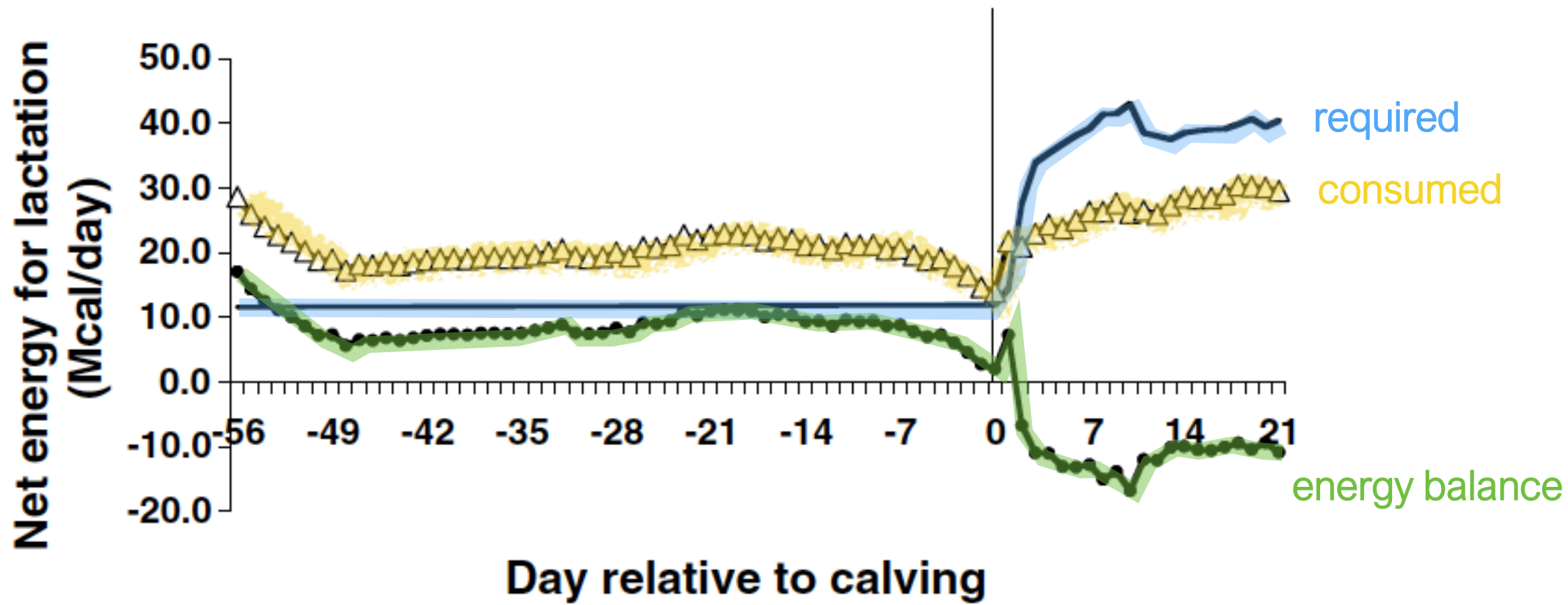


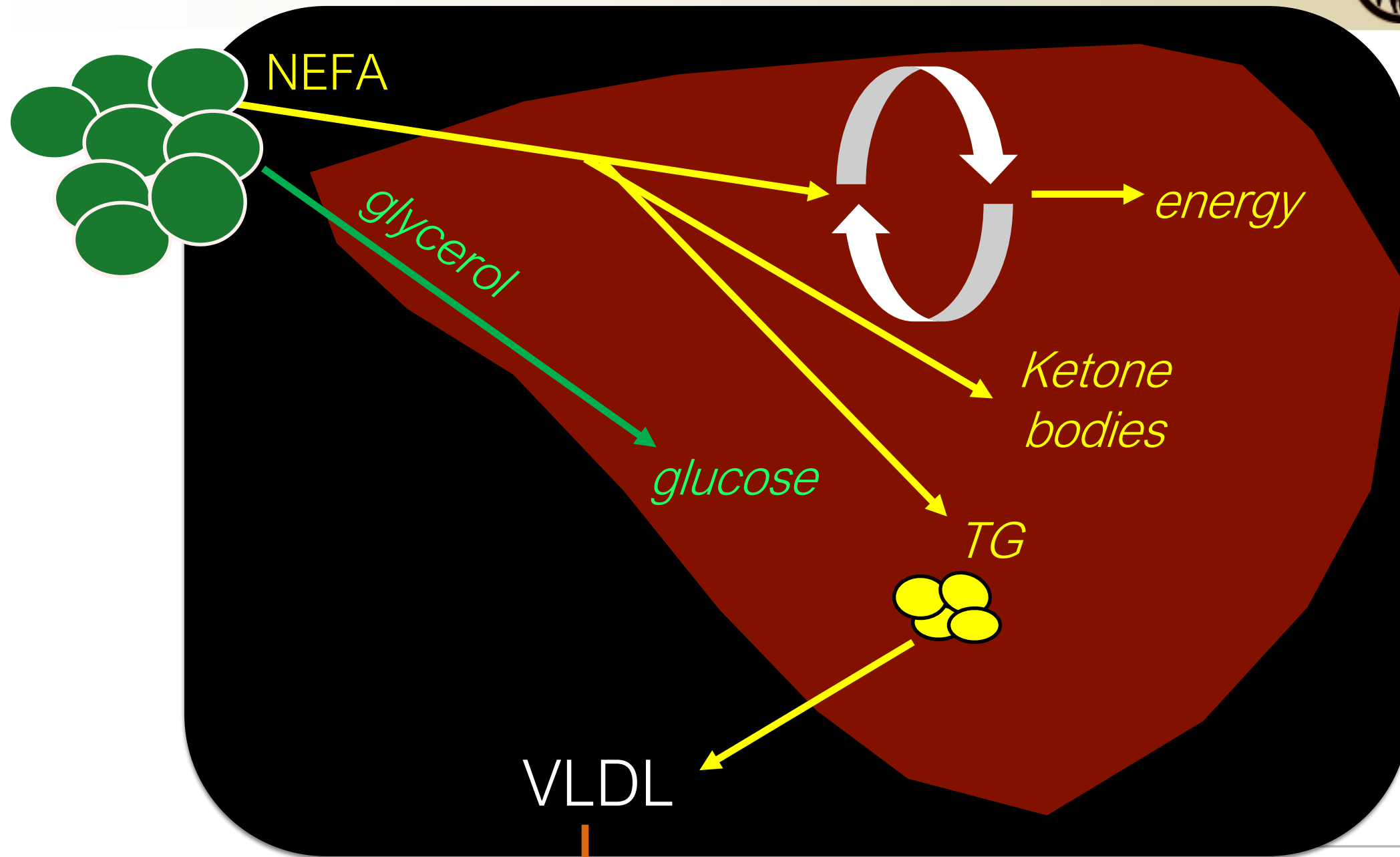
Knockdown of patatin-like phospholipase