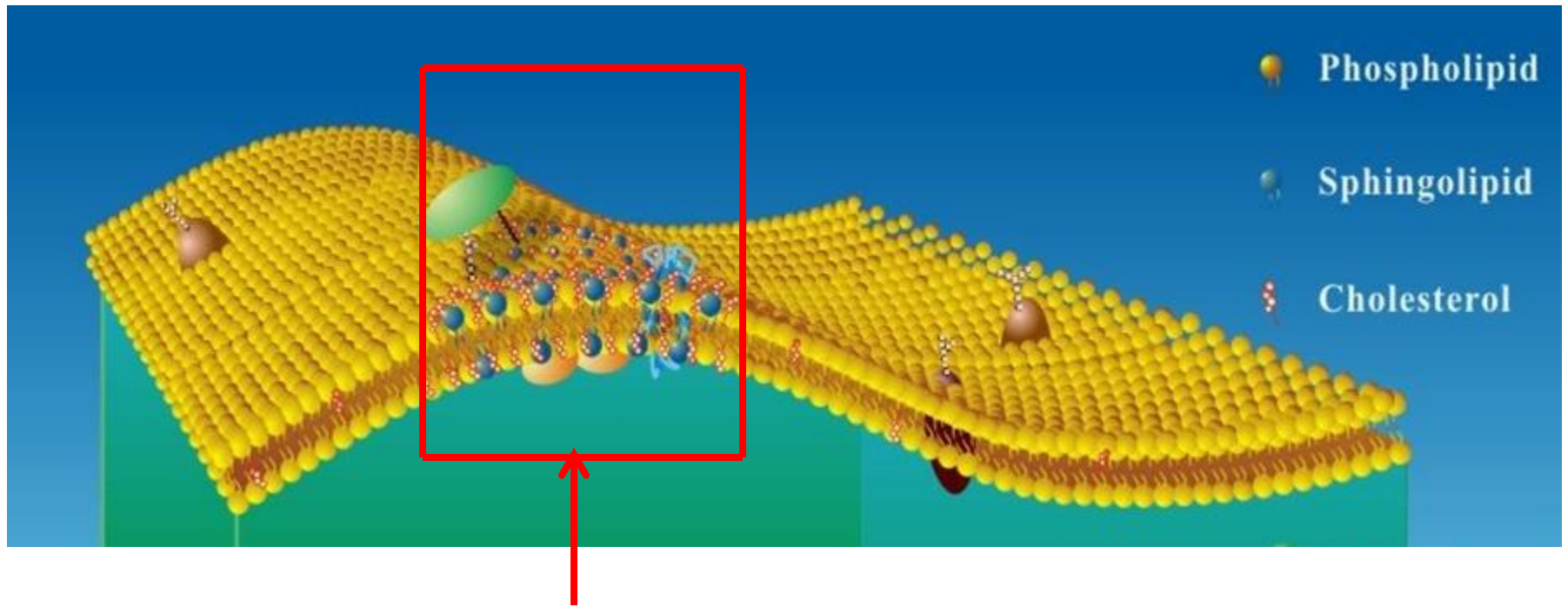


**Sphingolipid *de novo* synthesis:
its relevance to metabolic diseases
and cell polarity**

**Xian-Cheng Jiang
(shian-chen chiang)
SUNY Downstate Medical Center**

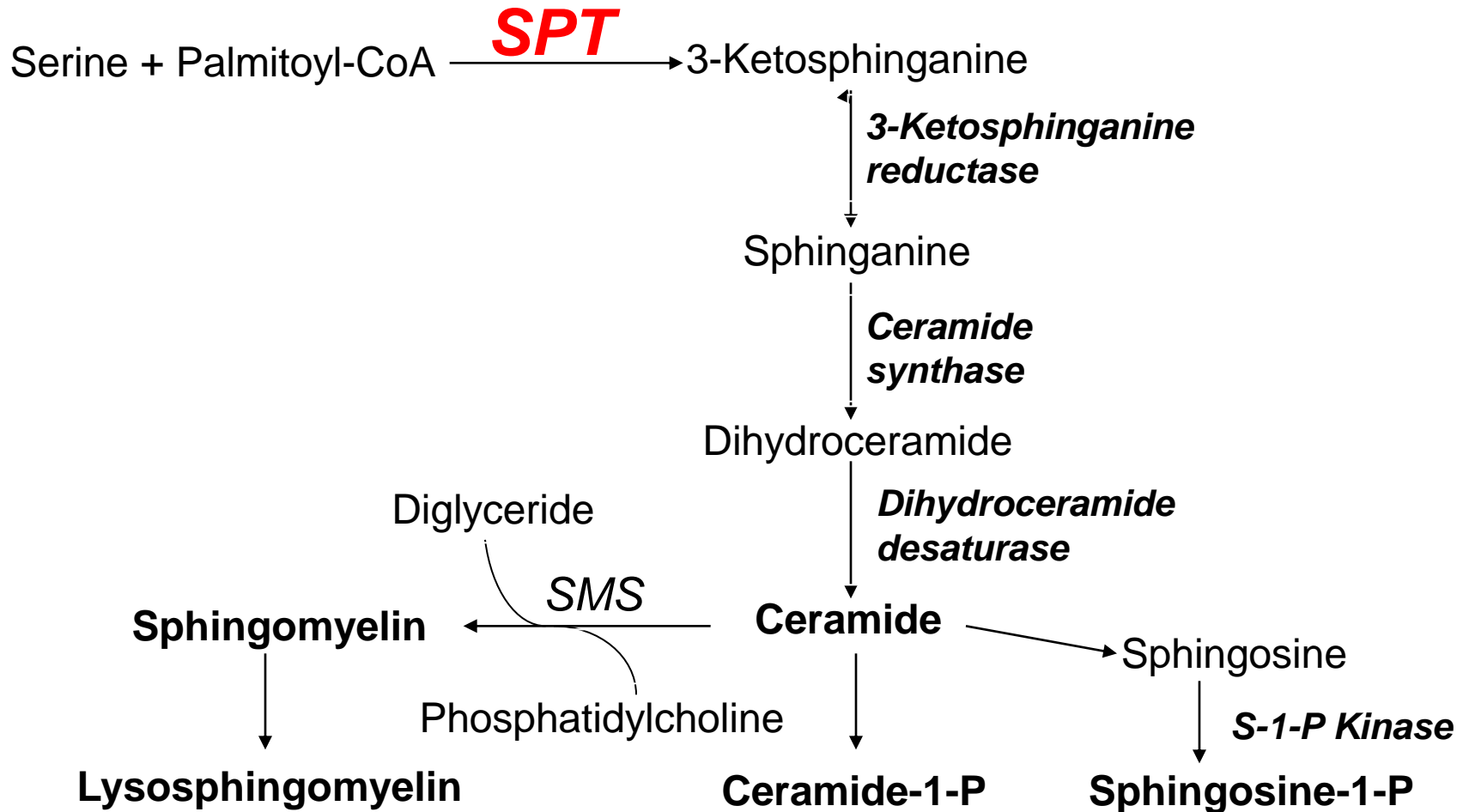
Cell plasma membrane



Lipid rafts

Sphingolipid = sphingomyelin, glycosphingolipid, and ceramide

Sphingolipid Biosynthesis



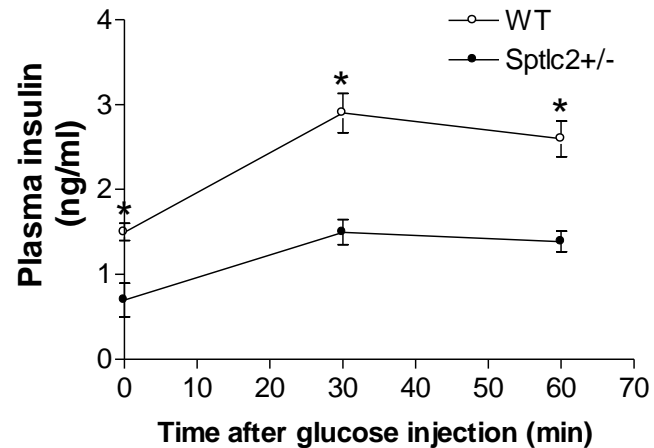
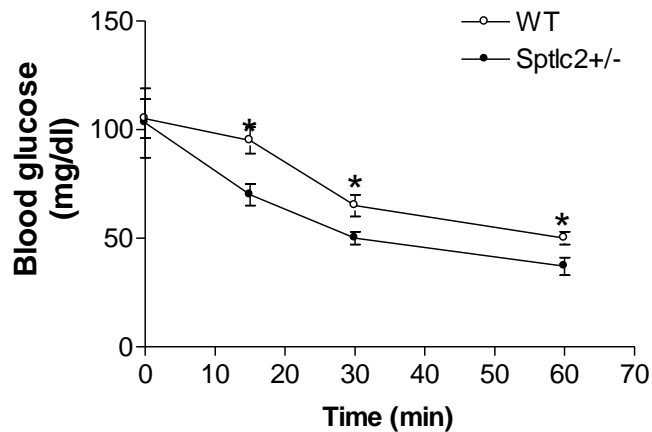
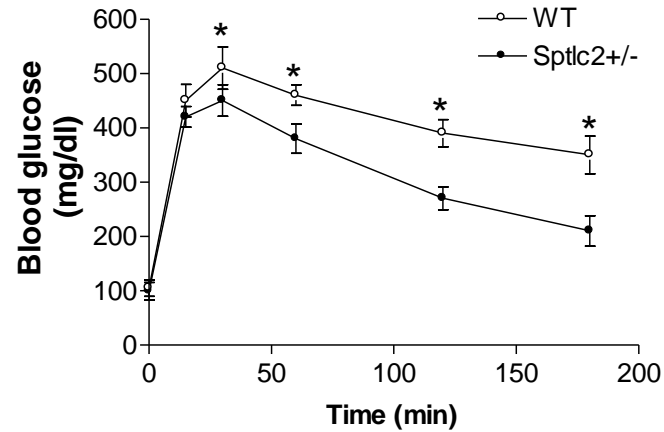
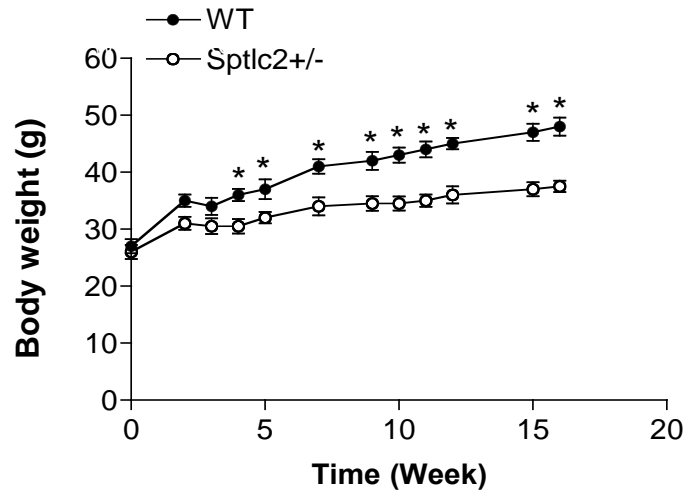
Serine Palmitol-CoA transferase (SPT)

1. SPT has subunits: Sptlc1 and Sptlc2/Sptlc3.
2. Sptlc1 and Sptlc2 total gene knockouts are embryonic lethal.

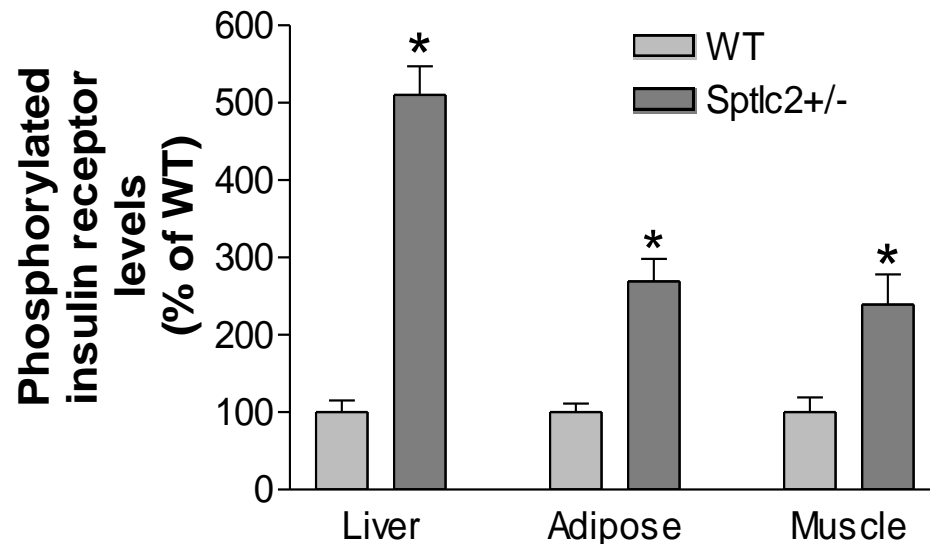
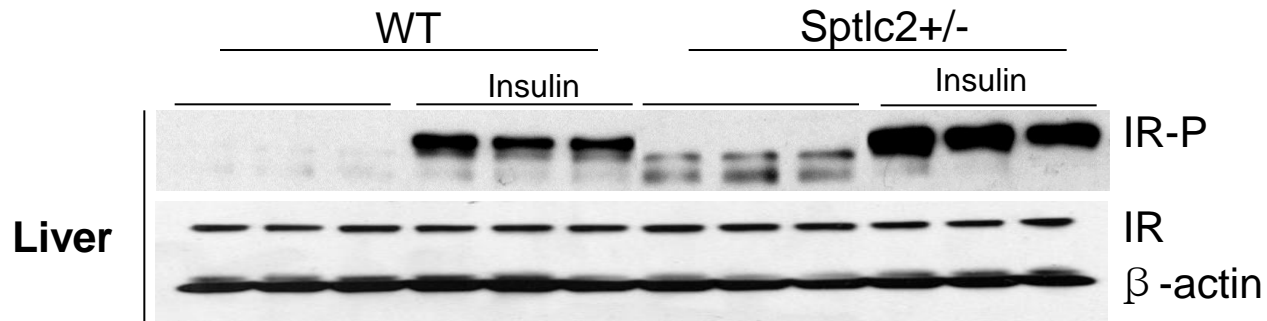
Subtopics

- ✓ *The relevance of plasma membrane sphingolipid changes to the metabolic diseases.*
- ✓ The effect of adenovirus associated virus (AAV)-Cre-mediated liver SPT deficiency on lipoprotein metabolism.
- ✓ The effect of albumin-Cre-mediated liver SPT deficiency on hepatocyte polarity.

