope. *p* values were determined using *Student's t* test. *, $p<0.05$, **, $p<0.01$, and no labeling means there was no significant difference (vs. WT). Scale bars, 5 μm.

Fig. S2. Confirmation of CRISPR-Cas9 gene knock out (KO) by immunoblotting.

Cell lysates were extracted from the indicated cells and subjected to immunoblotting with antibodies against ARL8a and ARL8b (**A**) or VPS39 and VPS41 (**B**). GAPDH serves as a loading control.

Fig. S3. NPC2 and cathepsin D were less associated with lysosomes in living cells with HOPS deletion.

The indicated cells were transfected with the plasmids encoding NPC2-mCherry (n=43 and 23 in the upper panel, and n=48, 45, and 21 in the lower panel) (**A**) or cathepsin D-RFP (n=31 and 43) (**B**) and stained with LysoTracker (1:10,000, ThermoFisher) in complete medium for 30 min. Cells were imaged with a confocal microscope and analyzed for NPC2/cathepsin D-LysoTracker colocalization. *p* values were determined using *Student's t* test. ***, $p<0.001$ (vs. WT). Scale bars, 5 μm. **C.** NPC2- and cathepsin D-positive lysosomes (labeled with LysoTracker) were quantified based on the images obtained from (**A**) and (**B**), and the percentages of NPC2- or cathepsin D-positive

lysosomes out of the total lysosomes are shown in the graph. *p* values were determined using *Student's t* test. ****, $p<0.0001$.

Fig. S4. Disruption of BORC/ARL8/HOPS did not change CD-MPR levels.
Cell lysates were extracted from the indicated cells and subjected to immunoblotting with the antibody against CD-MPR. Calnexin serves as a loading control. Quantification was from 3 independent experiments and normalized to WT cells. *p* values were determined using *Student's t* test. n.s., no significant difference (vs. WT).

Supplemental tables

Table. S1 cDNA constructs used in this study

Insert	Vector	Tag	Remarks
Target sequences of human Arl8a	pSpCas9(BB)-2A-GFP (PX458)	3X N-FLAG, c-GFP	
Target sequences of human Arl8b	pSpCas9(BB)-2A-GFP (PX458)	3X N-FLAG, c-GFP	
Target sequences of human VPS39	pSpCas9(BB)-2A-GFP (PX458)	3X N-FLAG, c-GFP	
Target sequences of human VPS41	pSpCas9(BB)-2A-GFP (PX458)	3X N-FLAG, c-GFP	
ARL8b-GFP	pcDNA3.1/CT-GFP	C-GFP	Gift from Dr. J. H. Brumell (University of Toronto)
NPC2	mcherry-N1	c-mCherry	Gift from Dr. P. Lobel (Rutgers University)
NPC1	N3	EGFP and His6	Addgene #53521
Cathepsin D	Modified Clontech plasmid	C-RFP	Gift from Dr. J. Bonifacino (NIH)

Table. S2 Antibodies used in this study

Antibody	Source	Product Number	Dilution	Applications
Arl8a	Proteintech	17060-1-AP	1:1000	WB
Arl8b	Proteintech	13049-1-AP	1:1000	WB
Calnexin	Cell Signaling Technologies	2679s	1:8000	WB
Cathepsin D	Proteintech	219361	1:4000	WB
CD-MPR	Developmental	22d4	1:50	WB

	Studies Hybridoma Bank			
CI-MPR	Homemade		1:4000/1:500	WB/IF
CI-MPR	Abcam	124767	1:200/1:2000	IF/WB
Diaskedin (C10orf32)	Millipor-Sigma	HPA037648	1:1000	WB
EEA1	BD Biosciences	610457	1:200	IF
EEA1	Cell Signaling Technologies	3288	1:100	IF
GAPDH	Proteintech	6004-1	1:5000	WB
GFP	ThermoFisher Scientific	A10262	1:1000	IF
LAMP1	Cell Signaling Technologies	9091s	1:500	IF
LAMP2	Santa Cruz	SC-18822	1:500	IF
LAMTOR4	Cell Signaling Technologies	13140S	1:200	IF
Lyspersin (C17orf59)	Agent	AP11018c	1:500	WB
mCherry	ThermoFisher Scientific	M11217	1:1000	IF
Myrlysin (LOH12CR1)	Proteintech	17169-1-AP	1:1000	WB
NPC1	AbCam	134113	1:1000	WB
NPC2	Gift from P. Lobel		1:10000	WB
PDI	Cell Signaling Technologies	3501S	1:10,000	WB
Rab5	Cell Signaling Technologies	3547	1:100	IF
Rab7a	Cell Signaling Technologies	9367	1:200	IF
RFP	ThermoFisher Scientific	R10367	1:1000	IF
Snapin	SYSY	148 002	1:1000	WB
SREBP2	Santa Cruz	SC-13552	1:500	WB
TGN46	BioRad	AHP500GT	1:500	IF
VPS39	Santa Cruz	SC-514762	1:500	WB
VPS41	Santa Cruz	SC-377118	1:500	WB