domain-containing protein 3 (PNPLA3) increased cellular triglyceride

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Department of Dairy Science
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Negative Energy Balance

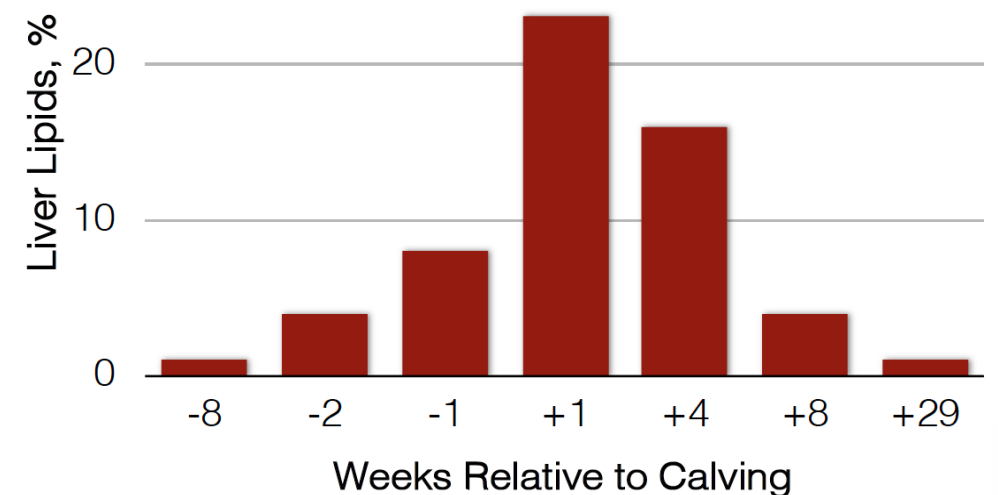


