



Graduate
Programme
Lille



**Precision
health**



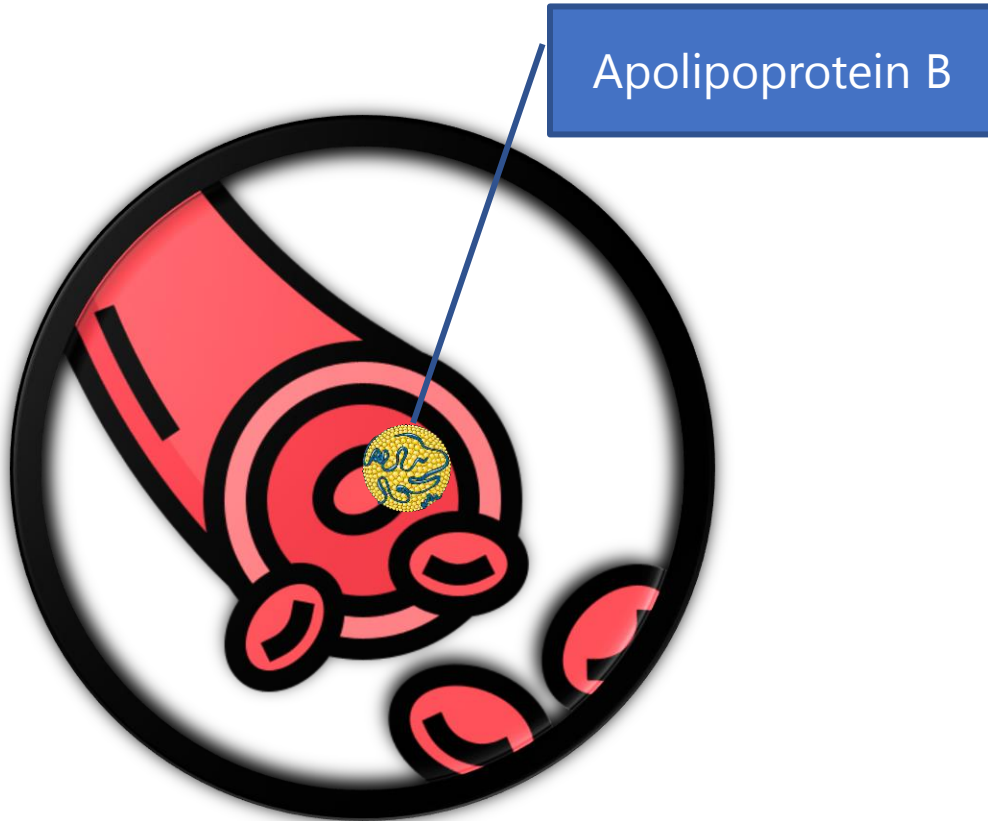
SMLRI: A NEW PROTEIN INVOLVED IN THE VLDL FORMATION WHERE AND HOW?

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Laboratory : Department of Pediatrics, UMCG, Groningen (NL)

HYPOCHOLESTEROLEMIA



Frequency : 1-2/1000¹

Clinical findings : Decreased CV risk

Hepatic steatosis

Molecular aetiology for 40% of those patients :

