

Supplemental Materials

Molecular Biology of the Cell

Liao et al.

Supplemental figure legends

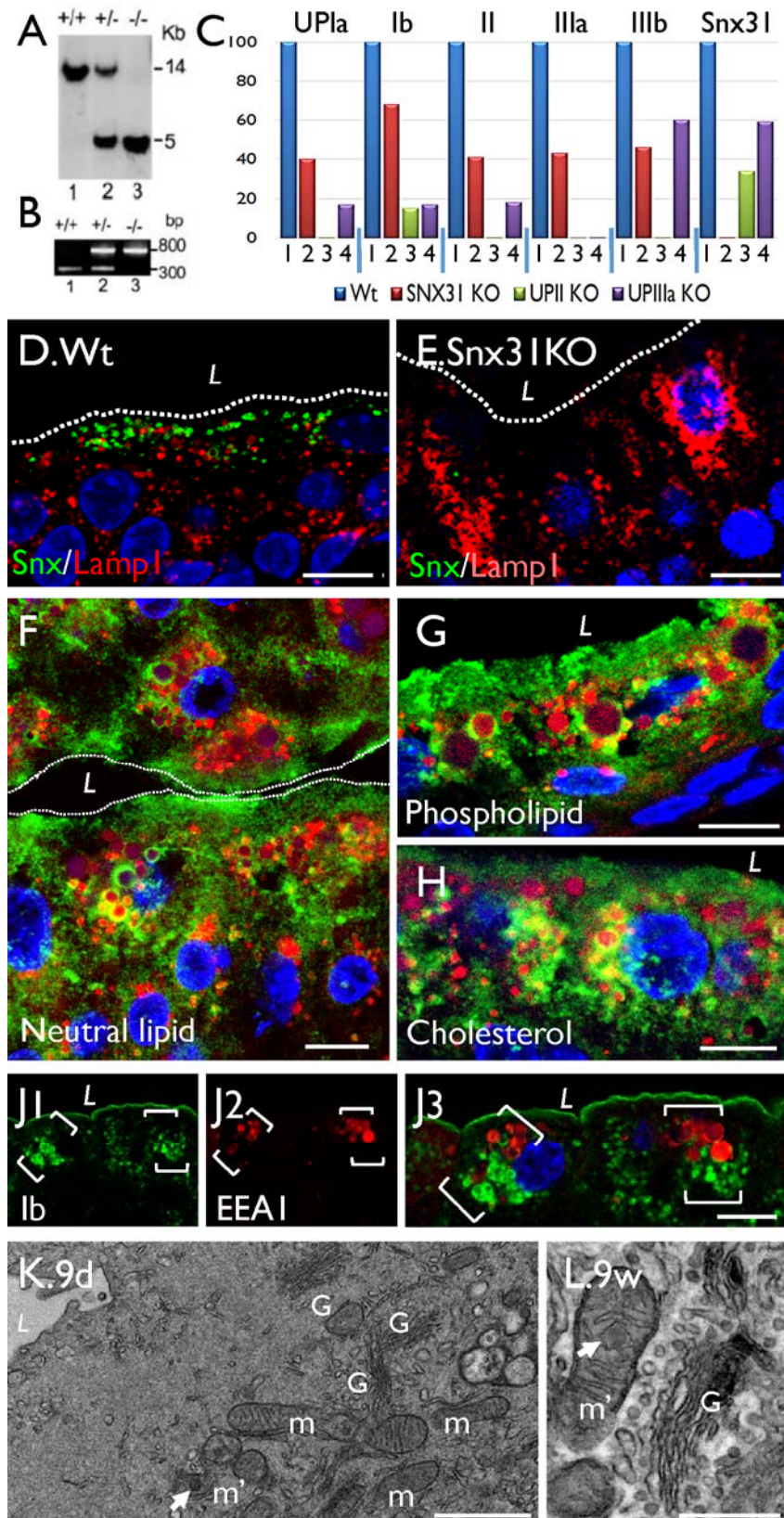
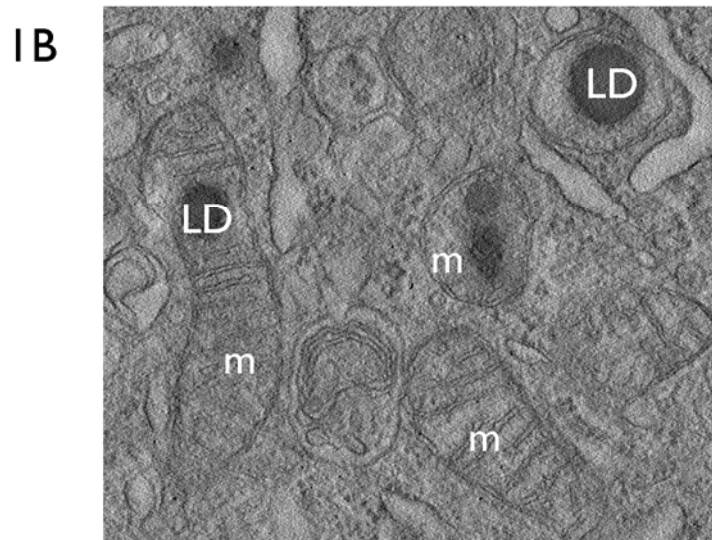
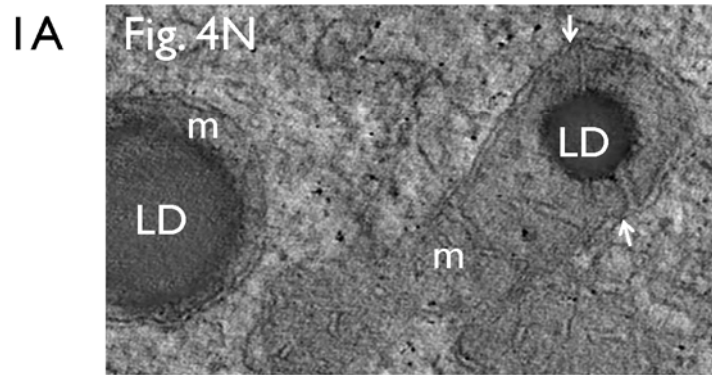