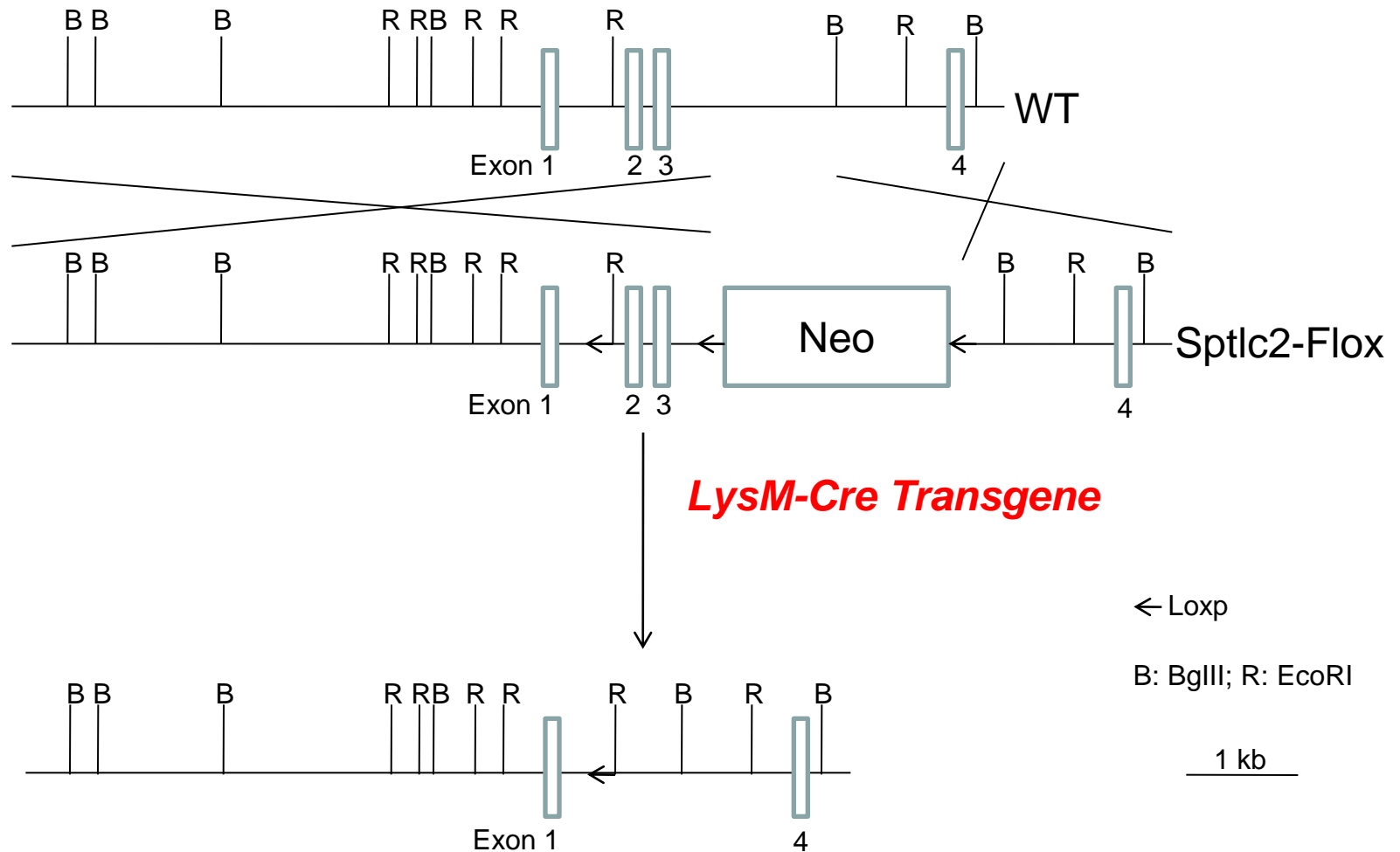
Sptlc2 partial deficiency increases insulin sensitivity



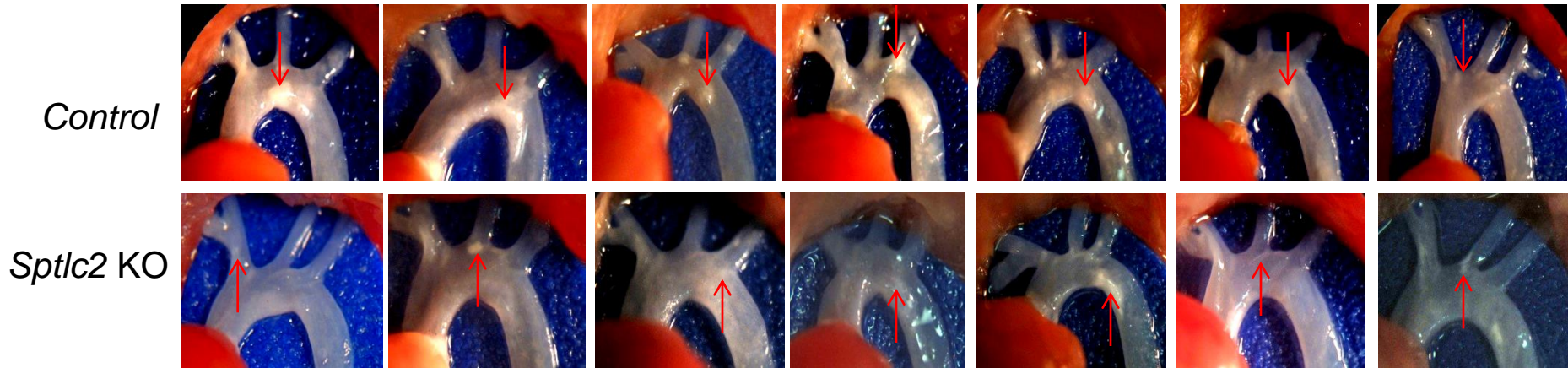
Sptlc2 partial deficiency increases insulin receptor phosphorylation



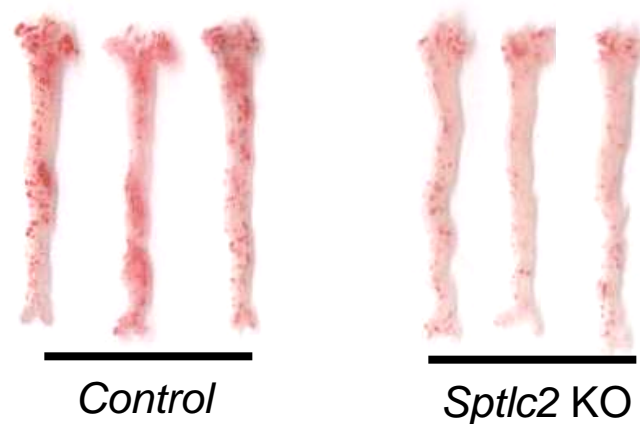
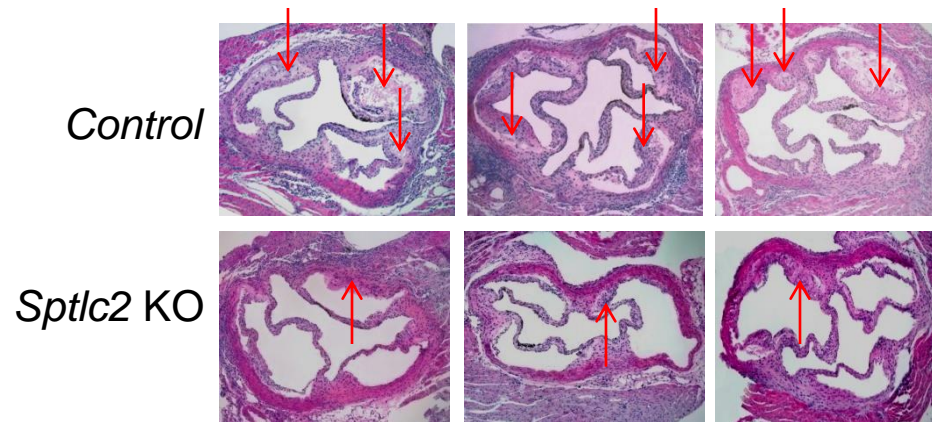
Myeloid cell-specific *Sptlc2* deficient mouse preparation



Myeloid cell-specific *Sptlc2* deficiency decreases atherosclerotic lesions (in arch)



Myeloid cell-specific *Sptlc2* deficiency decreases atherosclerotic lesions (root and *en face*)



Summary (1)

- Sphingolipid *de novo* synthesis related with the development of metabolic diseases.
- Inhibition of sphingolipid *de novo* synthesis may prevent metabolic diseases.

Subtopics

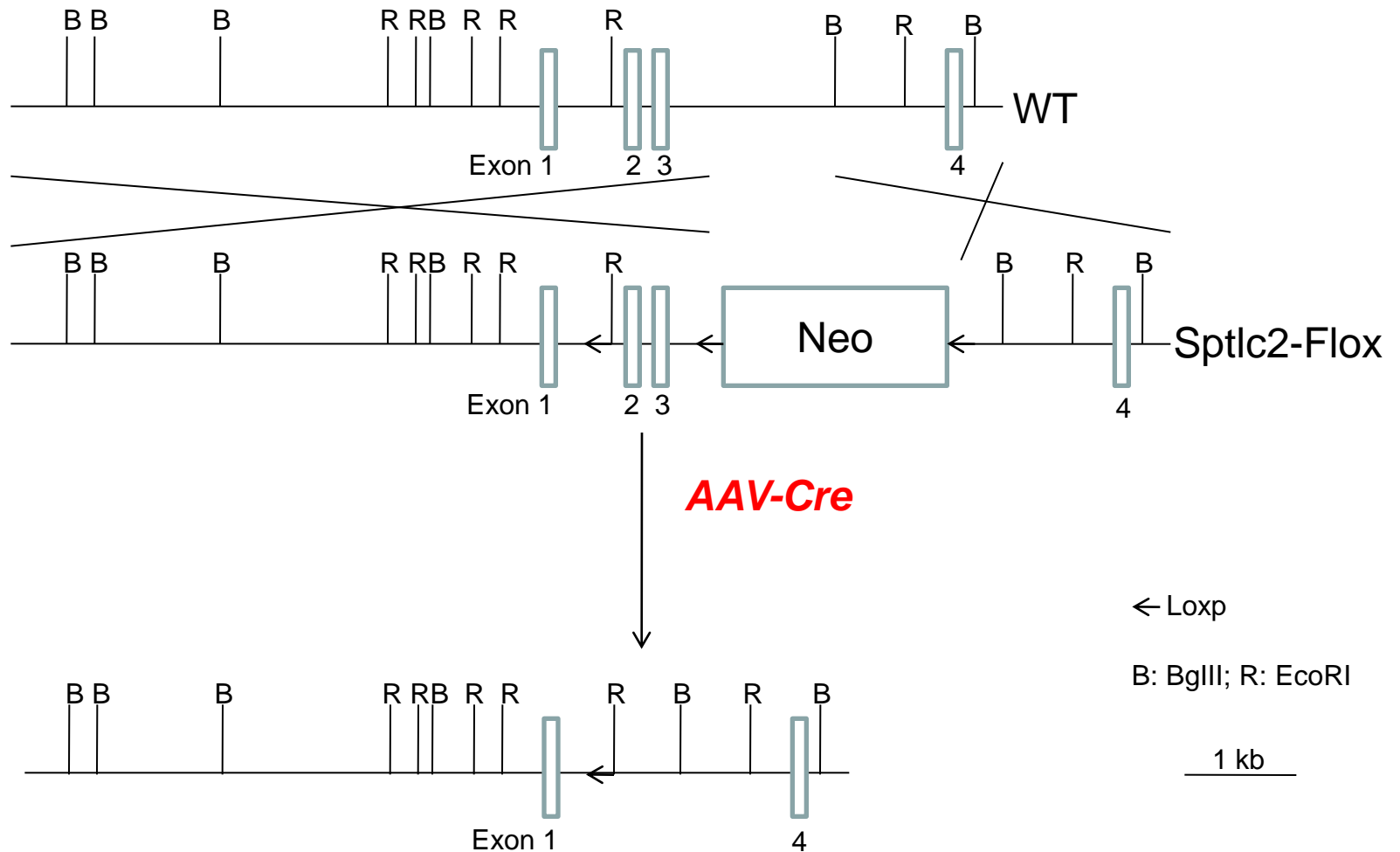
- ✓ The relevance of plasma membrane sphingolipid changes to the metabolic diseases.
- ✓ *The effect of adenovirus associated virus (AAV)-Cre-mediated liver Sptlc2 deficiency on lipid metabolism.*
- ✓ The effect of albumin-Cre-mediated liver Sptlc2 deficiency on hepatocyte polarity.

Liver-specific KO mouse preparation

1. AAV-Cre (for adult)
2. Albumin-Cre transgenic mice (for whole life)
3. Inducible-Cre (for adult)

AAV-Cre/Sptlc2-loxp mice

Liver-specific Sptlc2 KO mouse preparation (for adult)



Plasma lipid measurement in liver-specific Sptlc2 KO and WT mice (LC/MS/MS)

Mice	SM (μ M)	PC (μ M)	PC/SM	Cer (ng/ml)	Sph (ng/ml)	S-1-P (ng/ml)	DHS-1-P (ng/ml)
WT	96 \pm 9	1759 \pm 97	18 \pm 2	1000 \pm 85	23 \pm 2	264 \pm 21	114 \pm 14
Sptlc2+/-	61 \pm 5*	2096 \pm 89*	32 \pm 5*	462 \pm 42*	31 \pm 1*	242 \pm 13	99 \pm 13

Value: mean \pm SD; n=4-5. SM, sphingomyelin; Cer, Ceramide; Sph, Sphingosine; S-1-P, Sphingosine-1-phosphate; DHS-1-P, Dihydroxyl-sphingosine-1-phosphate. *P<0.01.

Plasma lipid measurement after AAV-Cre injection

	AAV-lacZ	AAV-Cre	Change	P value
Cholesterol (mg/dl)	104 ± 7.5	116 ± 21		
Phospholipid (mg/dl)	198 ± 17	211 ± 27		
Triglyceride (mg/dl)	47 ± 8	50 ± 7		
Sphingomyelin (mg/dl)	28 ± 5	19 ± 2	-32%	P < 0.01

Liver lipid measurement in liver-specific Sptlc2 KO and WT mice (LC/MS/MS)

Mice	<u>SM</u>	<u>PC</u>	<u>Cer</u>	<u>Sph</u>	<u>S-1-P</u>	<u>DHS-1-P</u>
	(μg/mg liver)		(ng/mg liver)			
WT	0.58±0.02	17±1	134±10	2.2±0.3	0.10±0.01	0.12±0.02
Sptlc2KO	0.42±0.01*	15±2	82±9*	1.8±0.2	0.11±0.02	0.13±0.01

Value: mean±SD; n=4-5. SM, sphingomyelin; PC, phosphatidylcholine; Cer, ceramide; Sph, sphingosine; S-1-P, sphingosine-1-phosphate; DHS-1-P, dihydroxyl-sphingosine-1-phosphate. *P<0.01.

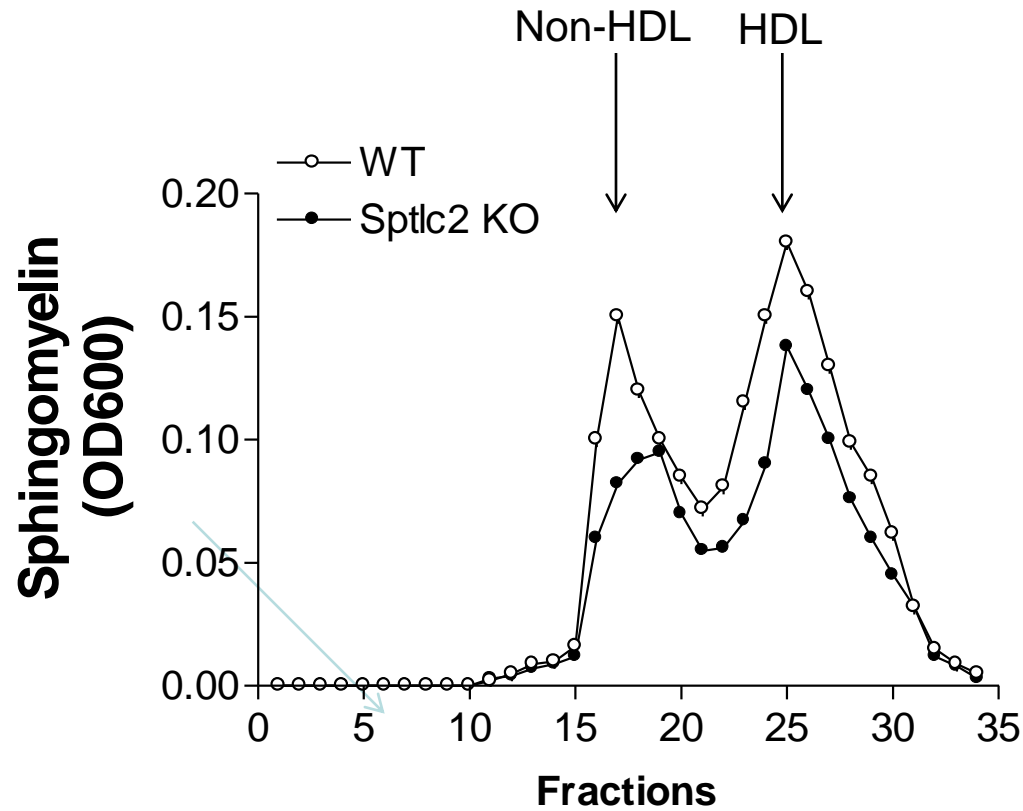
Plasma

AAV-LacZ

AAV-Cre



Plasma sphingomyelin distribution



Summary (2)