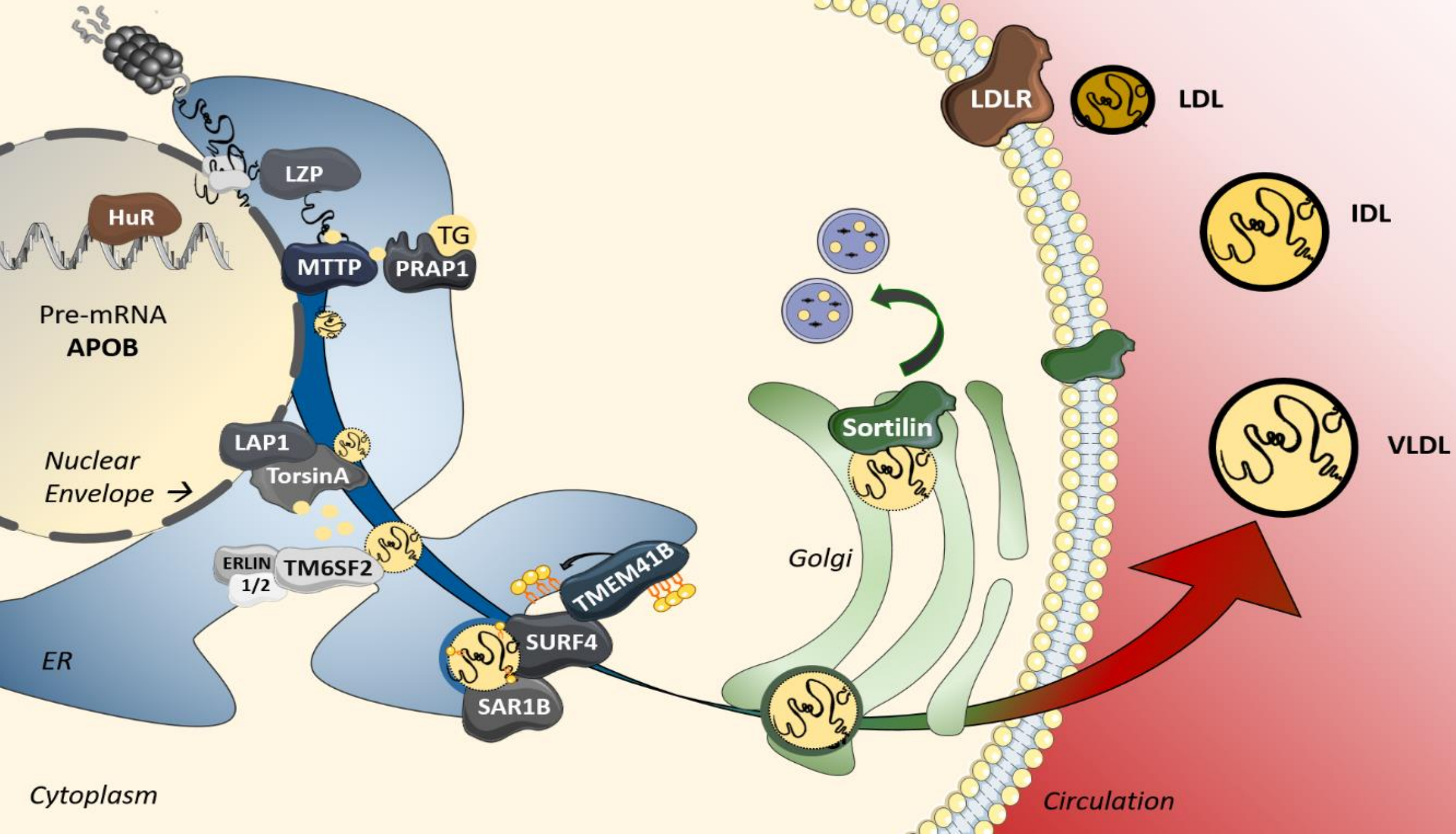
- *APOB* (Familial HypoBetaLipoproteinemia I),
- *MTTP* (Abetalipoproteinemia)
- *ANGPTL3* (Familial Hypolipidemia)

For the other patients (60-70%)

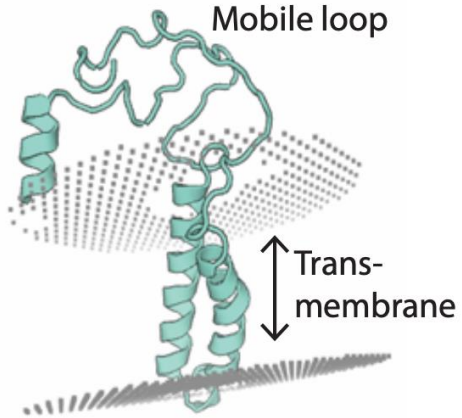
➔ Polygenic ?

➔ Alteration in other gene ?

1. Burnett JR, Hooper AJ, Hegele RA. APOB-Related Familial Hypobetalipoproteinemia. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJ, Mirzaa G, et al., editors. GeneReviews® [Internet].

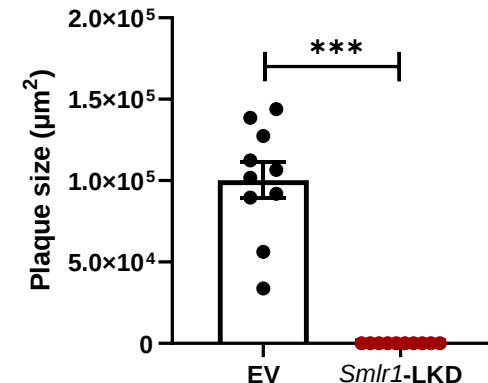
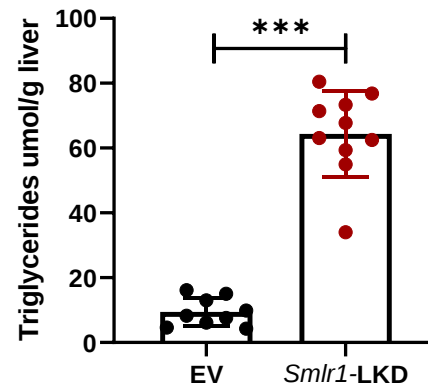
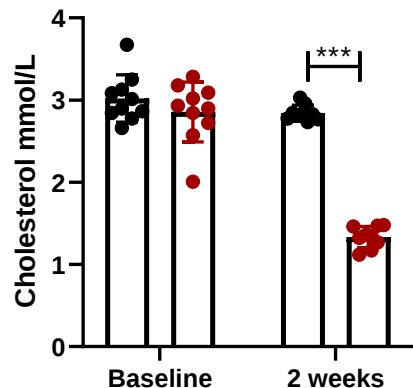