Figure S1. Generation of the *Snx31*-KO mice and LD accumulation (related to Fig. 1). (A) A 1070-bp fragment of the mouse *Snx31* gene (NM_025712), including Exon 3, which encodes part of the PX domain (39 amino acids out of 109), involved in binding phosphoinositides of vesicular membranes, was deleted using homologous recombination in embryonic stem cells (129SV background), resulting in a frame shift with a premature stop codon. (B) Southern blot showing the characterization of the +/+, +/-, and -/- mice. (C) Quantification of proteins shown in Fig. 8A. (D and E) Paraffin-sections of (D) wild type and (E) *Snx31*-KO mouse bladder were double-stained using antibodies to *Snx31* (green) and LAMP1 (red). Note in (D) the association of *Snx31* with the MVBs of wild type urothelium (Vieira et al., 2014), and in (E) the absence of *Snx31* (and the greatly increased LAMP1 staining) in the *Snx31*-KO (see text). (F-H) Frozen sections of the *Snx31*-KO mouse urothelium were double-stained for UPII (green) and (F) neutral lipid (HCS LipidTOX Green-NL), (G) Phospholipids (BODIPY HRC-PL), and (H) cholesterol (BODIPY Cholesteryl). Dashed line and L indicate the apical urothelial surface and luminal space, respectively. (J) Paraffin-section of *Snx31*-KO mouse bladder was double-stained using antibodies to UPIb (green) and EEA1, an early endosome marker (red). Note the uroplakin Ib-positive LDs are EEA1-negative. (K, L) TEM images showing mitochondria (m) and proximal Golgi apparatus (G) in the urothelia of 9-day (K) and 9-week (L) old *Snx31* KO mice. Note in (K) the newly initiated intramitochondrial LD (arrow). Scale bars = 0.5- μ m in (L); 1- μ m in (K); 10- μ m in (D-J).