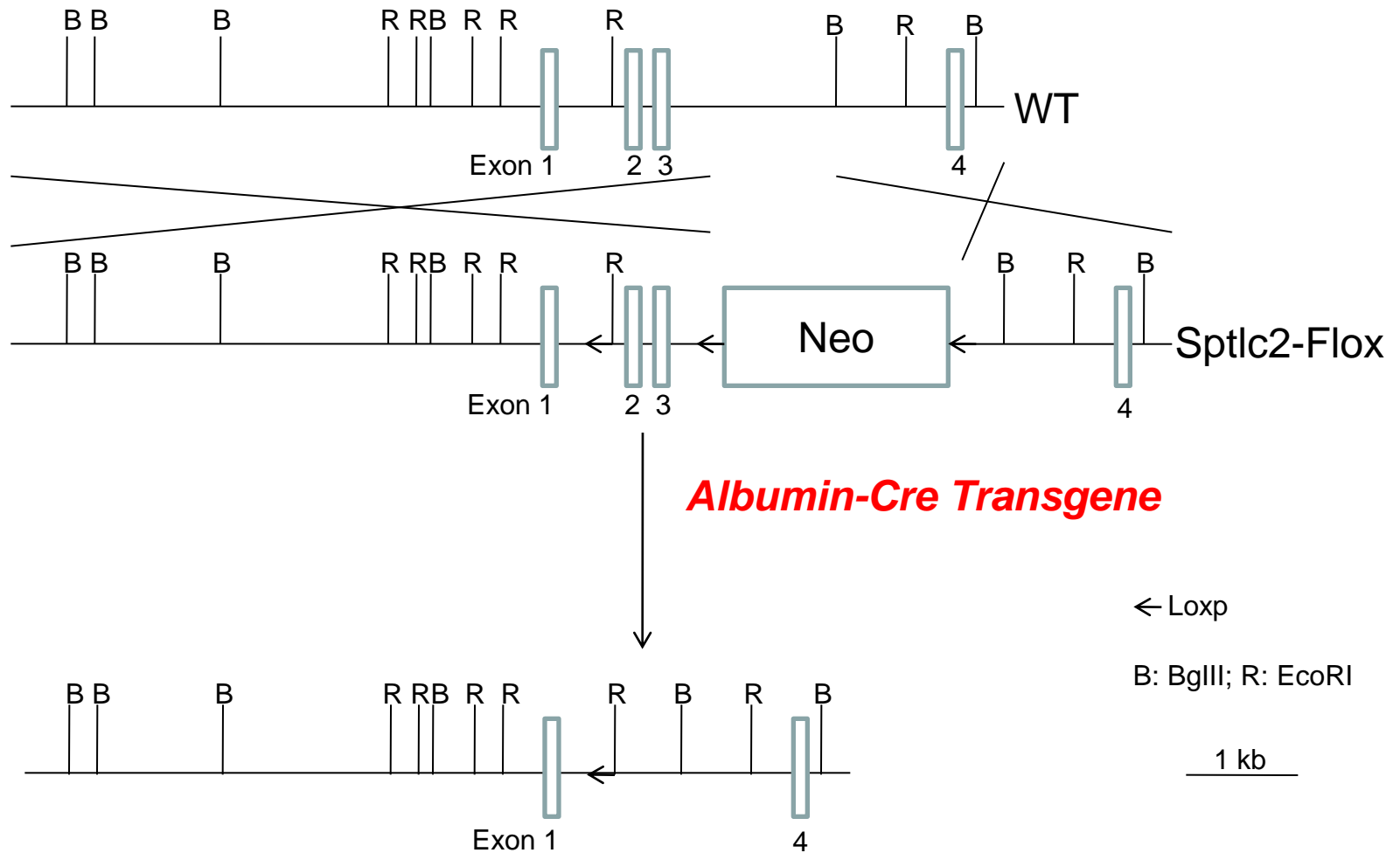
- As expected that AAV-Cre-mediated liver *Sptlc2* deficiency decreases plasma sphingomyelin levels.

Subtopics

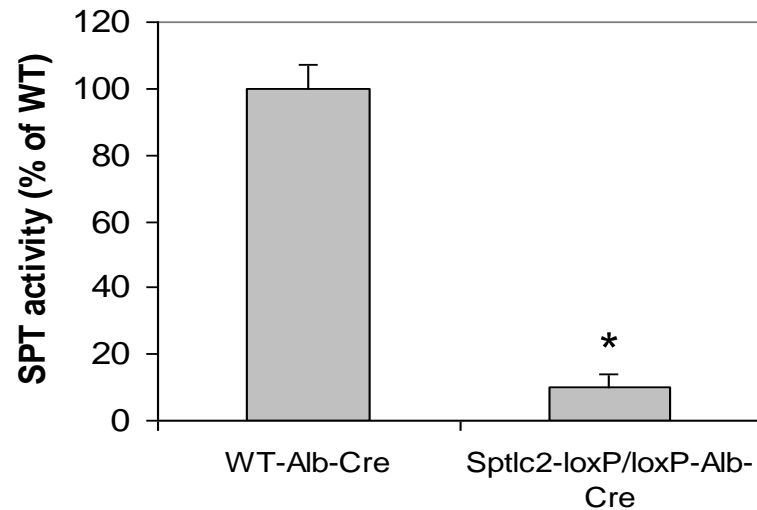
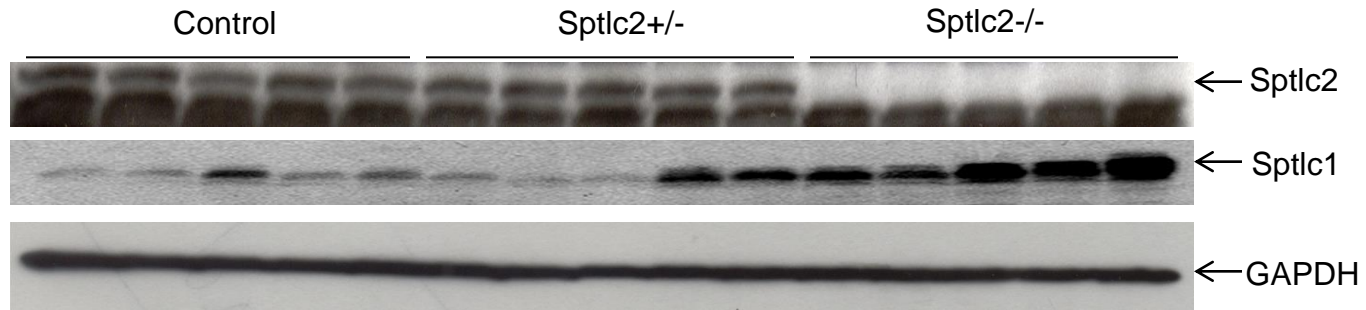
- ✓ The relevance of plasma membrane sphingolipid changes to the metabolic diseases.
- ✓ The effect of adenovirus associated virus (AAV)-Cre-mediated liver *Sptlc2* deficiency on lipoprotein metabolism.
- ✓ *The effect of albumin-Cre-mediated liver *Sptlc2* deficiency on hepatocyte polarity.*

Albumin-Cre/Sptlc2-loxp mice

Liver-specific Sptlc2 KO mouse preparation (for whole life)



Liver SPT activity and Sptlc1/Sptlc2 protein mass in liver-specific Sptlc2 KO mice

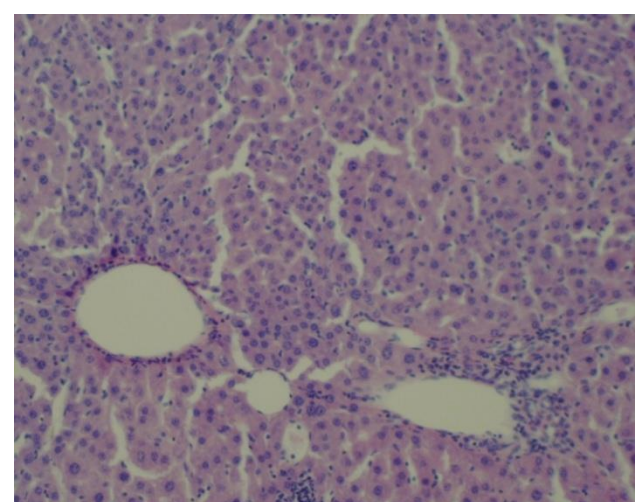
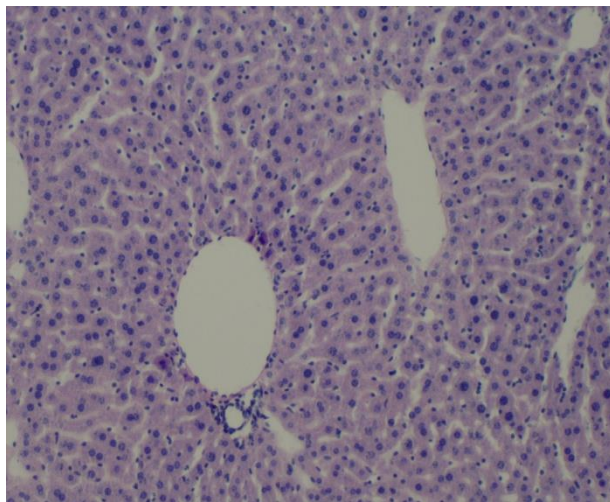
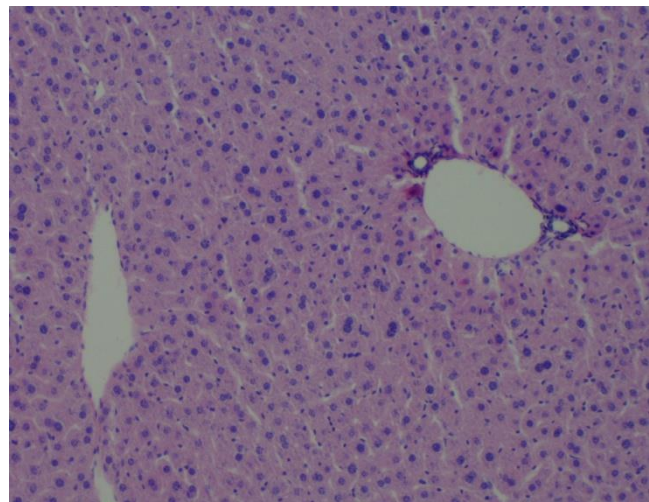


Bile ducts are few in Sptlc2 KO mouse liver (H&E staining, 2 months old)

Control

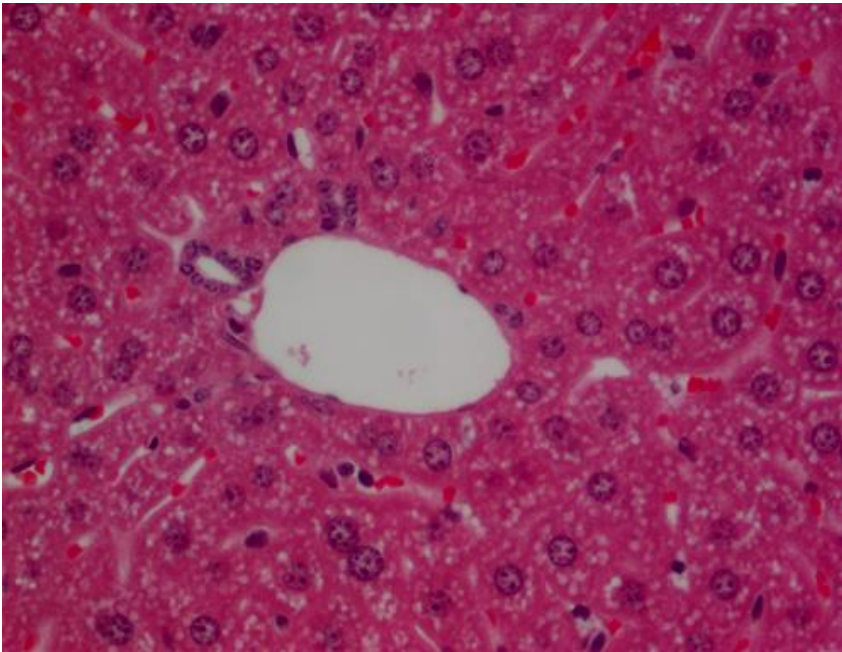
Hetero KO

Homo KO

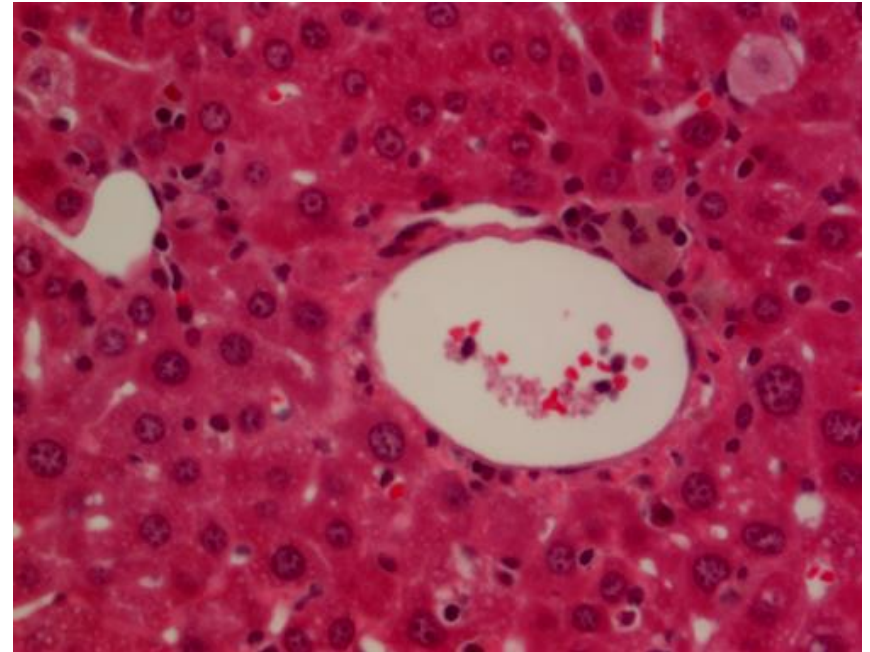


Bile ducts are few in *Sptlc2* KO mouse liver (H&E staining, 2 months old)

Control

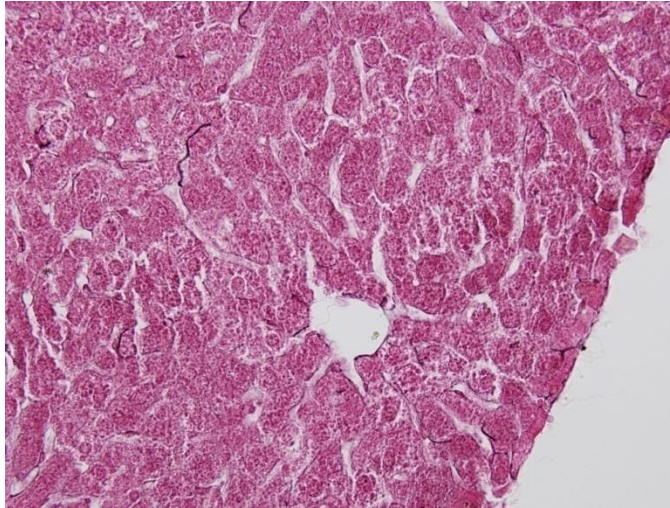


Sptlc2 KO

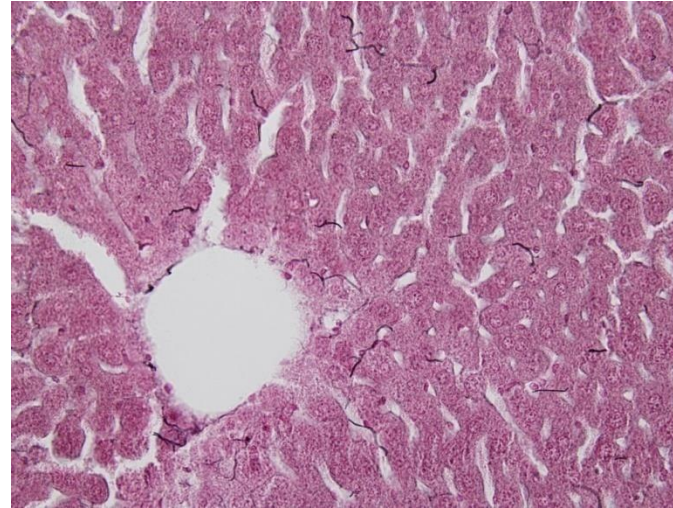


Reticular Fiber Staining for liver fibrosis (2 months old)