SMall Leucin-Rich protein I



- Identified thanks to co-expression profiling
- Size : 12.3 kDa, 107 amino acids
- Expressed in the liver and small intestine
- No information in the public domain
- No tools

- *SmlrI*-KO : lethal
- *SmlrI*-LKD : FHBL phenotype



ANGPTL3

A/CF; ADH6; AGXT; AMDHD1; CPN2;
CYP4F2; F11; ETNPPL; FMO5; HPD;
HRG; LEAP2; PIPOX; PLA2G12B; PLG;
REEP6; RNU1-70P; SERPINA6; SLC2A2;
SORD2P

ALB
APOH
PROC

MTTP

SmlrI

MTTP

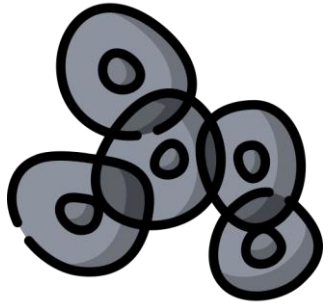
APOB

AMBIP; APOA2; C5;
CPB2; ENPPI; ETFB;
FOXA1; GPT2; IDH1;
KLB; MBL2; PON3;
SERPINA10; SERPINA7;
SERPINC1

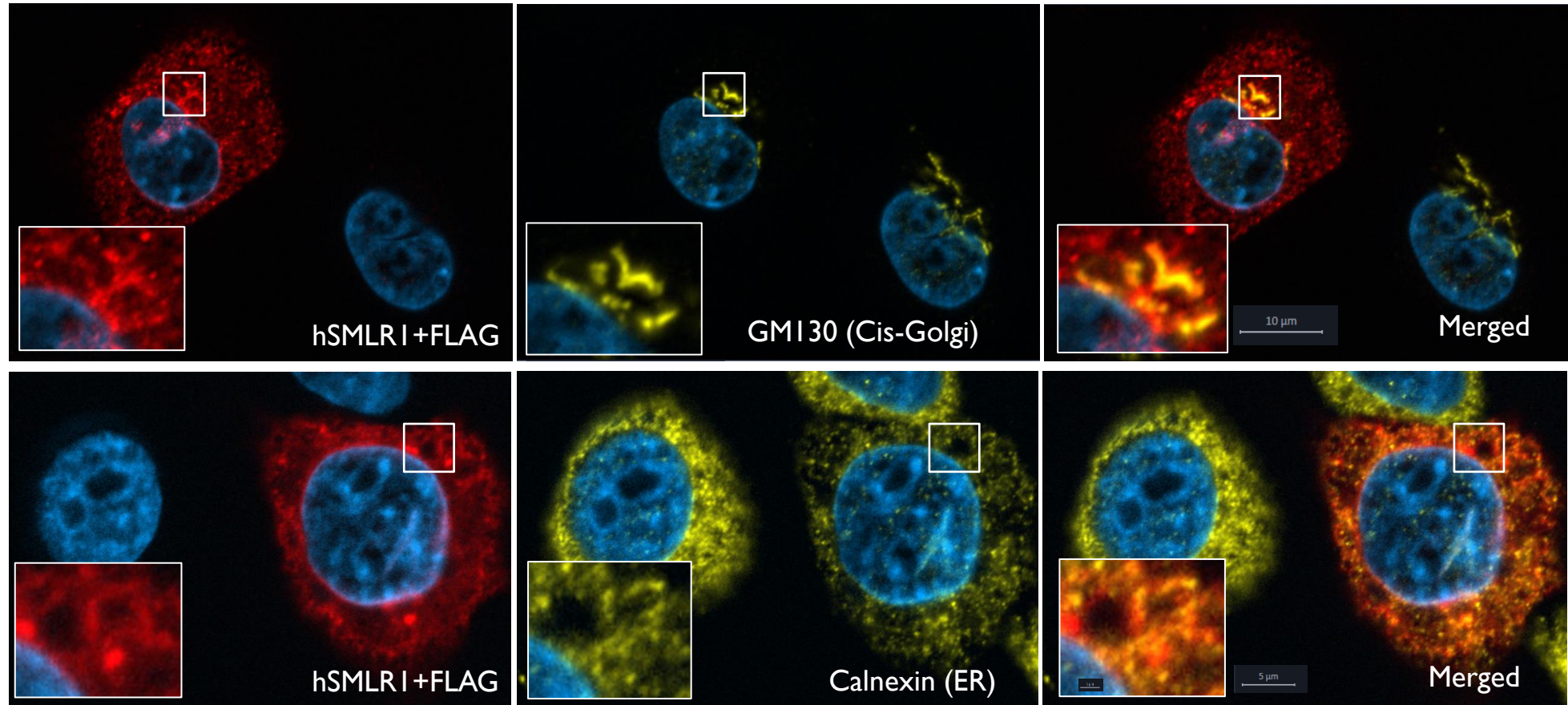
ADH4
AHSG
APOA1
APOC3
ASGR2
SULTA1

AADAC; AADACP
ABCG5; ASGR
C8G; CREB3L3; CRL
F10; FZD5; MOGA
MRPL35; **NPC1L**
NROB2; NR112;
NR5A2; SLC30A1
TM4SF4

Localization of SMLR I



Immortalized
Human Hepatic
cells,
overexpression
of *SMLR I*-FLAG

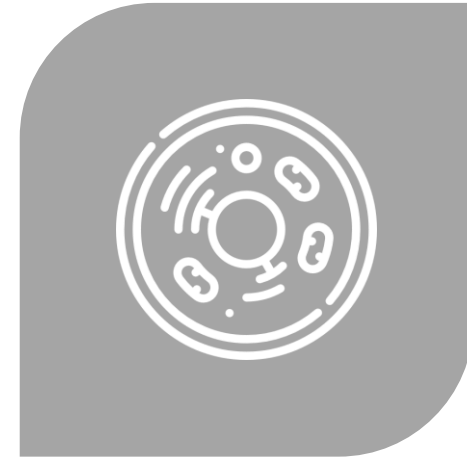


AIMS



Validate the subcellular localization of SMLR I

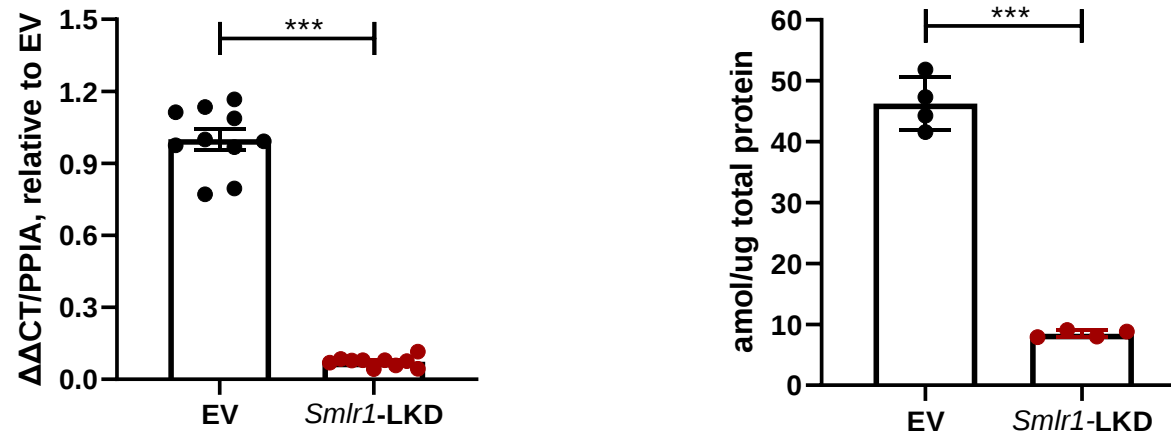