Hepatic Nutrient Partitioning

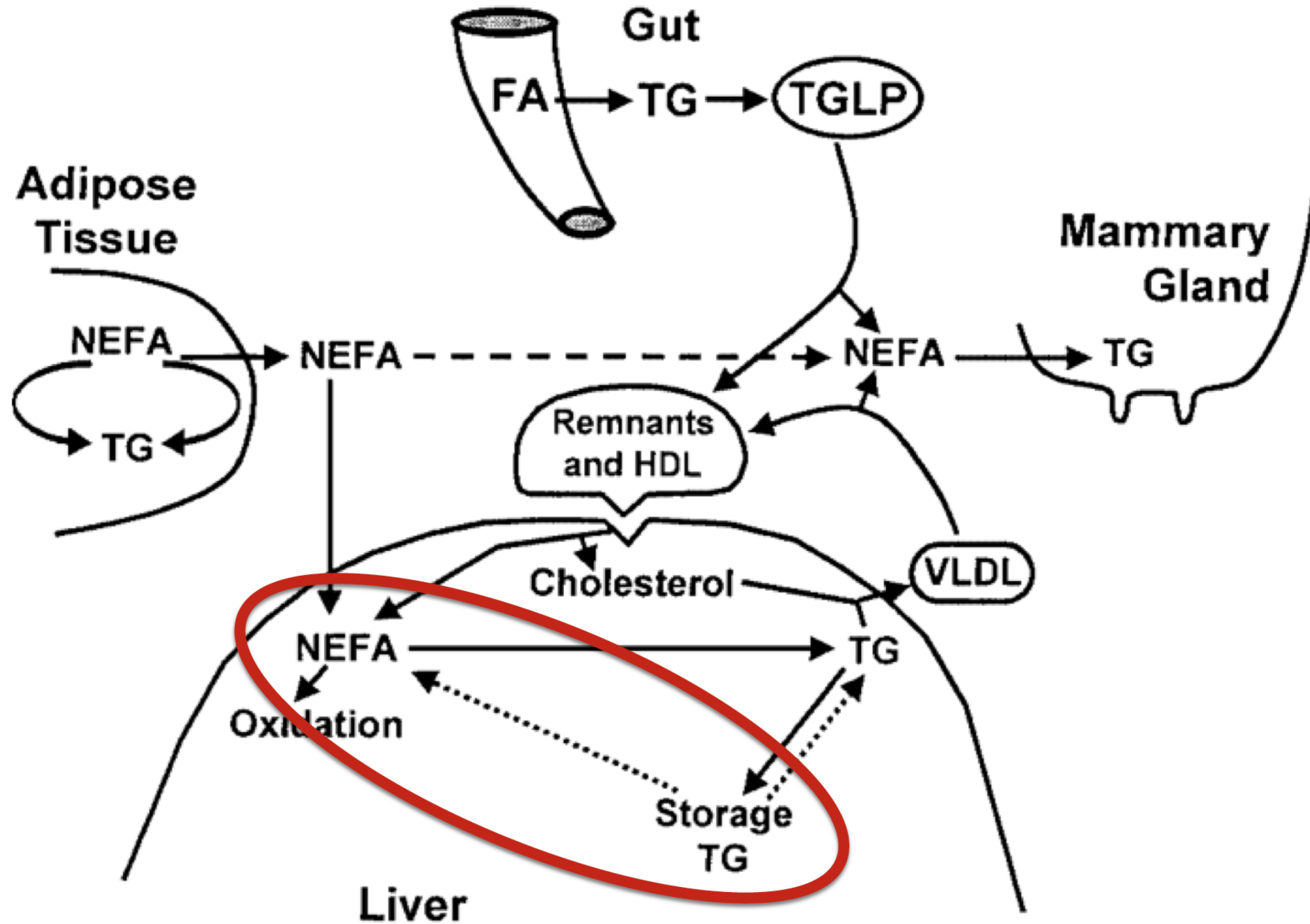


2 possibilities:

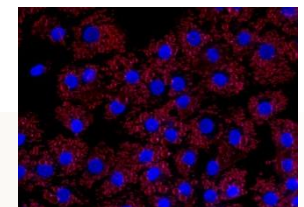