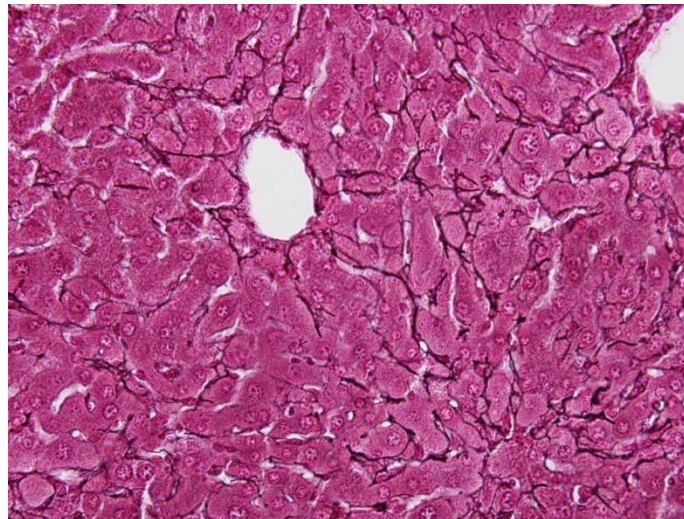
WT



Hetero KO



Homo KO



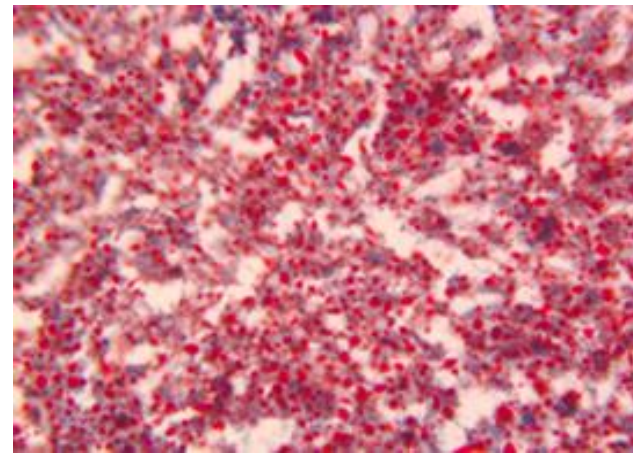
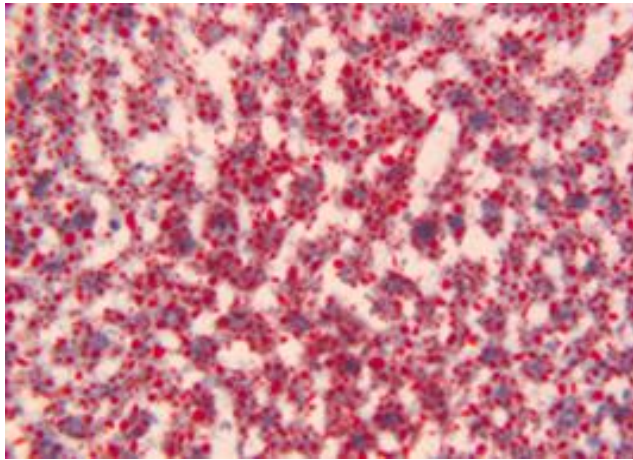
Silver staining (silver nitrate)

----Gordon-Sweets Method

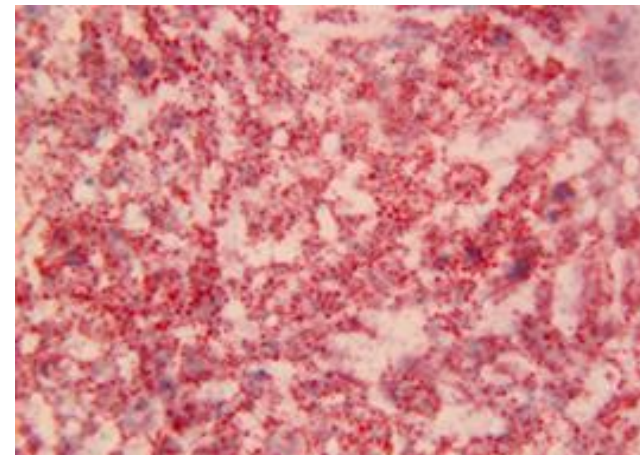
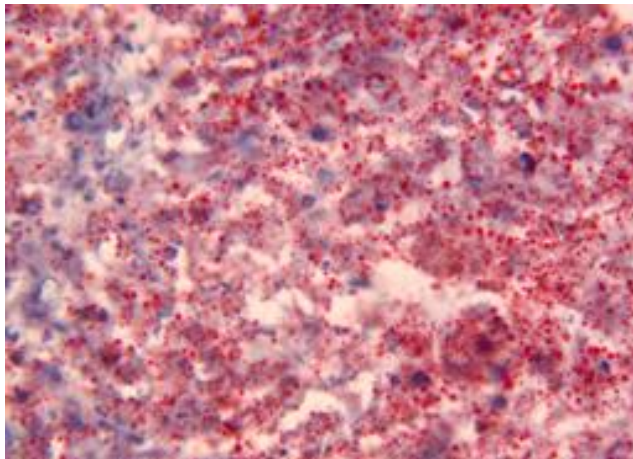
Sptlc2 deficiency does not cause lipid accumulation in the liver (2 months old)

Liver Oil red O staining

Control

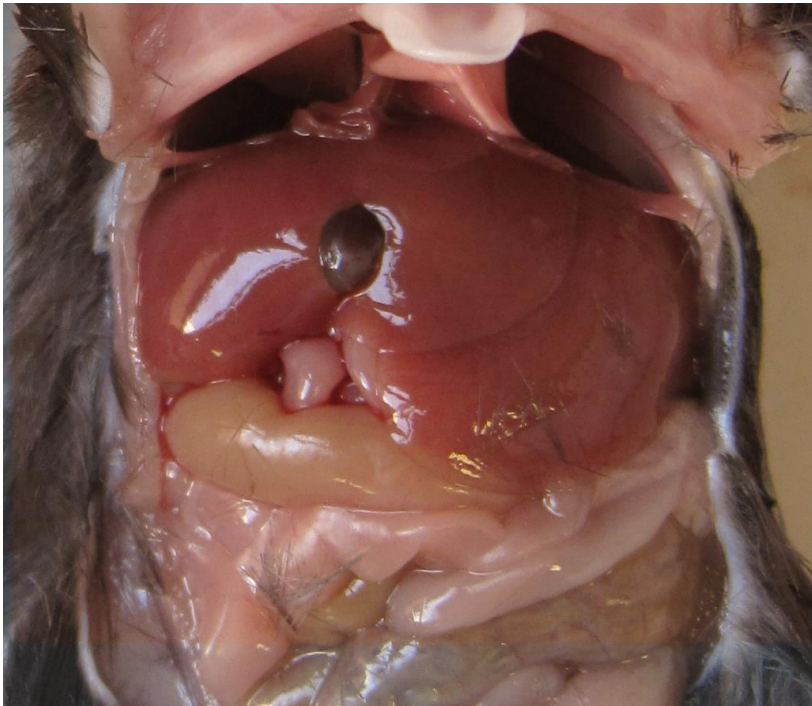


Sptlc2 KO

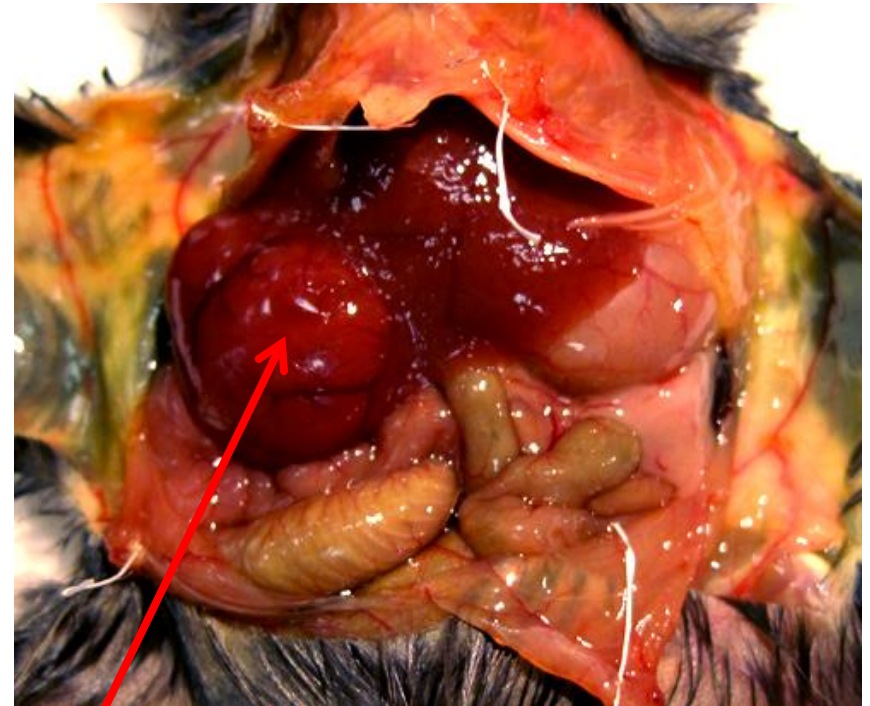


Sptlc2-deficiency-mediated liver tumor formation (8 months old)

Control



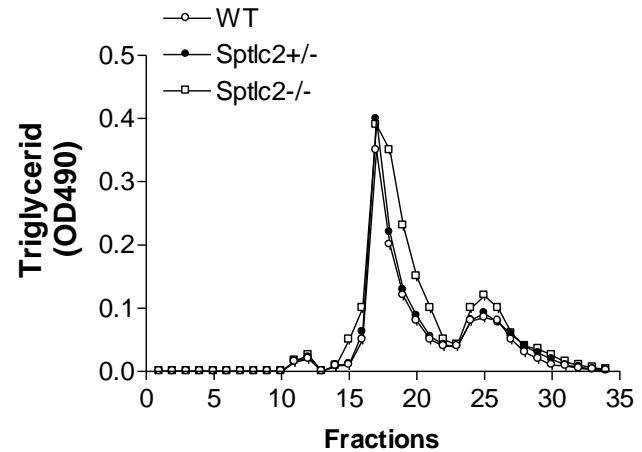
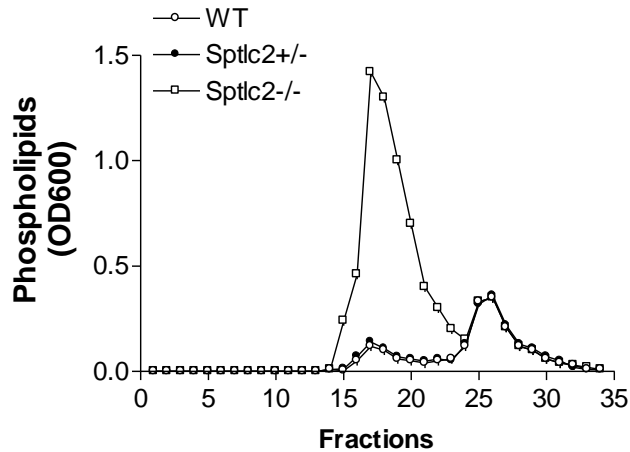
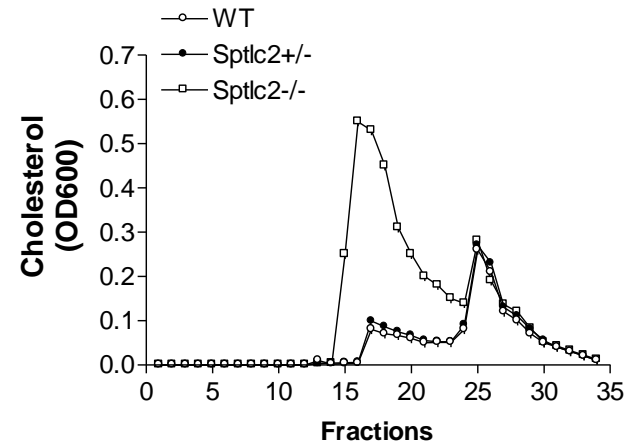
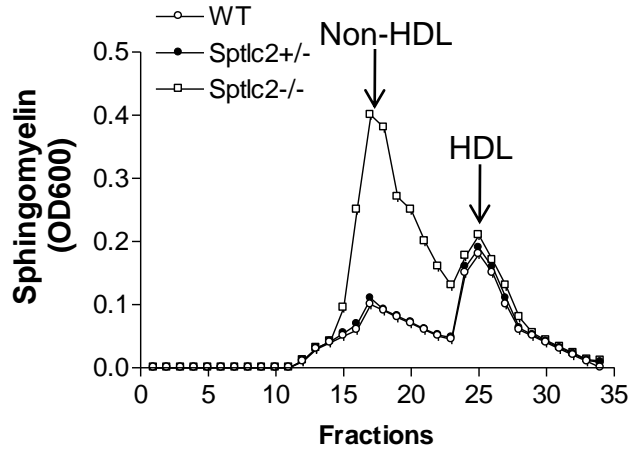
Sptlc2 KO



Plasma lipid measurement (2 months old)

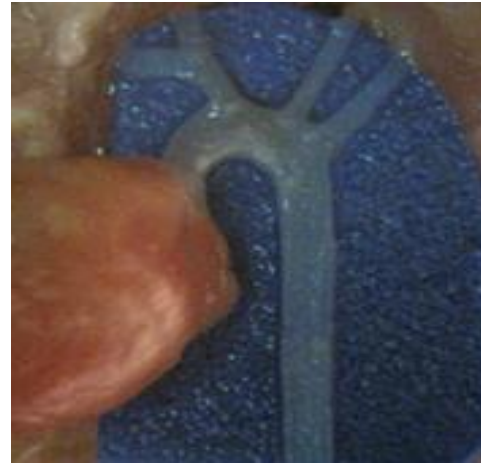
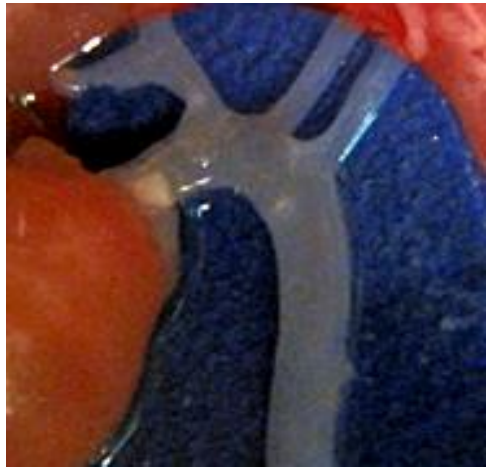
	WT	Homozygous KO	Heterozygous KO
Cholesterol (mg/dl)	80 ± 6	354 ± 59*	77 ± 14
Phospholipid (mg/dl)	217 ± 10	746 ± 19*	173 ± 44
Sphingomyelin (mg/dl)	14 ± 2	44 ± 10*	12 ± 2
Triglyceride (mg/dl)	56 ± 8	69 ± 10	58 ± 9

Plasma lipid distribution (FPLC) (chow diet)

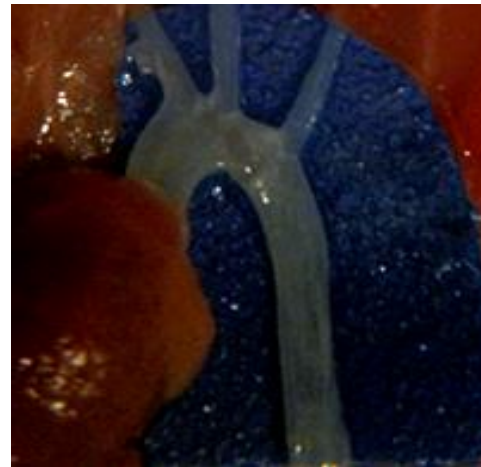
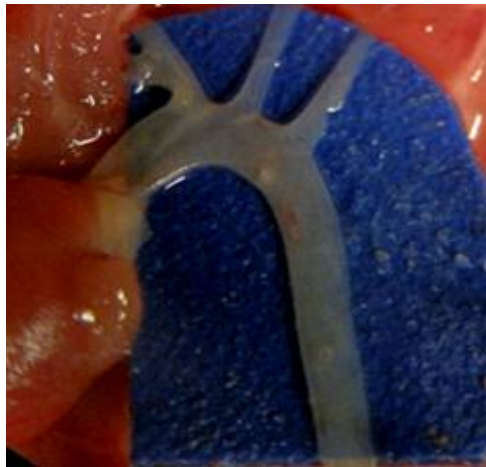