Method: Sucrose gradient +
custom-made SMLR I Ab's and LC-MS based
proteomics



Generate a *Smlr I* knock-out
cell line for further
molecular studies

SMLR I DETECTION

- **Targeted proteomics** shows 85 % reduction in Smlr I - liver-specific Smlr I knock-down mice



- Challenges :

- Endogenous SMLR I cannot be detected with targeted proteomic in cell lines (very low protein level)
- Generated antibodies do not work
 - in homogenates of cell lines and mouse liver
 - in immunofluorescence studies



MATERIALS & METHODS

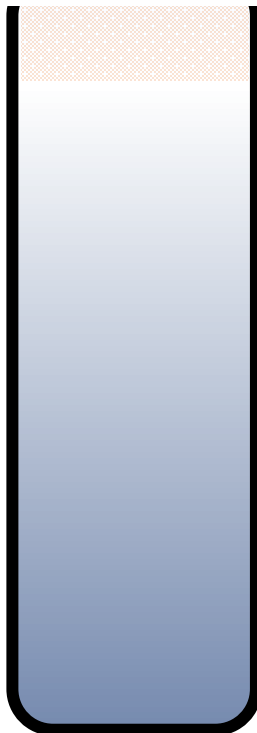
- Liver homogenates from mice with *Smlr1*-Liver Knock Down or WT
- Separation of organelles with **sucrose gradient**

SAMPLE = liver
homogeneization

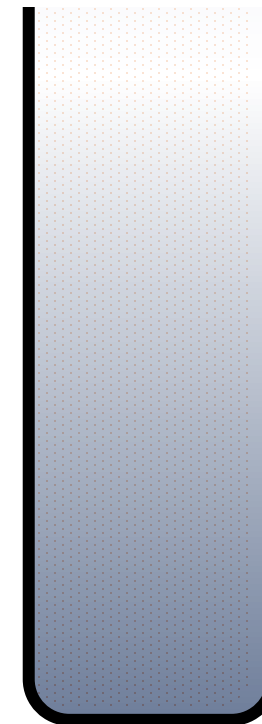
10%

SUCROSE

40%



Ultracentrifugation :
separation according sedimentation coefficient



Fraction 1
Fraction 2
Fraction 3
Fraction 4
Fraction 5
Fraction 6
Fraction 7
Fraction 8
Fraction 9
Fraction 10
Fraction 11
Fraction 12
Fraction 13
Fraction 14