Fig. S1

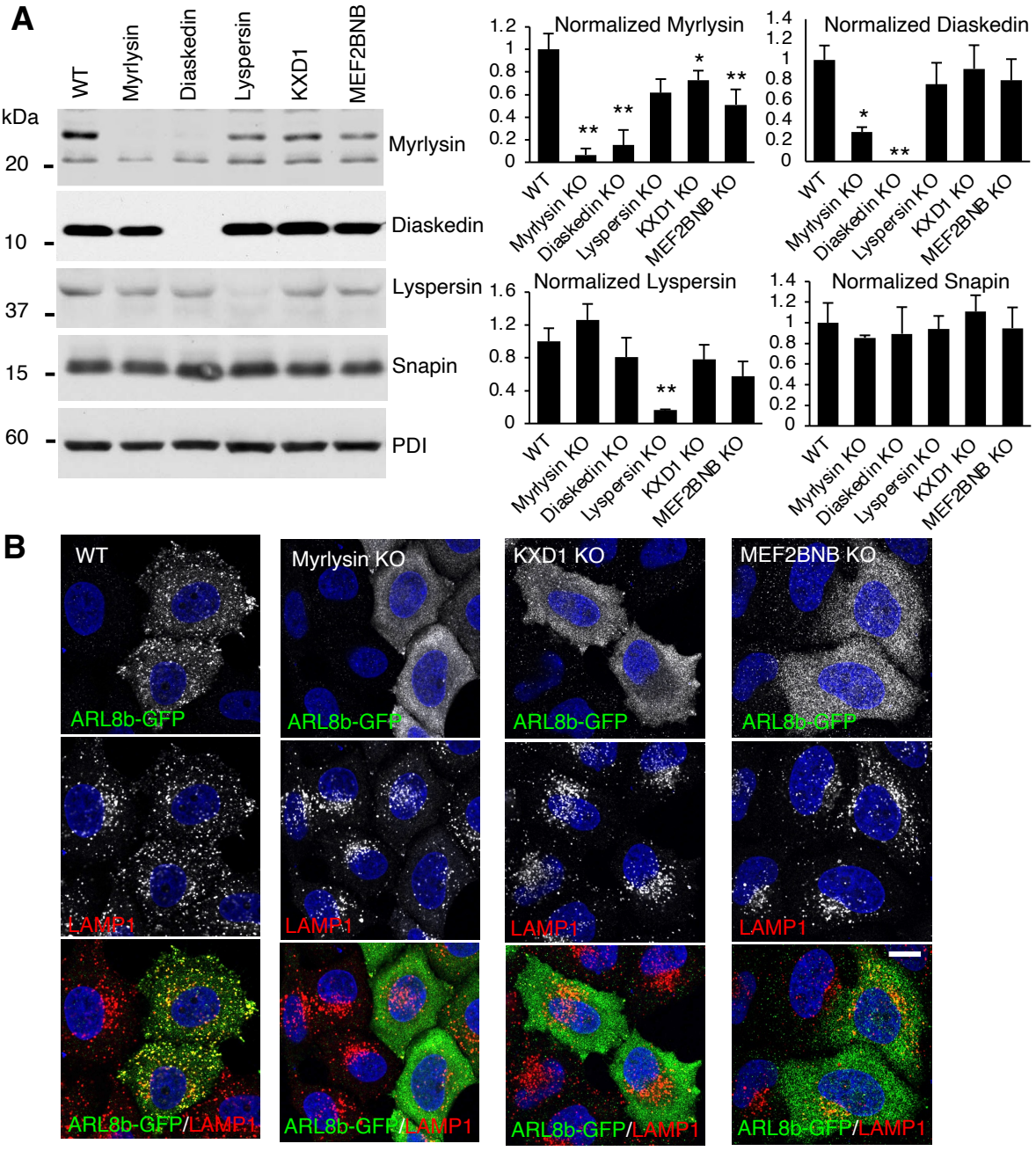

A
WT
Myrlysin
Diaskedin
Lyspersin
KXD1
MEF2BNB
kDa
20
10
37
15
60
Myrlysin
Diaskedin
Lyspersin
Snapin
PDI
Normalized Myrlysin
Normalized Diaskedin
Normalized Lyspersin
Normalized Snapin
WT
Myrlysin KO
Diaskedin KO
Lyspersin KO
KXD1 KO
MEF2BNB KO
B
WT
Myrlysin KO
KXD1 KO
MEF2BNB KO
ARL8b-GFP
LAMP1
ARL8b-GFP/LAMP1

Fig. S2

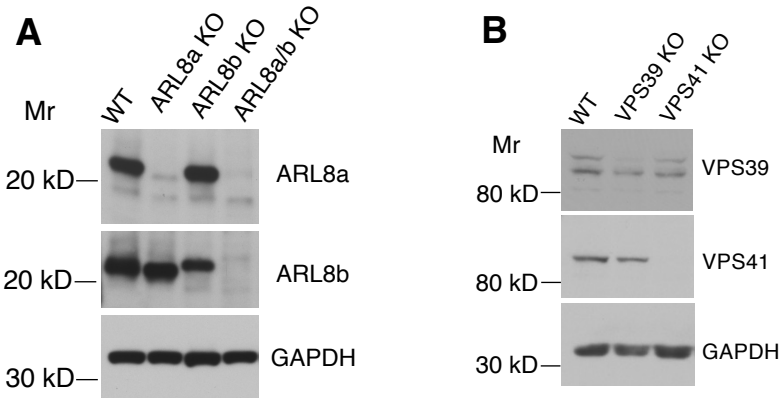

A
WT
ARL8a KO
ARL8b KO
ARL8a/b KO
Mr
20 kD
ARL8a
20 kD
ARL8b
30 kD
GAPDH
B
WT
VPS39 KO
VPS41 KO
Mr
80 kD
VPS39
80 kD
VPS41
30 kD
GAPDH