Higher cholesterol in liver Sptlc2 KO mice has no impact on atherosclerosis

WT



Sptlc2 KO

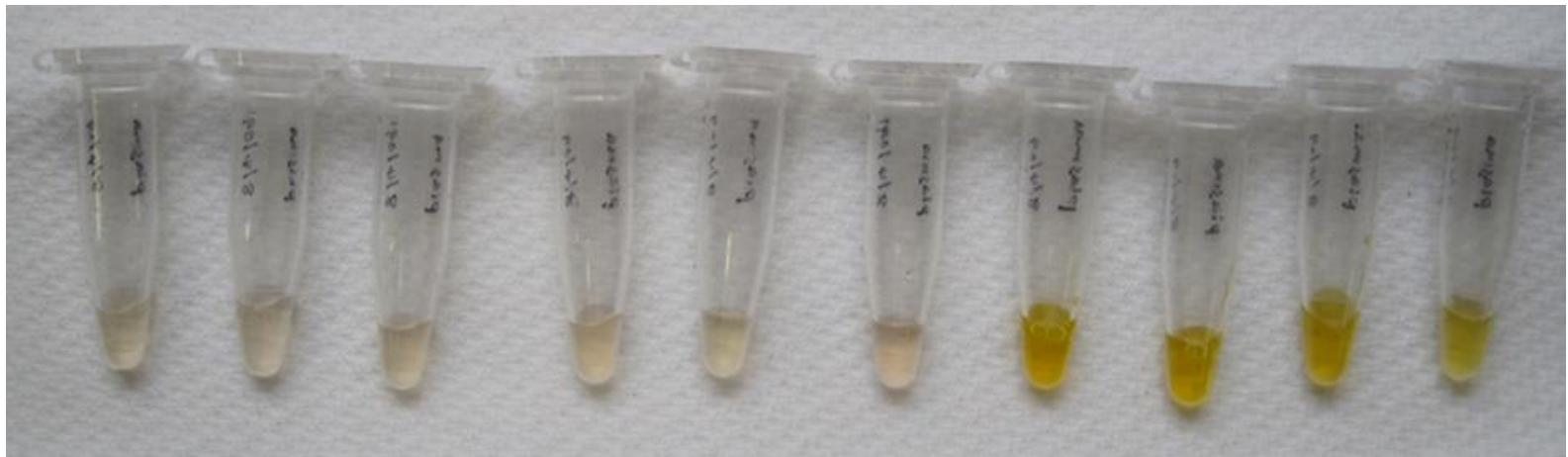


Liver Sptlc2 KO mice have severe jaundice

Control

Sptlc2^{+/-}

Sptlc2^{-/-}

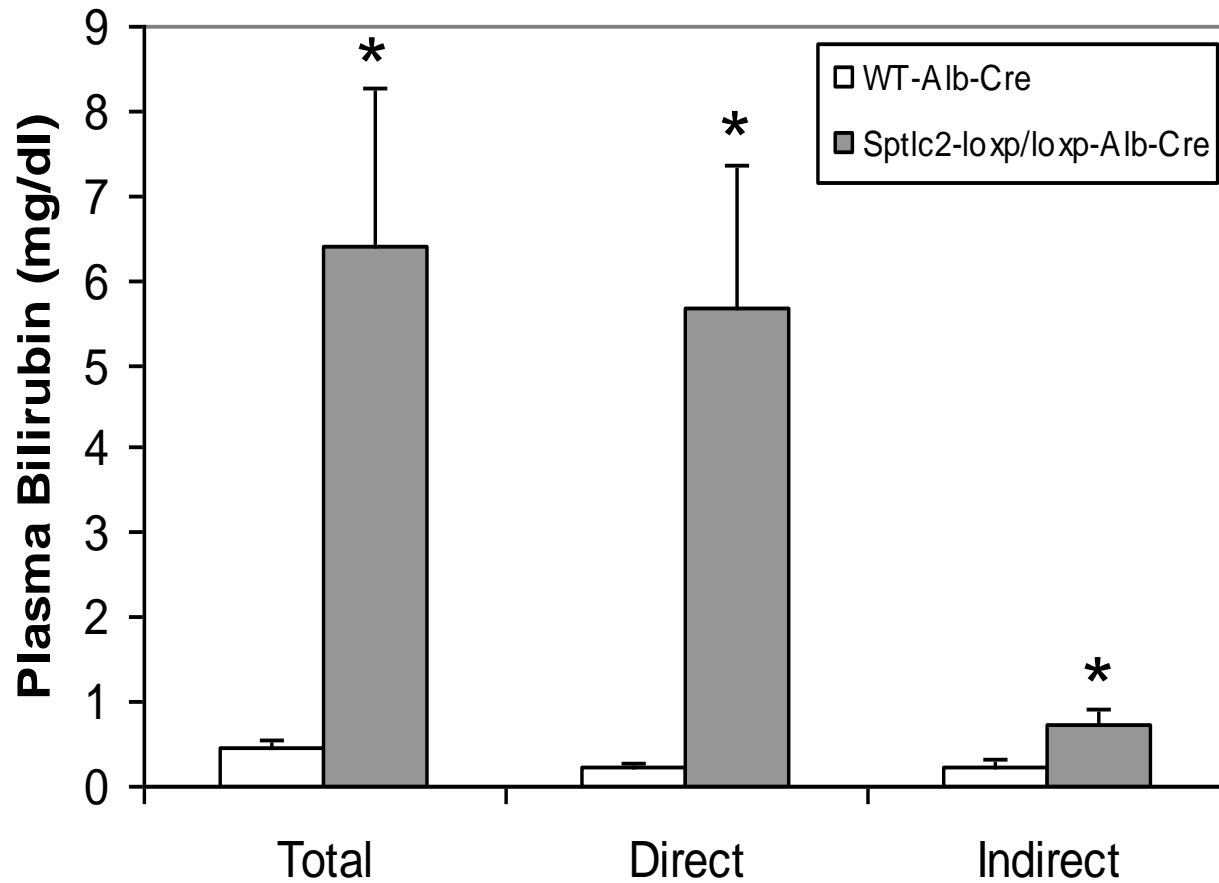


Cholestasis

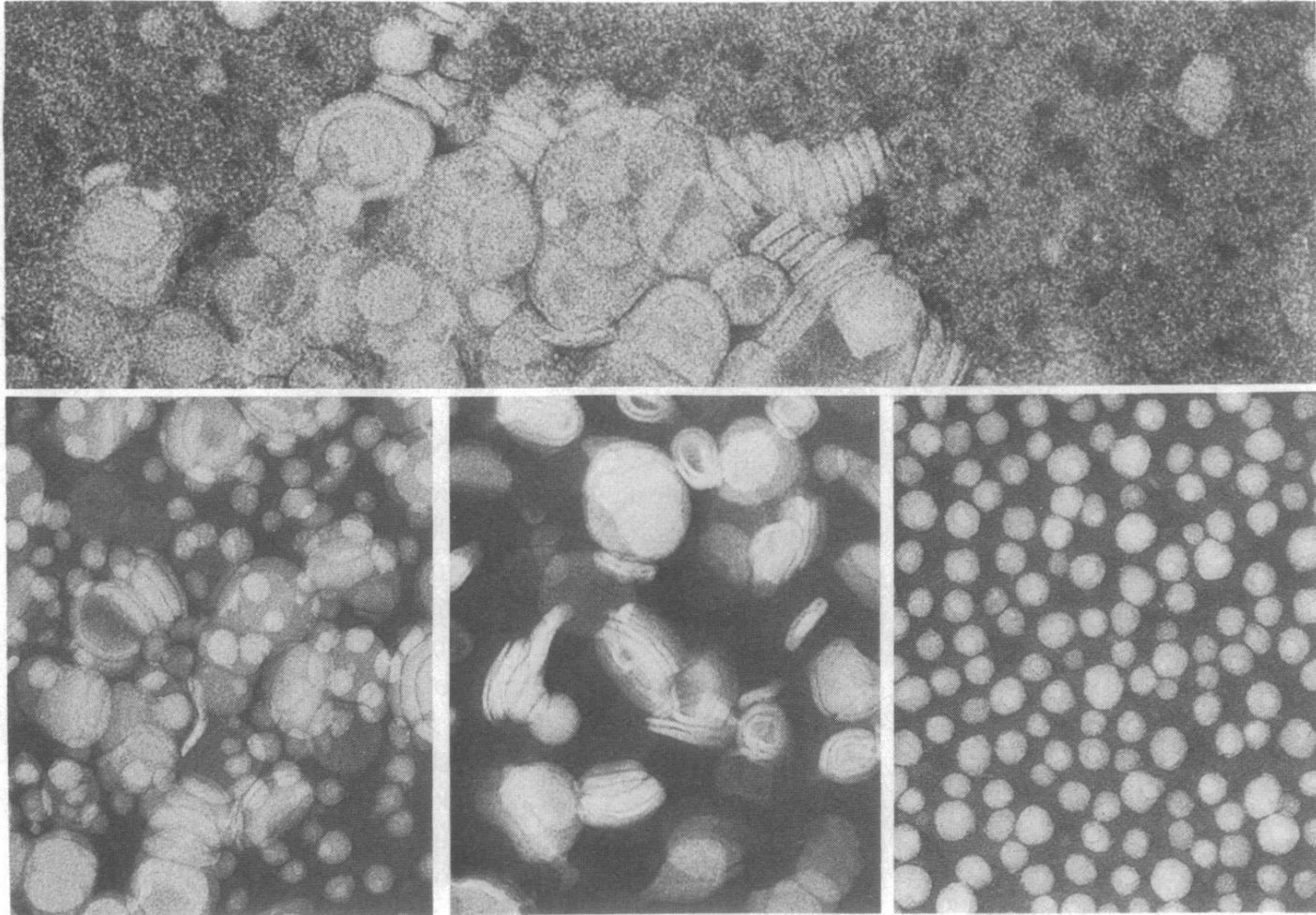
Cholestasis is a condition where bile cannot flow from the liver to the duodenum.

1. Gallstone or malignance
2. Genetic defects

Plasma Bilirubin

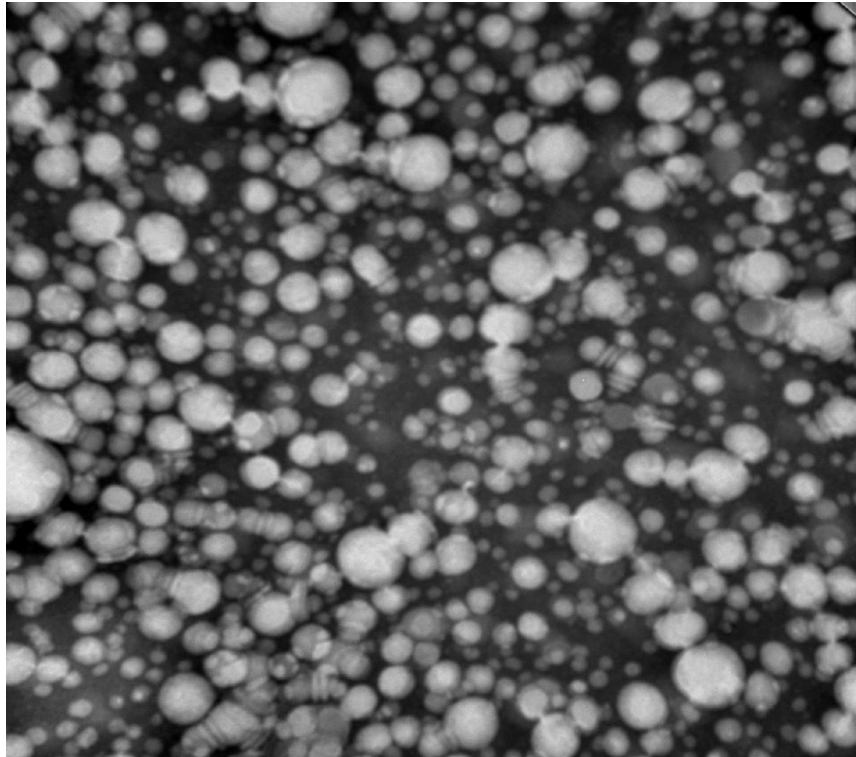


EM picture for lipoprotein-X



Accumulation of lipoprotein-X in Sptlc2 KO mouse plasma

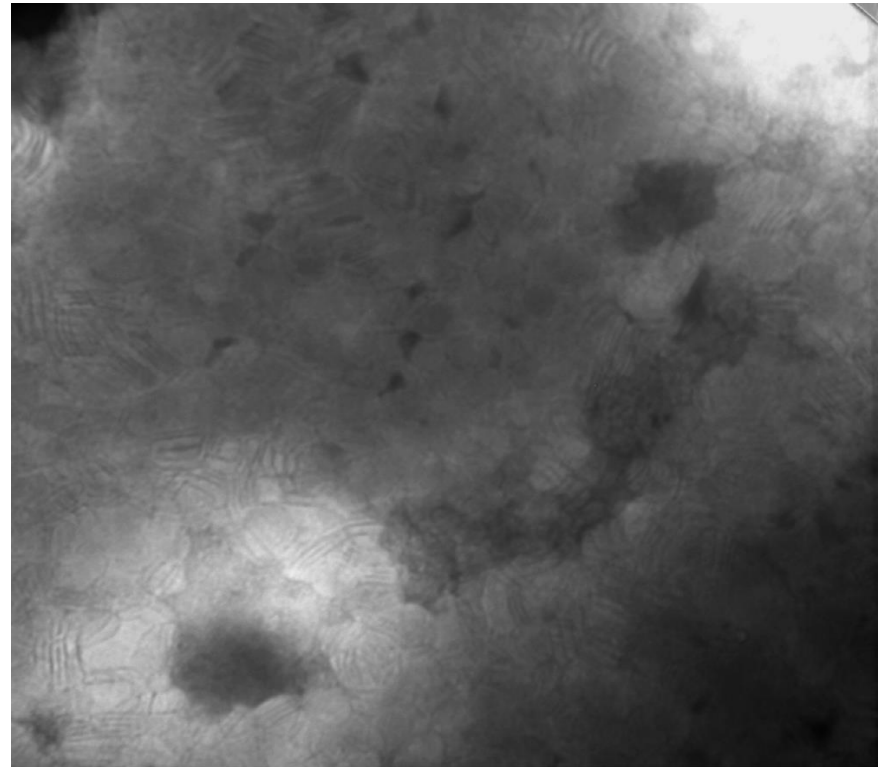
Control



Wei Quan.003.tif
WT-1
Print Mag: 170000x @ 7.0 in
TEM Mode: Imaging
Microscopist: Wei Quan

100 nm
HV=80kV
Direct Mag: 150000x
Institute for Basic Research

Sptlc2 KO

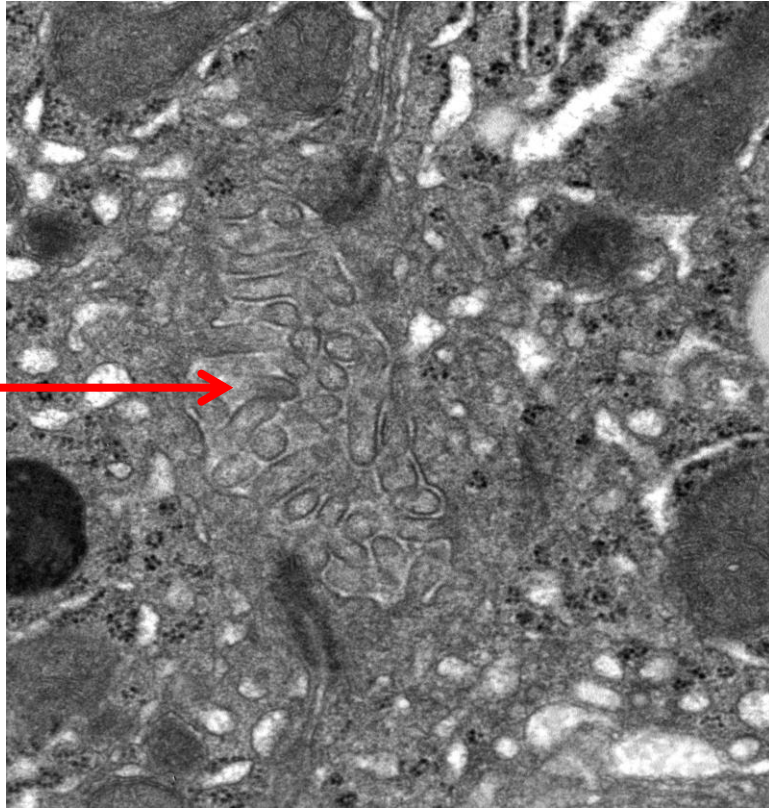


Wei Quan.033.tif
KO-1
50
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Microscopist: Wei Quan

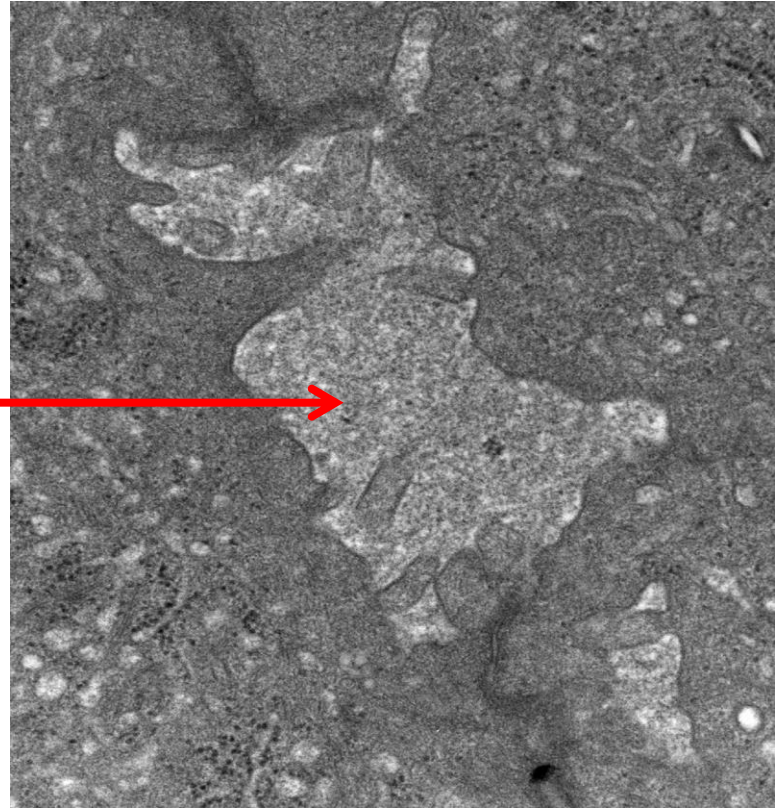
100 nm
HV=80kV
Direct Mag: 200000x
Institute for Basic Research