WB

proteomics



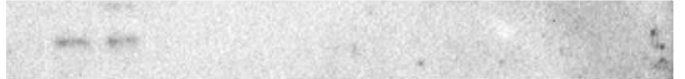
RESULTS : Sucrose gradient + Western blot

Sucrose density gradient from
liver of **WT mouse**

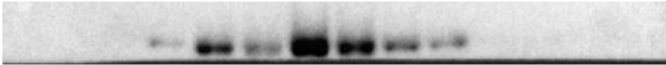
Sucrose density gradient from
liver of ***Smlr1*-LKD mouse**

SMLR1^{*\$} 

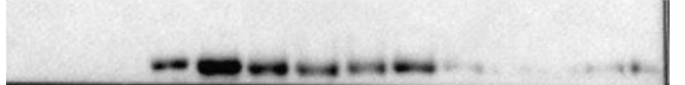
13kDa

SMLR1^{*\$} 

Golgi

Golgin97 

87kDa

Golgin97 

Endoplasmic retic

Calnexin^{*} 

90kDa

Calnexin^{*} 

Early endosome

EEA1 

160kDa

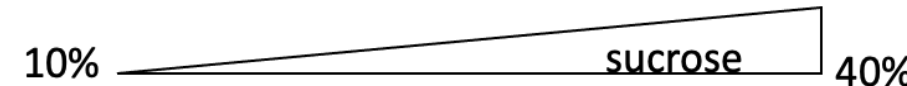
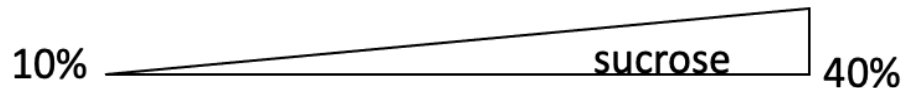
EEA1 

Lipid droplets

PLIN2 

49kDa

PLIN2 



* : replicated in another mouse liver \$: replicated with another antibody

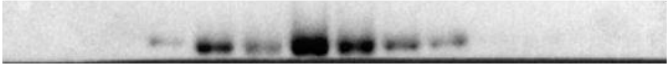


COMPARISON : Sucrose gradient + Western blot vs. ImmunoFluorescence

Sucrose density gradient from liver of **WT mouse**

SMLR1  13kDa

Golgi

Golgin97  87kDa

Endoplasmic retic Calnexin

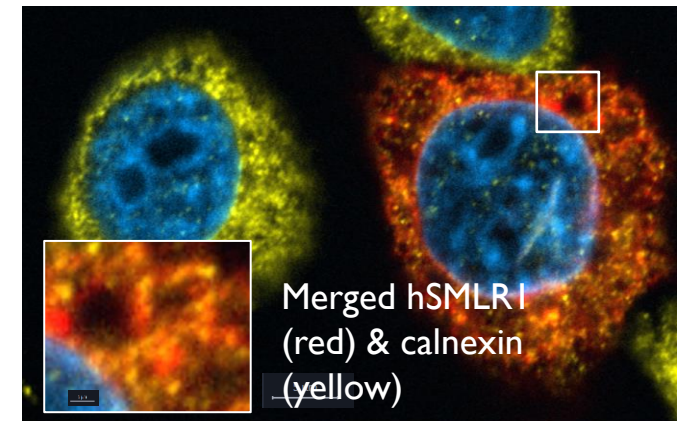
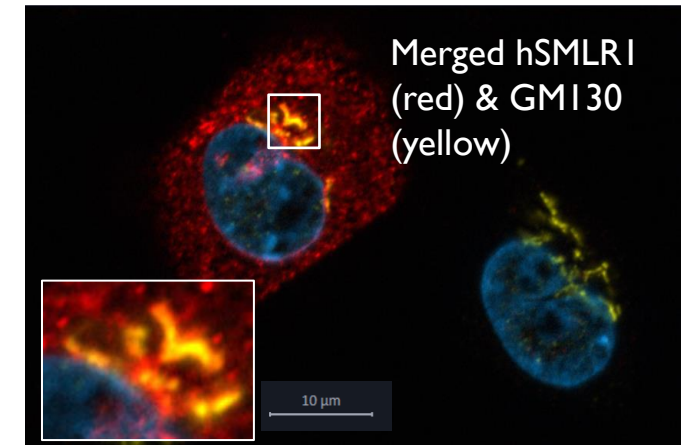
 90kDa

Early endosome

EEA1  160kDa

Lipid droplets

PLIN2  49kDa



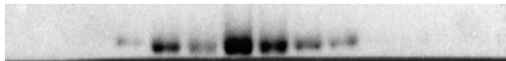
➔ Discordant results



RESULTS : Sucrose gradient + Proteomics

- Over 3 000 proteins in each fractions
- SMLR I was not detected
- Confirmation of the localization of the Golgi marker, calnexin and EEA1