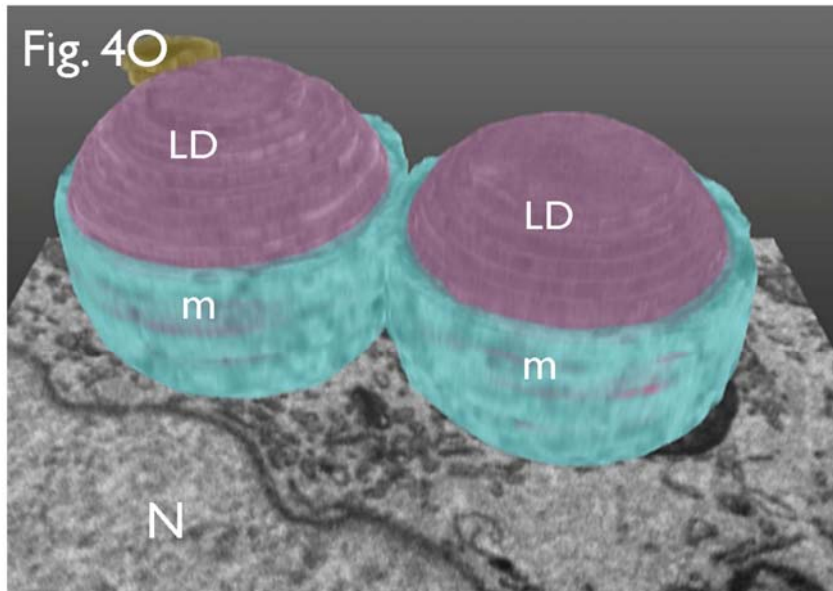
Supplemental video legends

Supplemental Video SVI (related to Fig. 4N)



Supplemental Video SV1A & B. Visualization of the intramitochondrial LDs by EM tomography (A is the basis for Fig. 4N). Serial images were collected from two different regions of an umbrella cells. Images were taken after the section was tilted every 2-degree from +70 to -70, and processed to generate a video. LD, m and arrows denote lipid droplet, mitochondria, and residual mitochondrial cristae, respectively. Note in (A) the intramitochondrial location of LDs (basis for Fig. 4N), and in (B) the relationship between the LDs and the mitochondrial cristae.

Supplemental Video SV2 (related to Fig. 4O)



Supplemental Video SV2. Visualization of intramitochondrial LDs by serial block-face SEM (basis for Fig. 4O). LD (purple), m (light blue) and N denote lipid droplet, mitochondria and nucleus, respectively. Note the intramitochondrial location of the LD.