Sptlc2 KO mice have much less microvilli in bile canaliculus

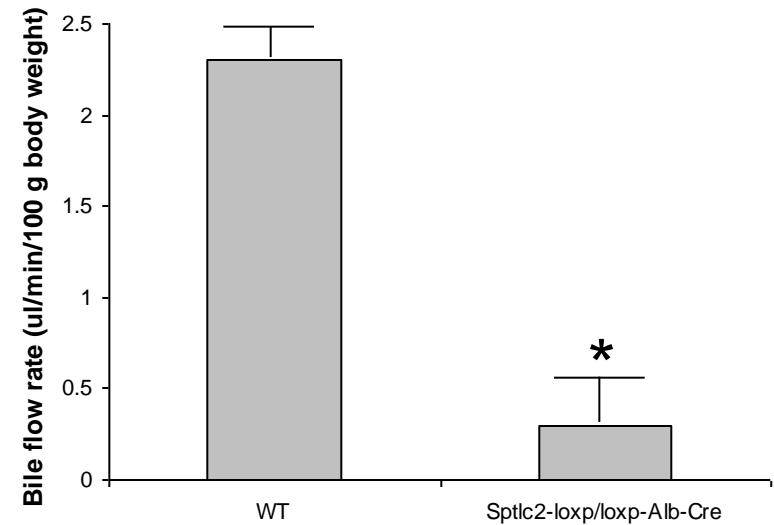
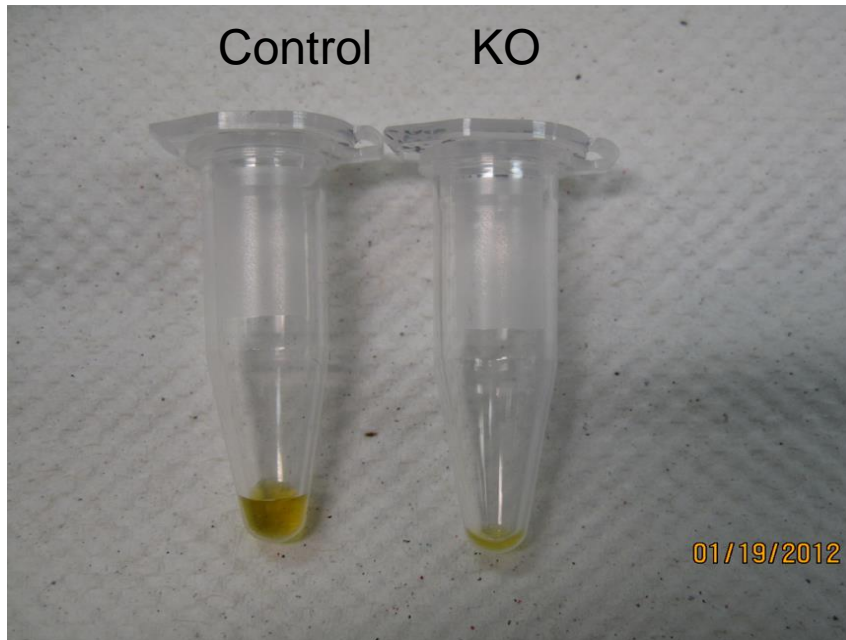
Control



Sptlc2 KO

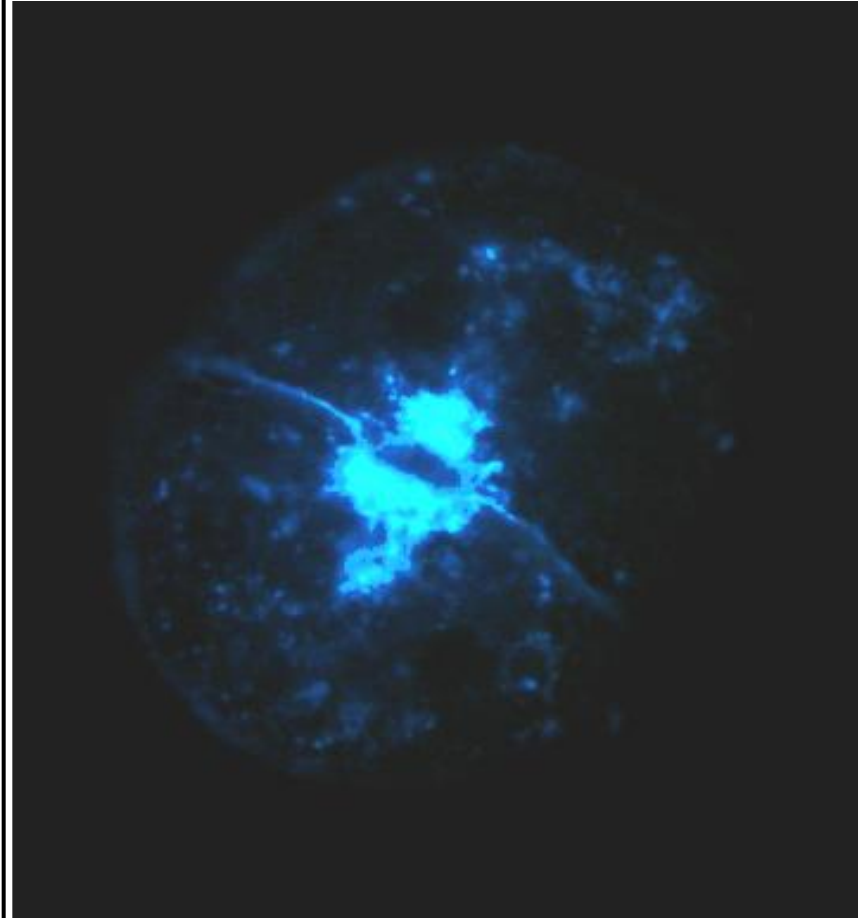
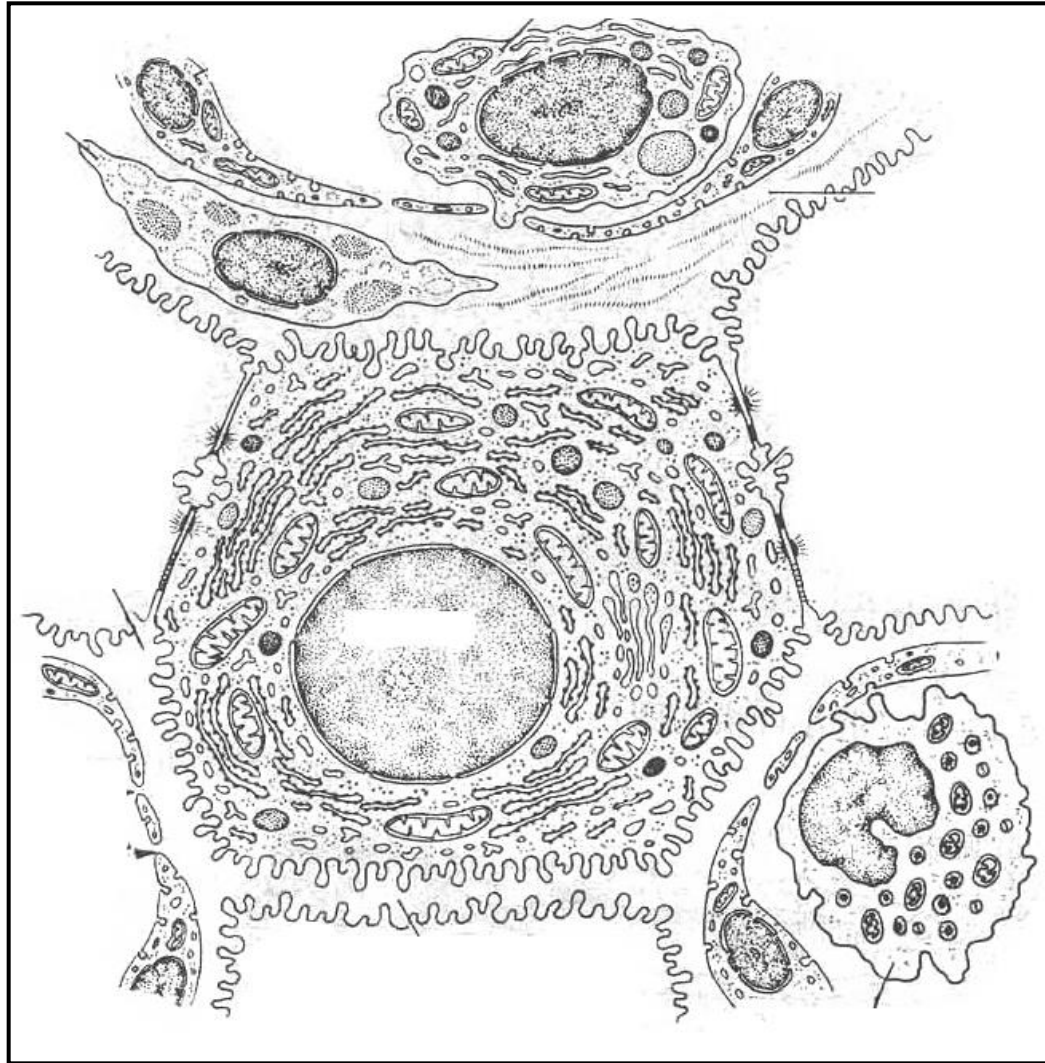


Liver Sptlc2 deficiency dramatically decreases flow rate of bile

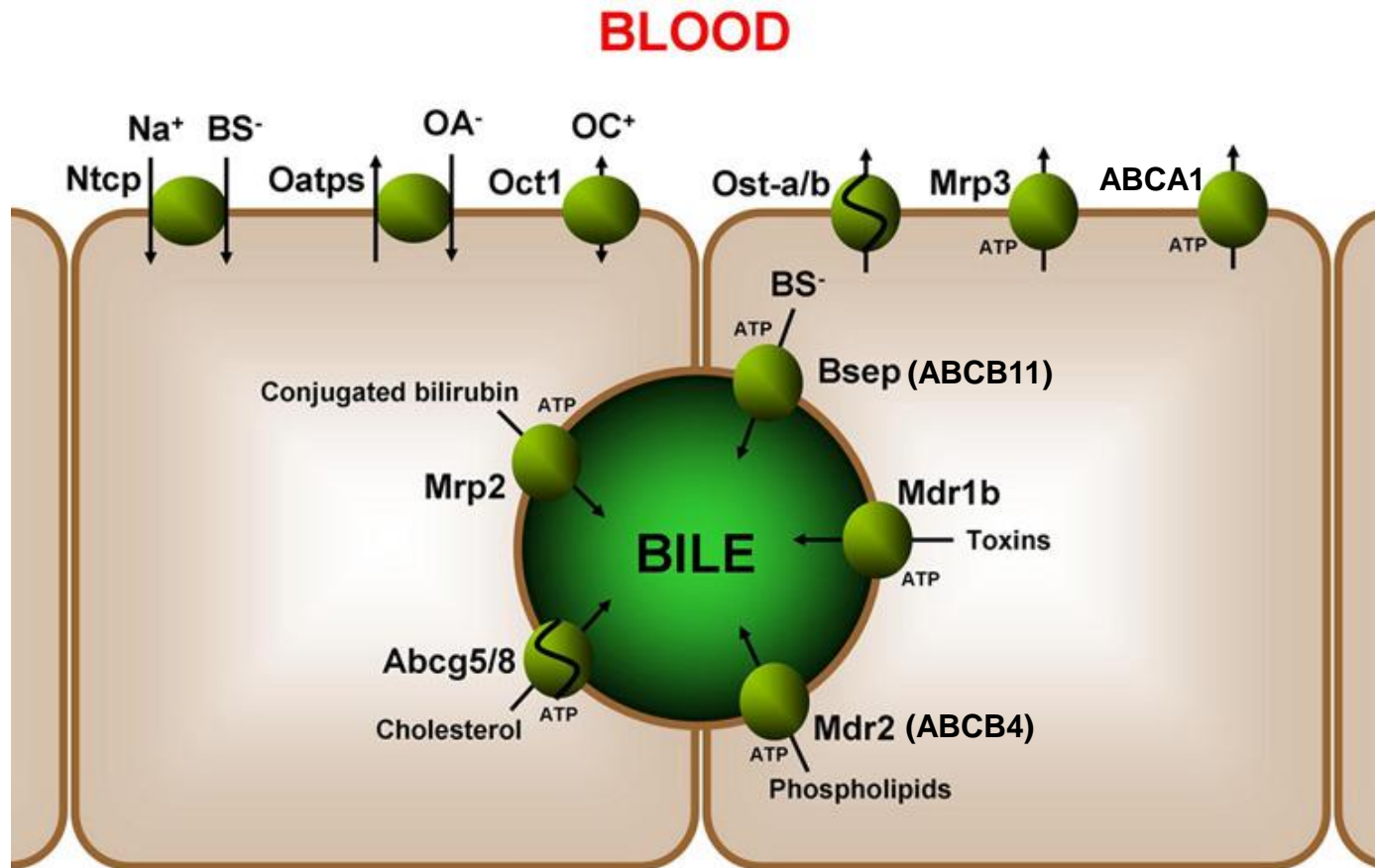


Collect bile for 1 hr

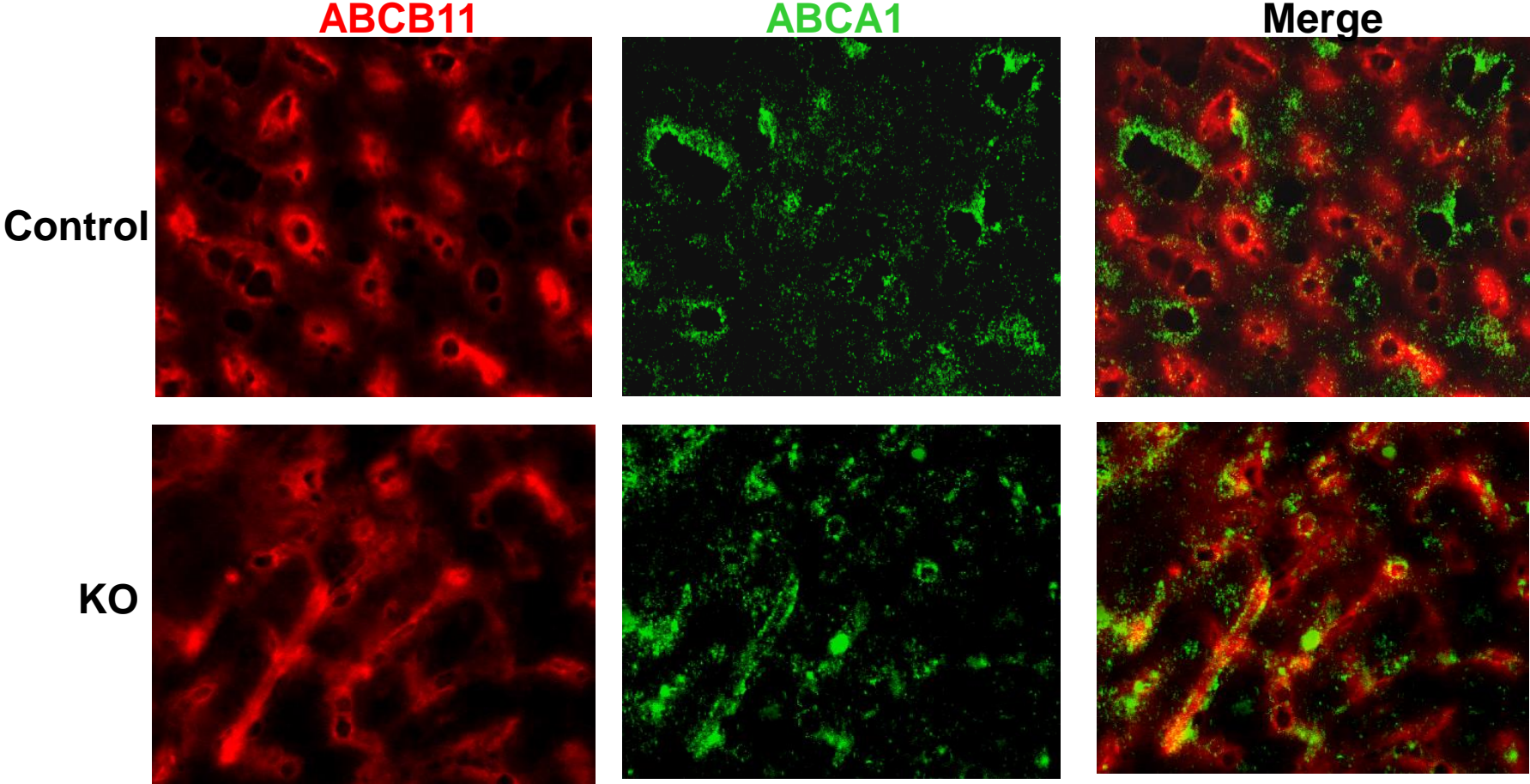
Hepatocyte polarity



Bile production

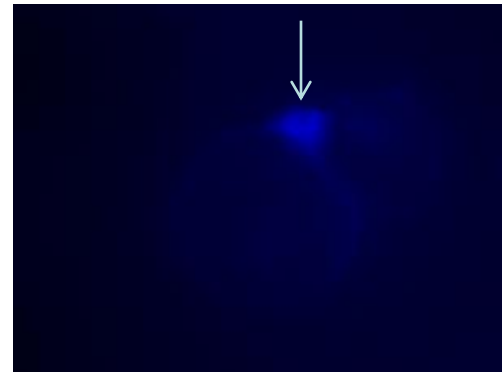
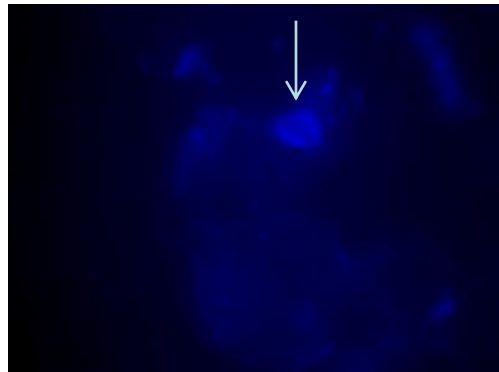


