# Lipid Distribution in Liver is Disrupted in the Translocator Protein (*Tspo*, 18-KDa) Knockout Mouse Model

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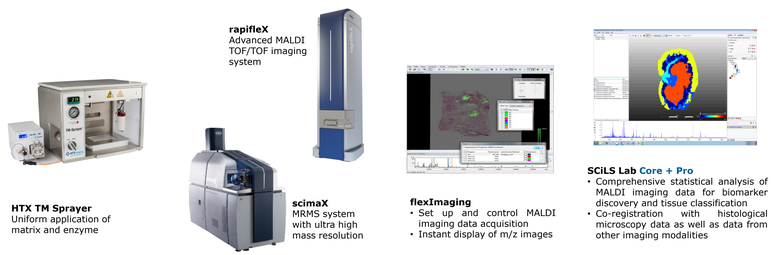
## Introduction

TSPO is a mitochondrial high affinity cholesterol-binding protein involved in steroid hormone biosynthesis, apoptosis, and cell proliferation. *Tspo* knockout (KO) mice show disturbances in neutral lipid homeostasis in adrenal, gonads and liver indicative of changes in cholesterol utilization. MALDI mass spectrometry imaging was used to examine changes in cholesterol distribution in *Tspo* KO liver in comparison to wild-type (WT) liver. Image acquisition in positive and negative ionization revealed accumulation and differential localization of various lipid classes involved in cholesterol metabolism including triglycerides, phospholipids and fatty acids in KO liver. MALDI imaging data allow us to understand dysregulation of the liver lipidome and facilitate characterization of liver diseases induced by deficiencies in the cholesterol uptake or utilization.

## Materials and Methods

- Freshly frozen livers from WT and *Tspo* KO mice were sectioned at 10-micron thickness using a Leica cryotome and the sections were thaw-mounted on conductive indium tin oxide glass slides.
- MALDI matrices were uniformly deposited onto the tissue sections using the HTX Sprayer.
- 2,5-dihydroxybenzoic acid (DHB) and 9-aminoacridine (9AA) were sprayed uniformly over liver sections using TM Sprayer from HTX Technology.
- RapifleX MALDI TOF/TOF and ScimaX mass spectrometers from Bruker were used for data acquisition
- Lipids were imaged at 50 µm spatial resolution and MS/MS fragmentation was used for molecular identification.
- MS imaging data analysis was performed using FlexImaging and SCiLS Lab software from Bruker.




## Results

### Mapping cholesterol distribution in liver

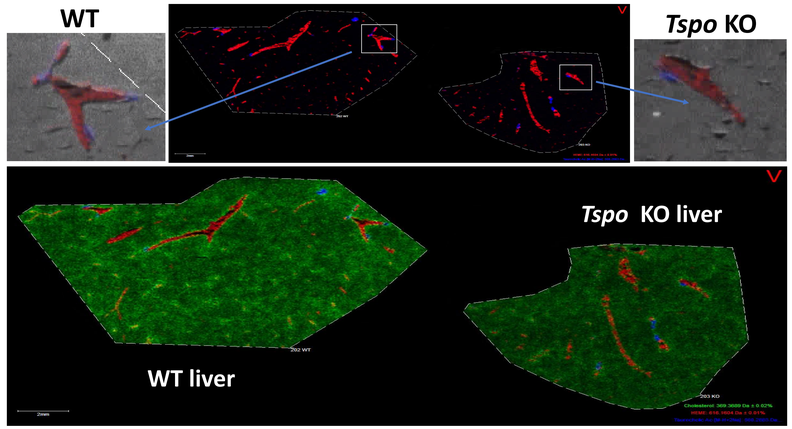



**Figure 1.** Coregistration of MS image for known tissue markers with the optical image shows morphological features: in red - distribution of heme molecule (m/z 616.5) from red blood cells reveals vasculature, in blue – localization of taurocholic acid (m/z 560.3) reveals the bile ducts. In green – cholesterol presents perfused throughout the liver tissue with slight accumulation in the hepatic lobules and around vasculature.

### Triacylglycerides (TAG) are upregulated in *Tspo* KO liver

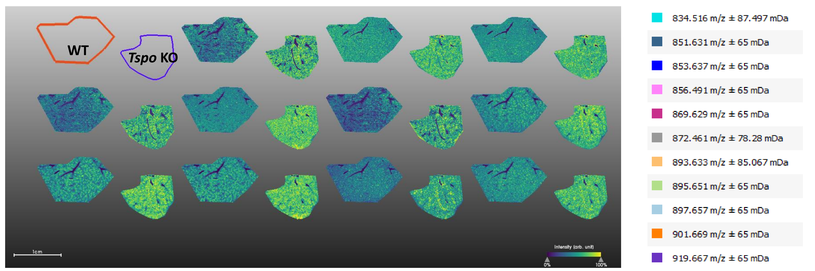
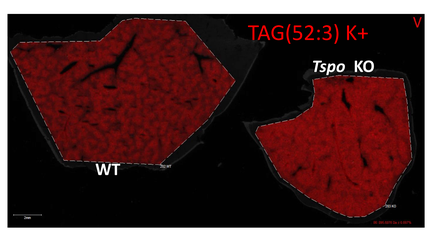



**Figure 2.** Statistical analysis using Receiver Operating Characteristic ROC in SCiLS Lab shows TAGs to be upregulated in the *Tspo* KO liver in comparison to WT. TAGs are highly localized to the hepatic lobules in WT liver and abundant and nonspecifically distributed throughout the *Tspo* KO liver.




## Results

### Lipids accumulate along the blood vessels in *Tspo* KO liver

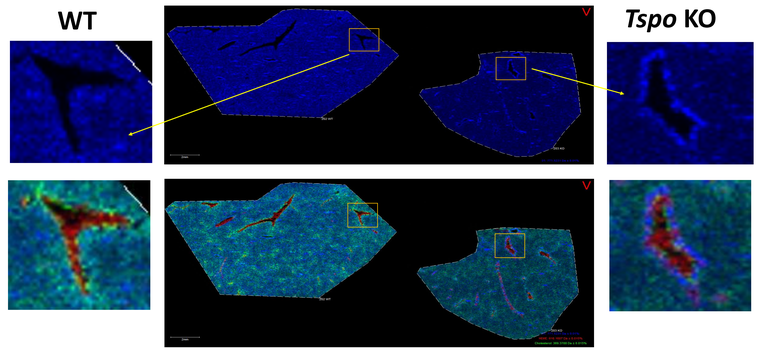



**Figure 3.** Distribution of m/z 771.5, in blue, shows clear blood vessels in the WT liver and accumulation of this molecule along the vasculature from the *Tspo* KO liver. This lipid was tentatively identified as phosphatidylglycerol. Coregistration with cholesterol distribution, in green, shows a cholesterol gradient around the vasculature from WT liver and very little cholesterol perfusion in the *Tspo* KO liver

## Conclusions

- Our results confirm the dysregulation of cholesterol clearance mechanism and show lipid deposits to be associated with blood vessels in the *Tspo* KO liver.
- Tissue distribution and expression patterns of lipid biomarkers visualized by MALDI imaging facilitate understanding of metabolic disease progression in liver.

## References / Acknowledgements

- Fan J, Campioli E, Sottas C, Zirkin B, Papadopoulos V. Amhr2-Cre-Mediated Global *Tspo* Knockout. J Endocr Soc. 2020 Jan 12;4(2):bvaa001. doi: 10.1210/jendso/bvaa001. eCollection 2020 Feb 1.
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