Supplemental Table S1. Sources of reagents

Antibody / probe	Source	Catalog #	Host*	Dilution
active Caspase 3	Novus	NB100-56113	Rb	IF (10 µg/ml)
ADFP	Novus	NB110-40877	Rb	IF (10 µg/ml)
AIF	R&D	AF5824	Shp	IF (10 µg/ml)
Beclin1	Abcam	ab62472	Rb	IF (10 µg/ml)
BiP	Dr. Andrew Hu	N/A	Rb	IF (1:100)
Calnexin	Abcam	ab112995	Ms	IF (10 µg/ml)
Cox4	NOVUS	NB110-39115	Rb	IF (10 µg/ml)
EEA1	Bioss	bs-11250R	Rb	IF (10 µg/ml)
GM130	GeneTex	GTX61445	Rb	IF (10 µg/ml)
LAMP1	DSHB	1D4B-f	Rat	IF (1:100)
LC3	Cell Signaling	12741	Rb	IF (10 µg/ml)
PDI	Dr. Andrew Hu	N/A	Rb	IF (1:100)
Perilipin	Novus	NB100-60554	Gt	IF (10 µg/ml)
PINK1	Novus	NBP2-36488	Ms	IF (10 µg/ml)
SEC23A	GeneTex	GTX109488	Rb	IF (10 µg/ml)
SNX31 (2660)	Sun lab (Vieira et al, 2014)	N/A	Rb	IF, IEM (1:100), WB (1:1000)
TGN38	Novus	NB300-575	Ms	IF (10 µg/ml)
α-Tubulin	Sigma	T5168	Ms	IF (1:10,000)
UPIa (AU-Ia-3, clone 3O13)	Sun lab (Liao et al, 2018)	N/A	Ms	IF (1:20), WB (1:200)
UPIa (128)	Sun lab (Liao et al, 2018)	N/A	Rb	IF, IEM (1:200)
UPIa (4867)	Sun lab (Liao et al, 2018)	N/A	Rb	IF, IEM (1:200), WB (1:2000)
UPIb (AU-Ib-2, 2N23)	Sun lab (Liao et al, 2018)	N/A	Ms	IF (10 µg/ml), WB (1 µg/ml)
UPIb (7472)	Sun lab (Liao et al, 2018)	N/A	Rb	IF, IEM (1:100)
UPII (S3045)	Sun lab (Liao et al, 2018)	N/A	Rb	IF (1:100), WB (1:1000)
UPIIIa (AU-IIIa-1, clone AU1)	Sun lab (Liang et al, 2001)	N/A	Ms	IF, IEM (1:20), WB (1:200)
UPIIIa (35804)	Sun lab (Liao et al, 2018)	N/A	Rb	IF (10 µg/ml)
UPIIIb (AU-IIIb-1, clone C362)	Sun lab (Liao et al, 2018)	N/A	Ms	IF (1:20), WB (1:200)
UPIIIb (6177)	Sun lab (Liao et al, 2018)	N/A	Rb	IF(1:100), WB (1:1000)
XBP1	Santa Cruz	sc-8015	Ms	IF (10 µg/ml)
β-BODIPY™ FL C12-HPC	Thermo Fisher	D3792	N/A	IF (1:1000)
CholEsteryl BODIPY™ FL C12	Thermo Fisher	C3927MP	N/A	IF (1:1000)
HCS LipidTOX™ Green Neutral Lipid	Thermo Fisher	H34475	N/A	IF (1:1000)
Click-iT™ Plus TUNEL, Alexafluor594	Thermo Fisher	C10618	N/A	See Manual
4',6-diamidino-2-phenylindole (DAPI)	Thermo Fisher	D1306	N/A	IF (1:500)

Donkey anti-rabbit IgG (H+L), Alexafluor 488	Thermo Fisher	A-21206	Dk	IF (1:200)
Donkey anti-rabbit IgG (H+L), Alexafluor 568	Thermo Fisher	A-10042	Dk	IF (1:200)
Donkey anti-mouse IgG (H+L), Alexafluor 488	Thermo Fisher	A-21202	Dk	IF (1:200)
Donkey anti-mouse IgG (H+L), Alexafluor 568	Thermo Fisher	A-10037	Dk	IF (1:200)
Donkey anti-goat IgG (H+L), Alexafluor 488	Thermo Fisher	A-11055	Dk	IF (1:200)
Donkey anti-rat IgG (H+L), Alexafluor 647	Jackson ImmunoResearch	712-605-153	Dk	IF (1:200)
Donkey anti-sheep IgG (H+L), Alexafluor 568	Thermo Fisher	A-21099	Dk	IF (1:200)
Goat anti-rabbit IgG (H+L), 18nm colloidal gold	Jackson ImmunoResearch	111-215-144	Gt	IEM (1:100)
Donkey anti-Mouse IgG HRP	Jackson ImmunoResearch	715-035-150	Dk	WB (1:10000)
Donkey anti-Rabbit IgG HRP	Jackson ImmunoResearch	711-035-152	Dk	WB (1:10000)
Hoechst 33258	Thermo Fisher	H3569	N/A	IF (1:500)

* Abbreviations: Hosts include Dk (donkey), G (goat), Ms (mouse), Rb (rabbit) and Shp (sheep); IF (immunofluorescent staining); IEM (immune-EM); IB (immune-blot);