Golgin97



87kDa

Calnexin

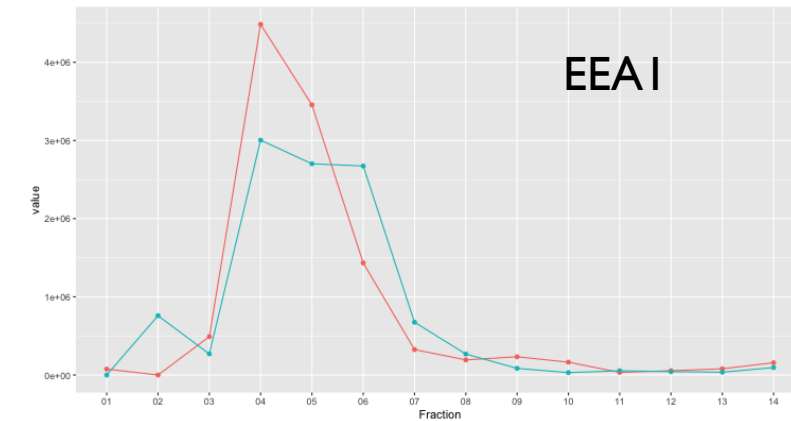
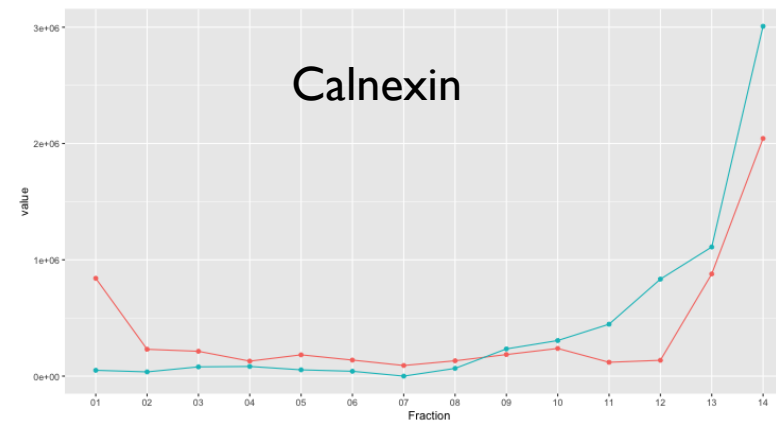
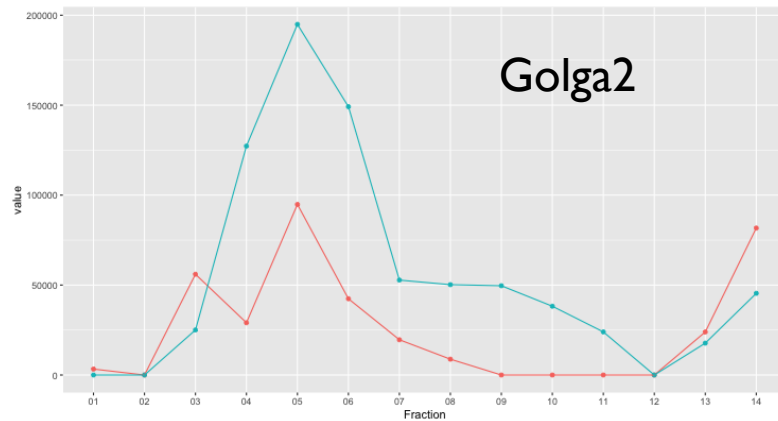


90kDa

EEA1



160kDa

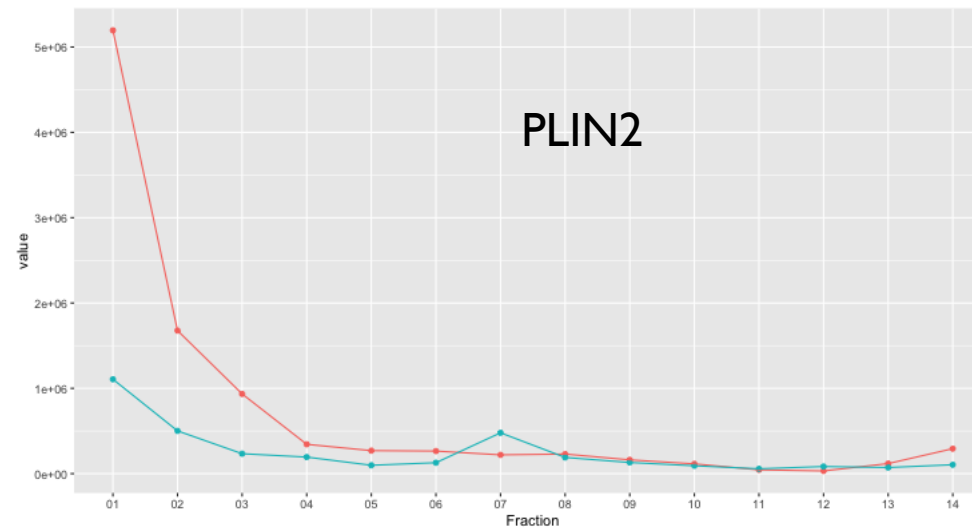


- mouse WT (I24, blue)
- *Smlr I*-LKD (I23, red)