Fig. S3

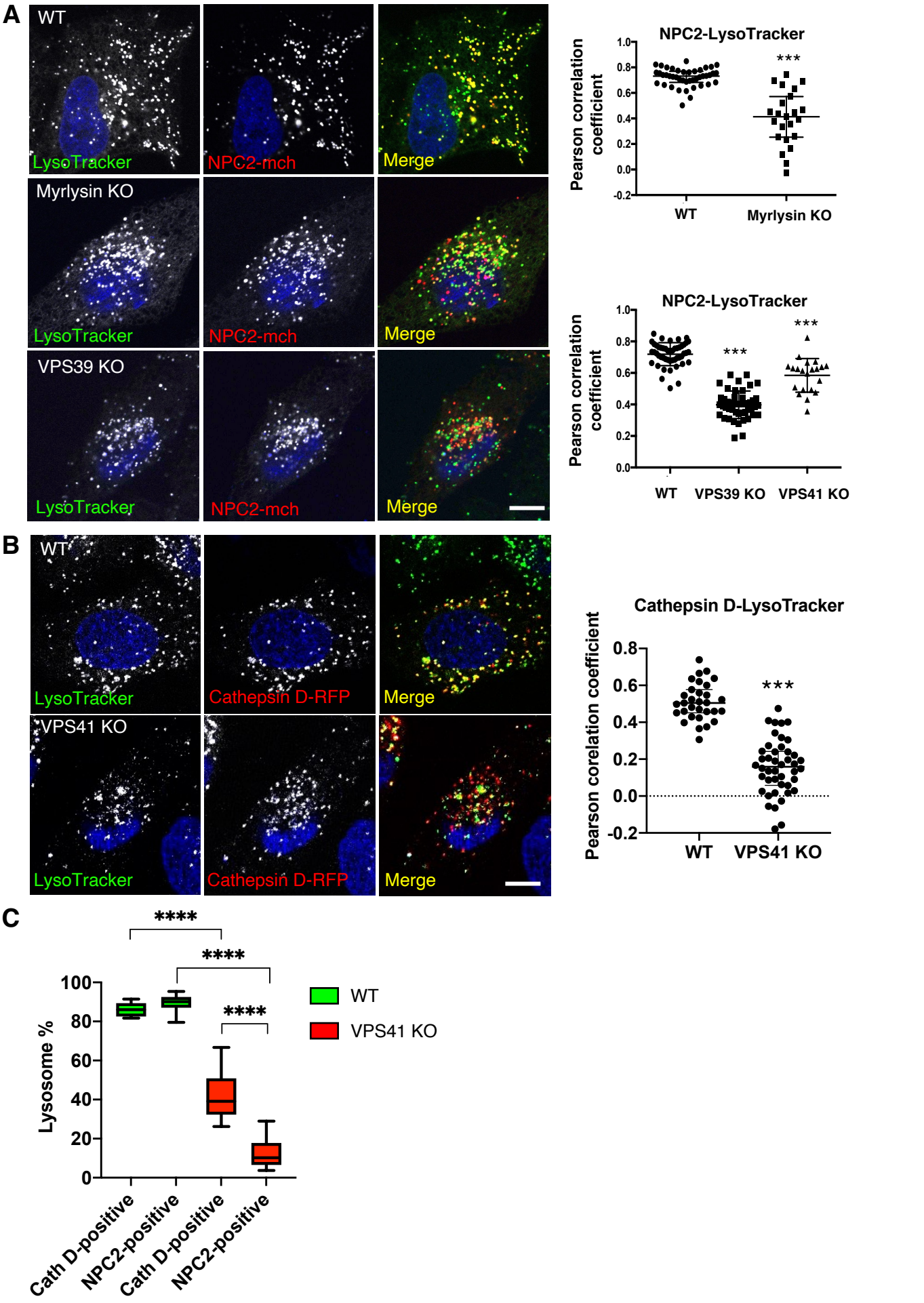
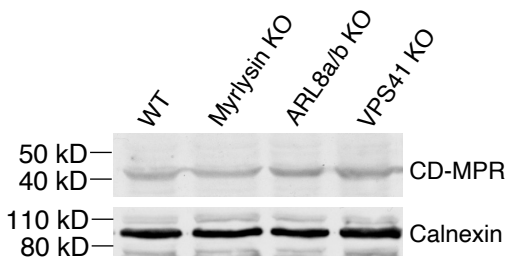
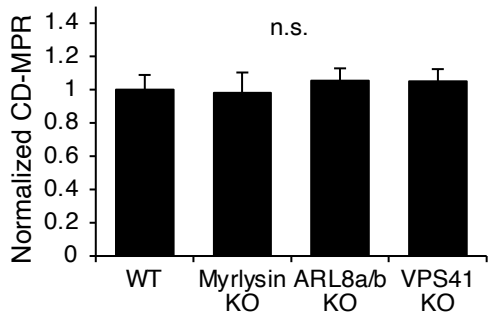

A
WT
LysoTracker
NPC2-mch
Merge
Myrlysin KO
LysoTracker
NPC2-mch
Merge
VPS39 KO
LysoTracker
NPC2-mch
Merge
NPC2-LysoTracker
Pearson correlation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
***
WT
Myrlysin KO
NPC2-LysoTracker
Pearson correlation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
***
***
WT
VPS39 KO
VPS41 KO
B
WT
LysoTracker
Cathepsin D-RFP
Merge
VPS41 KO
LysoTracker
Cathepsin D-RFP
Merge
Cathepsin D-LysoTracker
Pearson corelation coefficient
0.8
0.6
0.4
0.2
0.0
-0.2
***
WT
VPS41 KO
C
****
****
Lysosome %
100
80
60
40
20
0
WT
VPS41 KO
Cath D-positive
NPC2-positive
Cath D-positive
NPC2-positive

Fig. S4


WT
Myrlysin KO
ARL8a/b KO
VPS41 KO
50 kD
40 kD
CD-MPR
110 kD
80 kD
Calnexin


n.s.
Normalized CD-MPR
1.4
1.2
1
0.8
0.6
0.4
0.2
0
WT
Myrlysin KO
ARL8a/b KO
VPS41 KO