Sptlc2 KO mice lose hepatocyte polarity

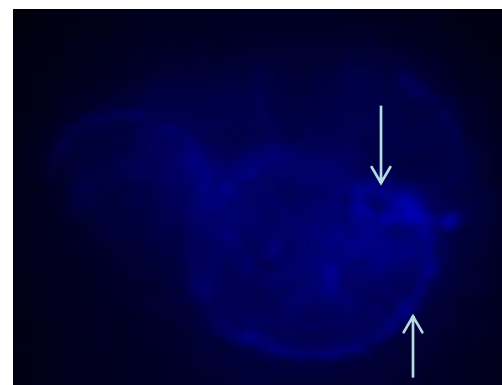
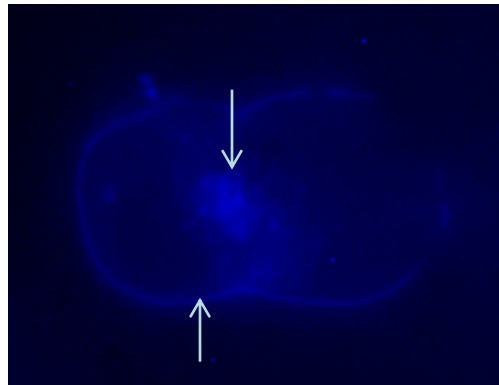


Sptlc2 KO mice lose hepatocyte polarity (Filipin staining)

Control



Sptlc2 KO

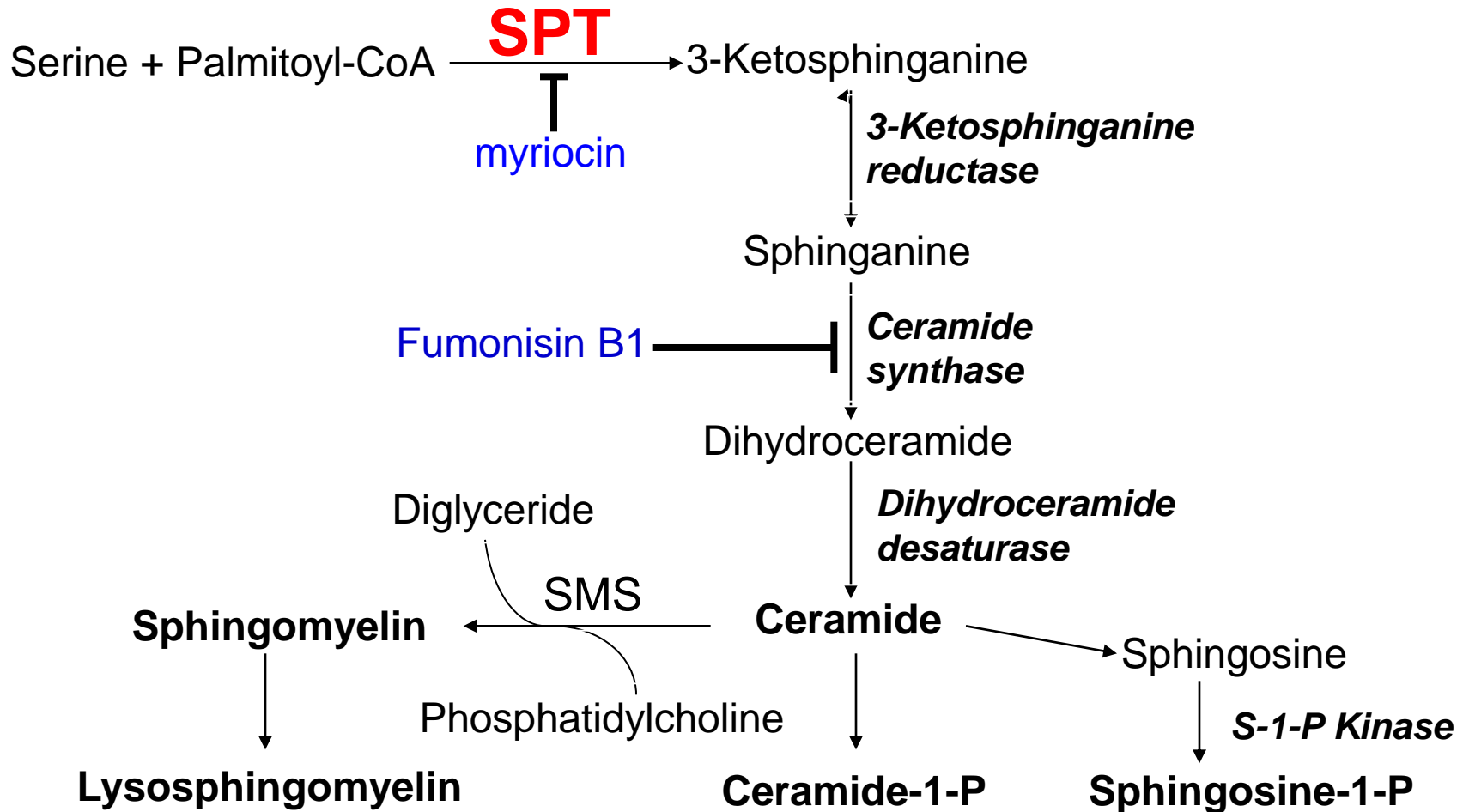


Comparison between liver LKB1 KO and liver Sptlc2 KO mice

<u>LKB1-Flox/albumin-Cre</u> (Woods et al. BJ 2011)	<u>Sptlc2-Flox/albumin-Cre</u>
LpX accumulation in the blood	LpX accumulation in the blood
Defective targeting of ABCB11	Defective targeting of ABCB11
Jaundice	Jaundice
Abnormal bile canaliculus	Abnormal bile canaliculus
Died at age 30 days	Normal life span with liver tumors

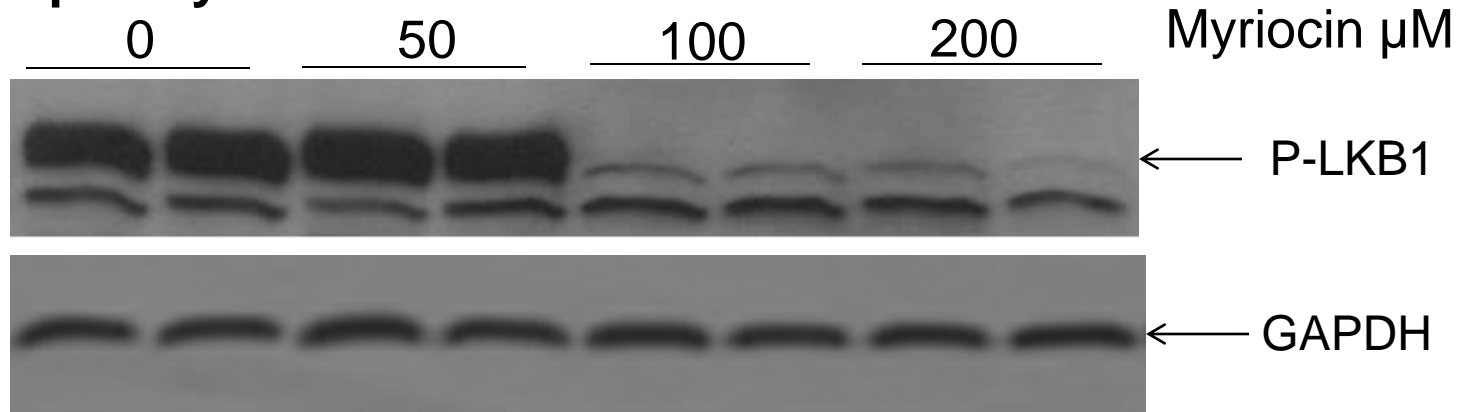
Above abnormalities do not exist in AdV-Cre-mediated LKB1 liver deficiency (Shaw et al. Science, 2005) and AAV-Cre-mediated Sptlc2 liver deficiency (Li et al. JBC, 2009).