RESULTS : Sucrose gradient + Proteomics

- Refinement of the organelle distribution across the gradient : PLIN2 for LD

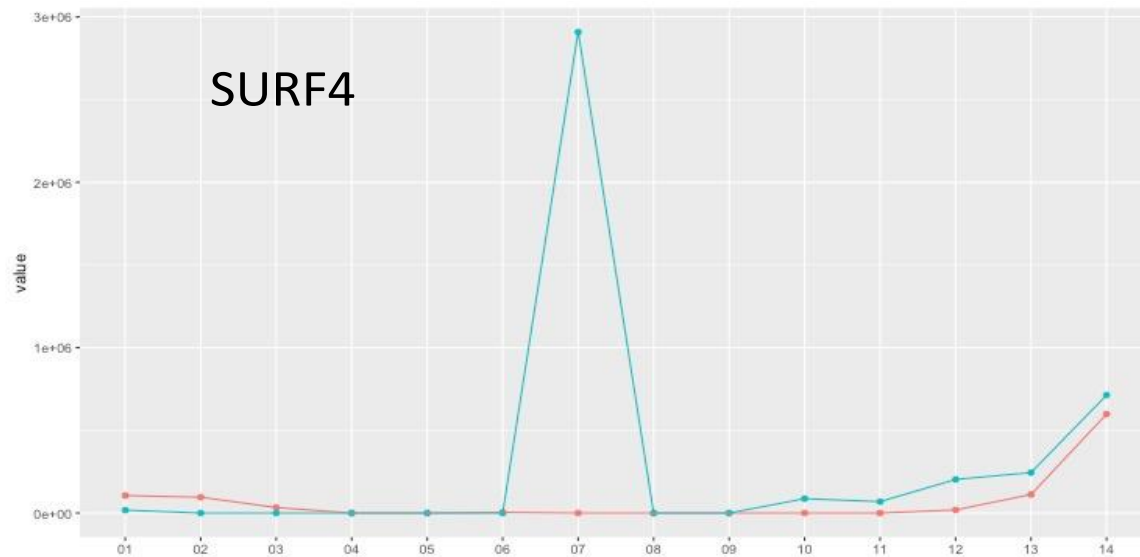


- mouse WT (I24, blue)
- Smlr1*-LKD (I23, red)

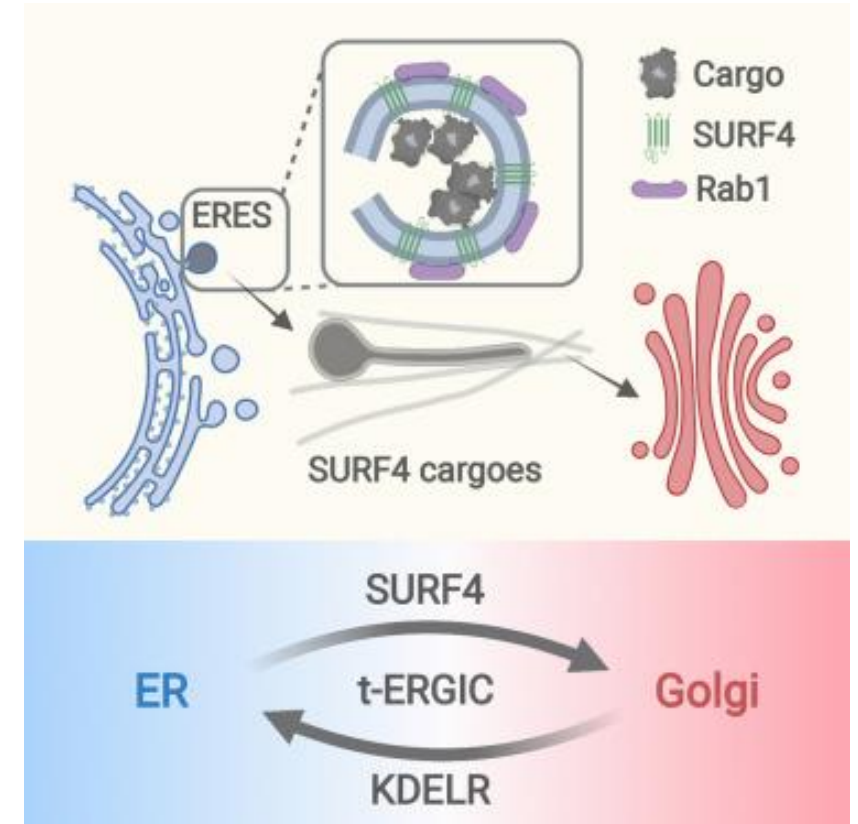


RESULTS : Sucrose gradient + Proteomics

- Modification of protein distribution in *Smlr1*-LKD sample vs.WT : SURF4



- mouse WT (I24, blue)
- Smlr1*-LKD (I23, red)



Rui Yan et al. SURF4-induced tubular ERGIC selectively expedites ER-to-Golgi transport, *Developmental Cell*, Volume 57, Issue 4, 2022.



CONCLUSIONS OF SUCROSE GRADIENT ANALYSIS

FINDING FROM WESTERN BLOT ON SUCROSE FRACTION

- SMLRI lies on fraction 2-3
- SMLRI do not co-localizes with calnexin
- SMLRI do not co-localize with Golgi markers

Thus, my work does not validate previous findings using IF.

FINDING FROM LC-MS ON SUCROSE FRACTION

- SMLRI cannot be detected
- No detection of SURF4 in case of SMLRI deficiency

DISCUSSION

- SMLR I localisation : contradictory results between sucrose gradient and IF data
- SMLR I Antibody : 2 custom-made Ab's gave the same results, but unable to find SMLR I in proteomics data

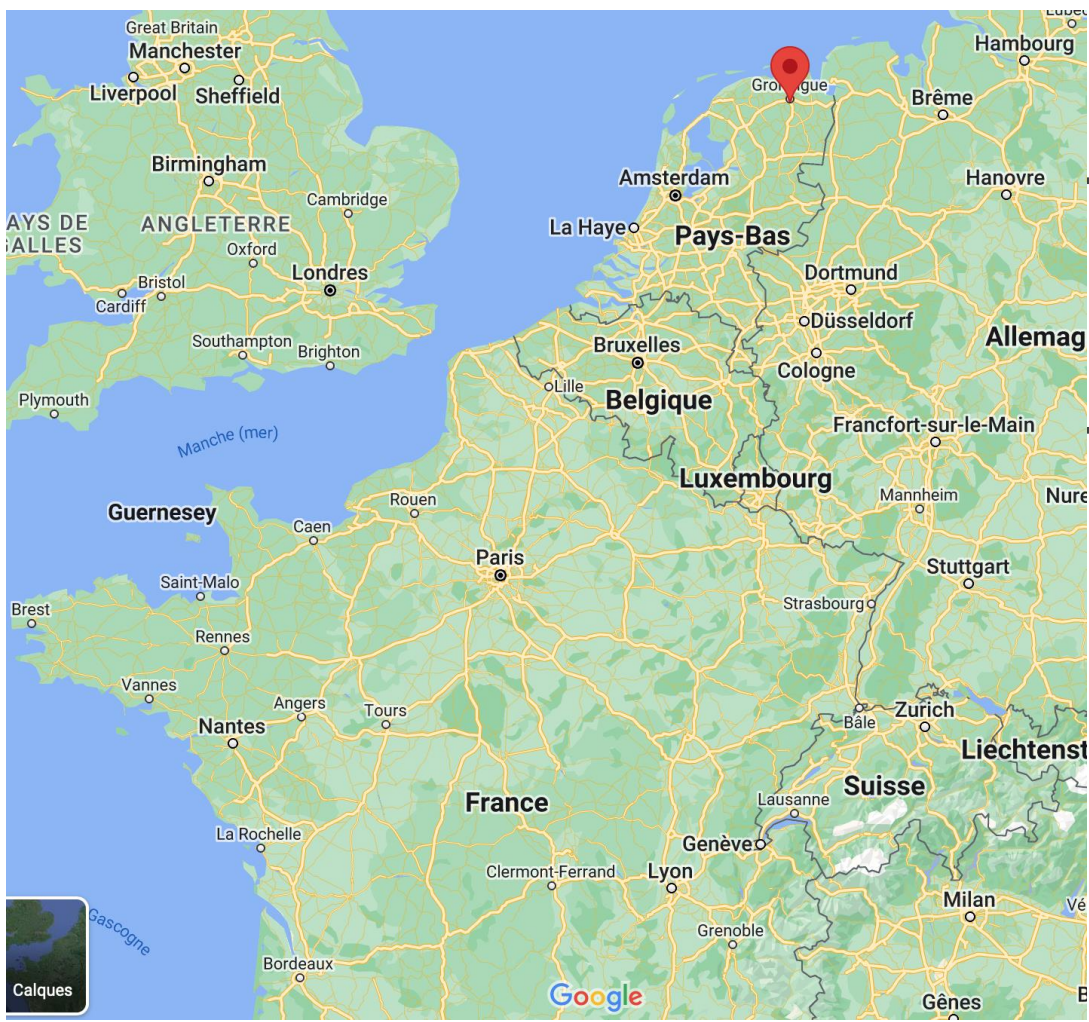
GeneScript AB / 13kDa



Yenzym AB / 13kDa



- Need to replicate proteomics analyses with samples of additional couples of mice.



PAYS BAS - GRONINGEN