Sphingomyelin Biosynthesis

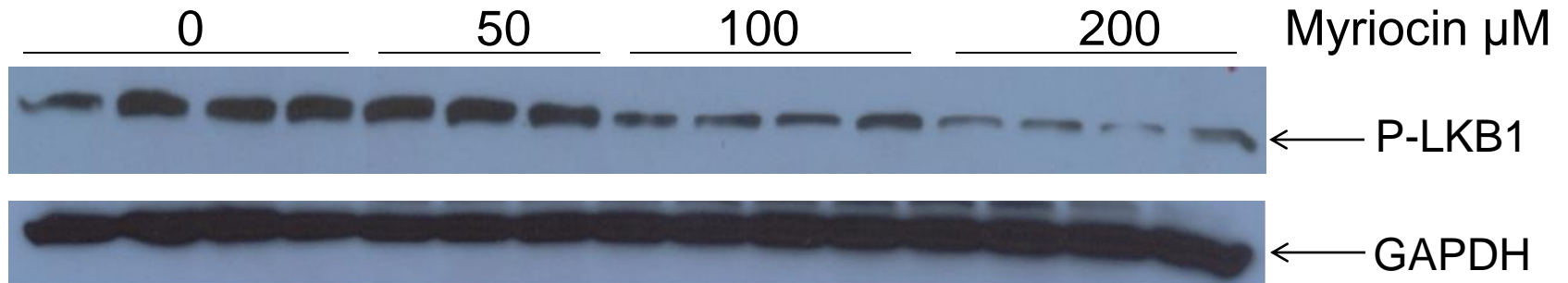


Myriocin treatment attenuates LKB1 activation

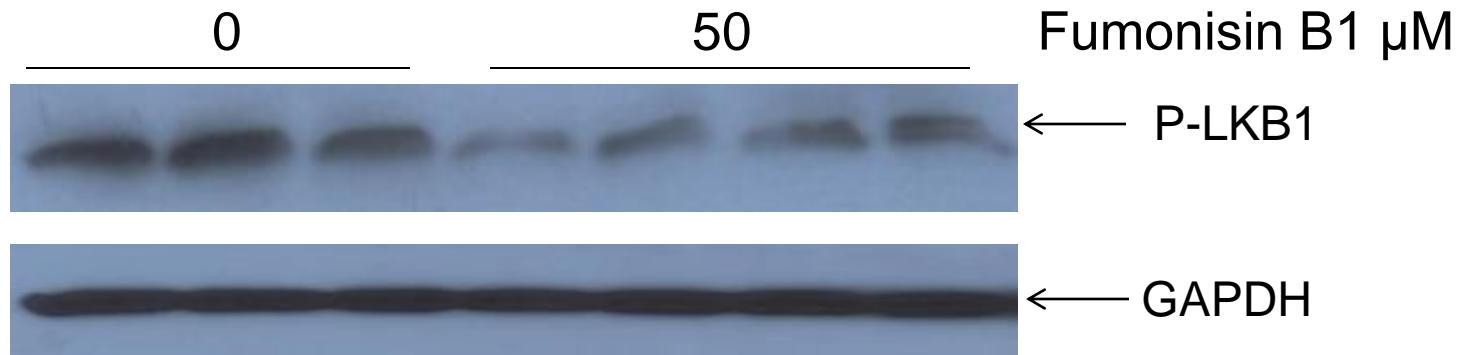
Primary hepatocytes



Huh7 Cells

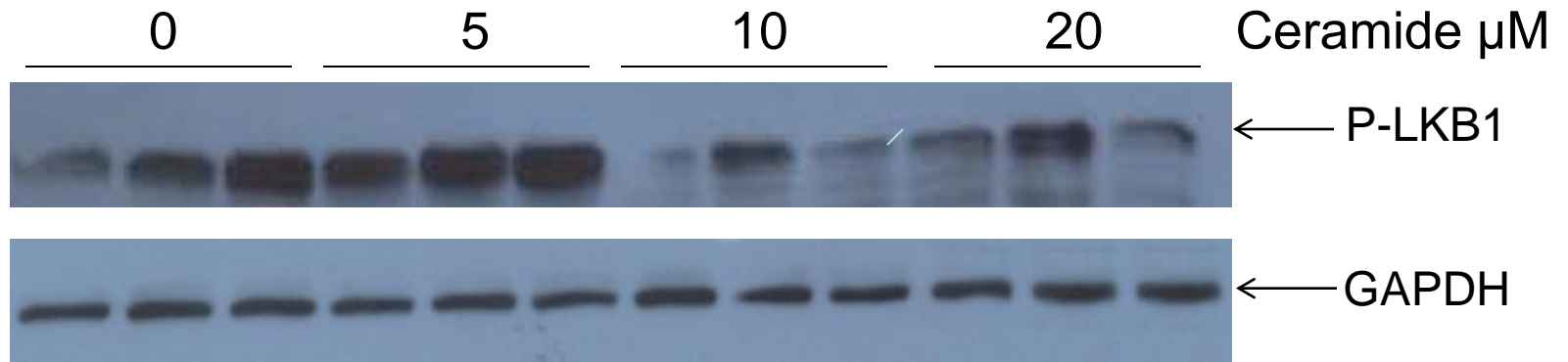


Fumonisin B1 treatment attenuates LKB1 activation in primary hepatocytes

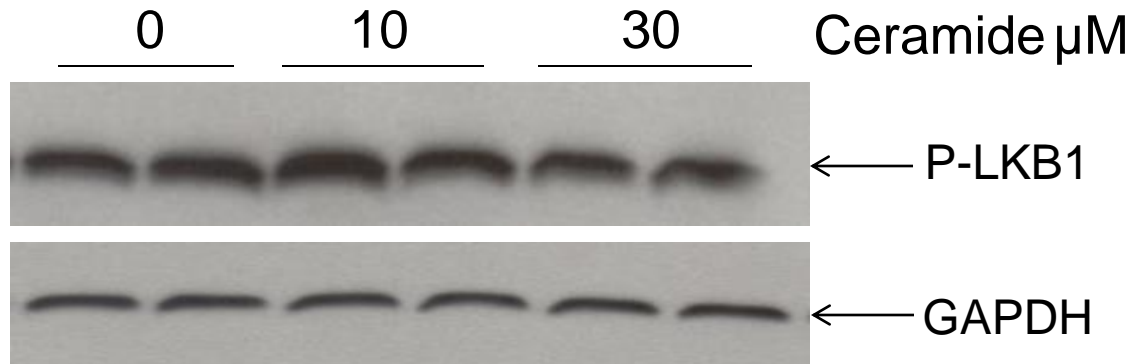


Ceramide treatment attenuates LKB1 activation in primary hepatocytes

Primary hepatocytes

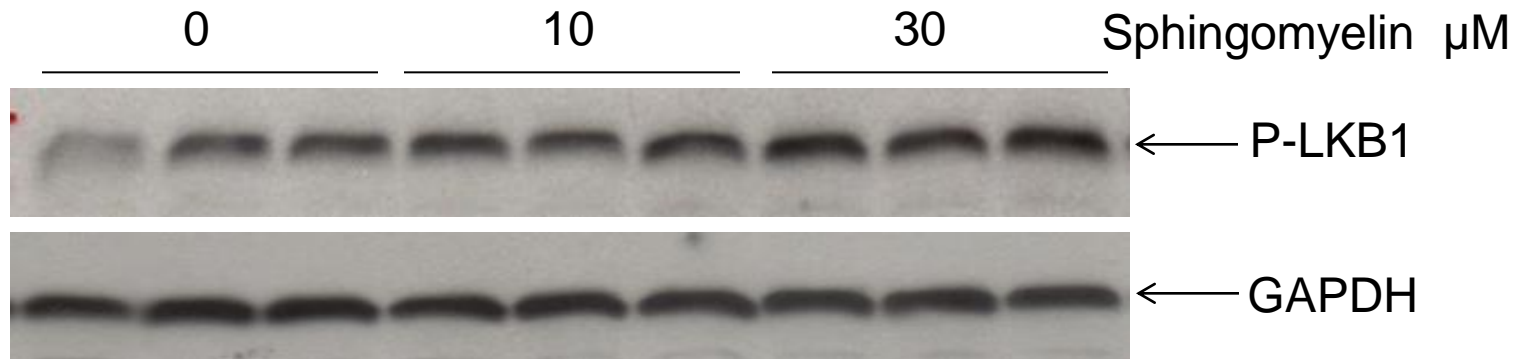


Huh7 Cells



Sphingomyelin treatment promotes LKB1 activation

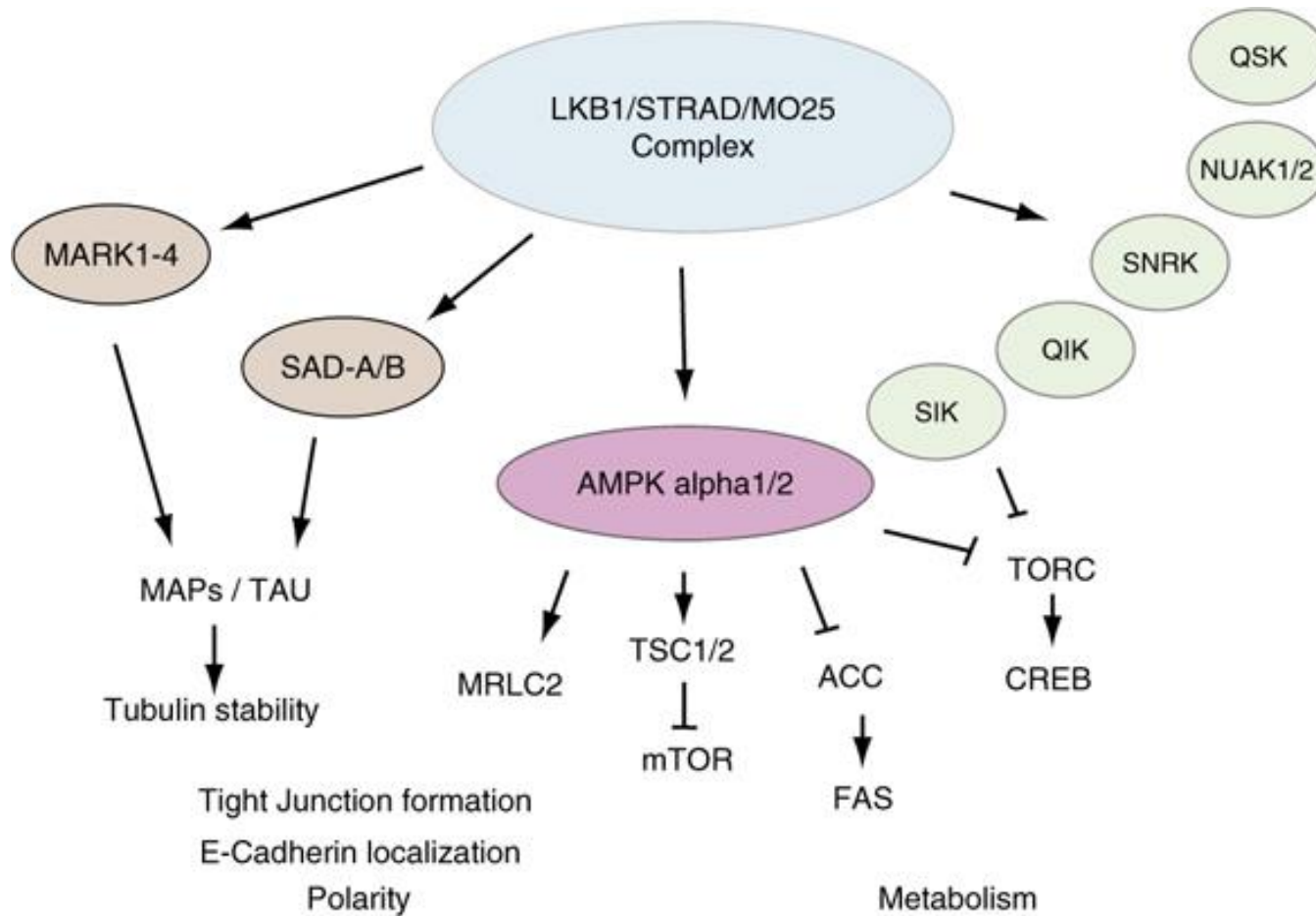
Huh7 Cells



Summary (3)

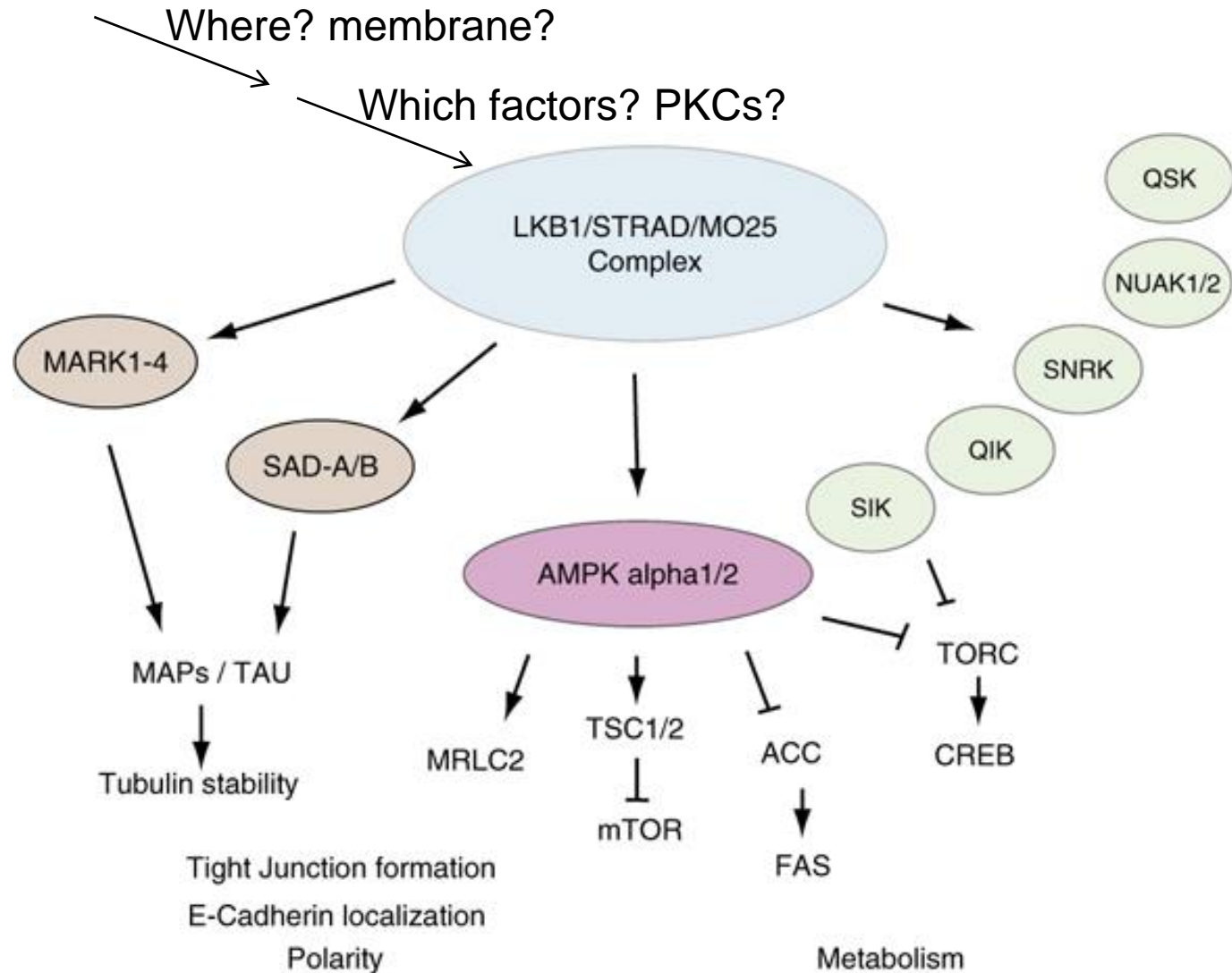
- In the early stage of life, Sptlc2 is involved in the formation of liver cell polarity.
- In adult livers, cell polarity formation would have progressed normally through development. Sptlc2 deficiency in adulthood do not influence cell polarity.
- Both LKB1 and Sptlc2 deficiencies in the liver have similar phenotype, in terms of cell polarity.
- Do LKB1 and Sptlc2 have a linkage?

LKB1: cell polarity and metabolism

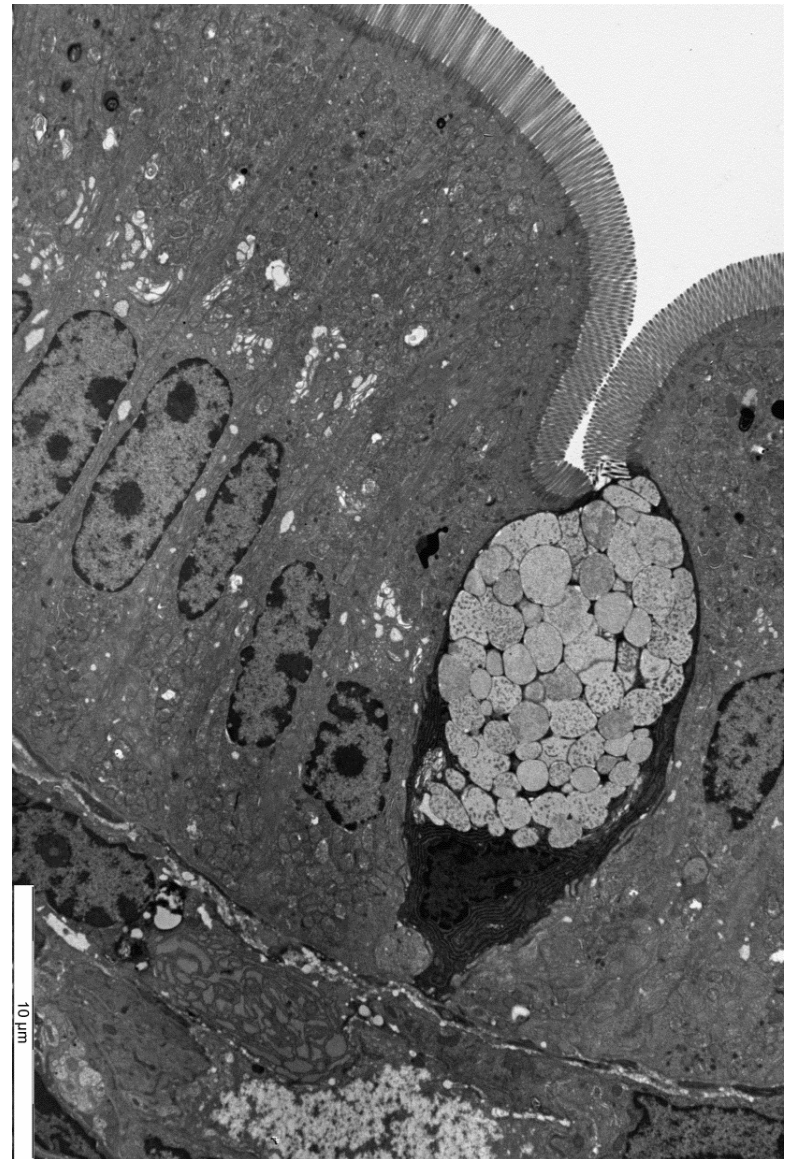
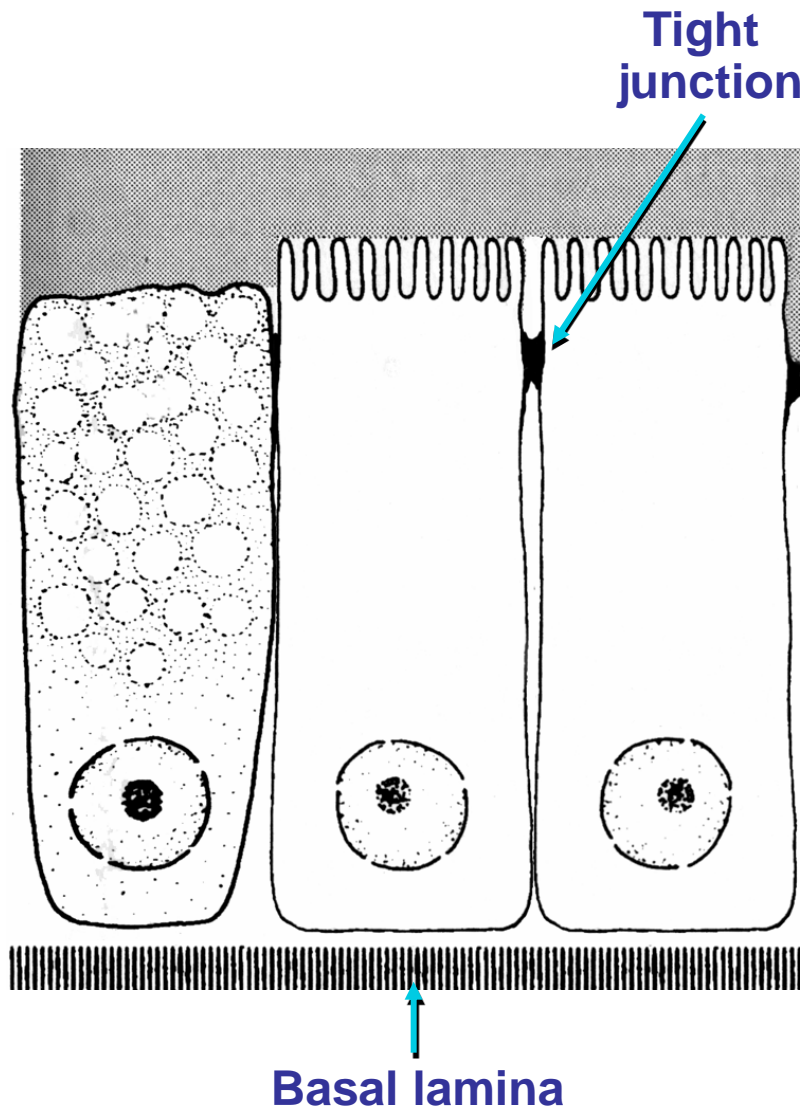


Questions:

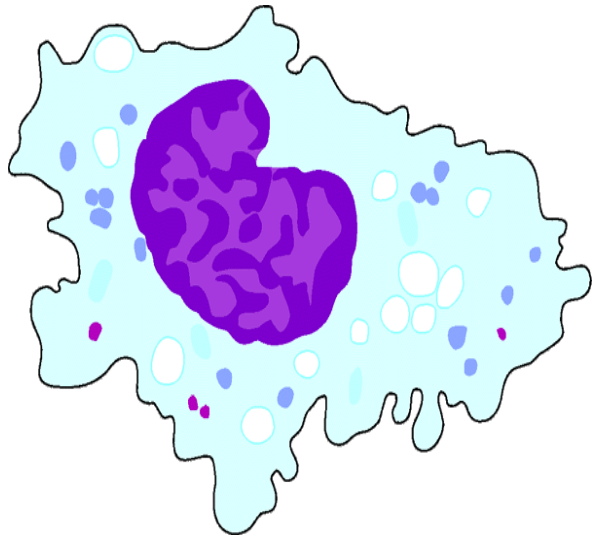
Sphingolipid *de novo* synthesis



Enterocyte polarity



Macrophage polarity





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