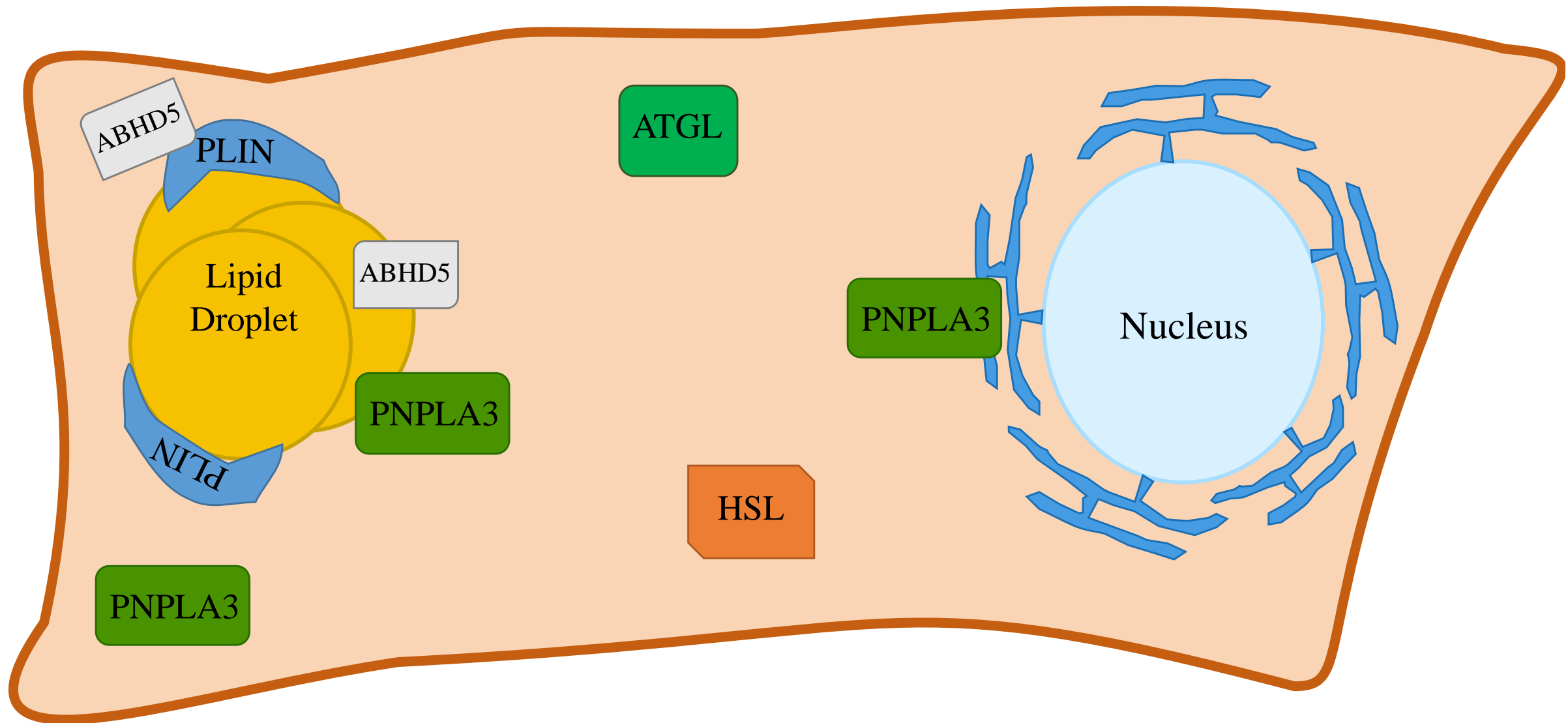
1. Lipolysis
2. Export



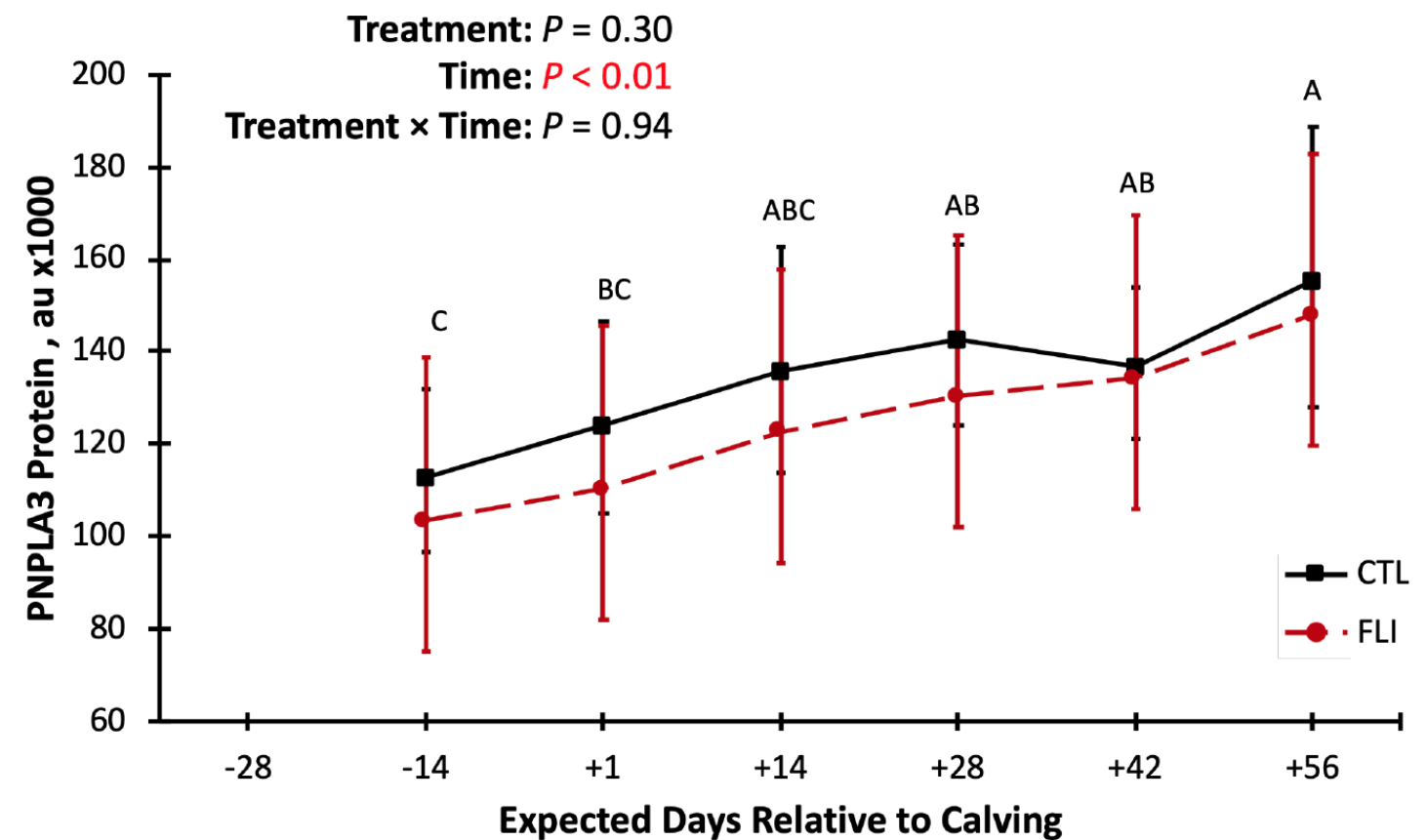
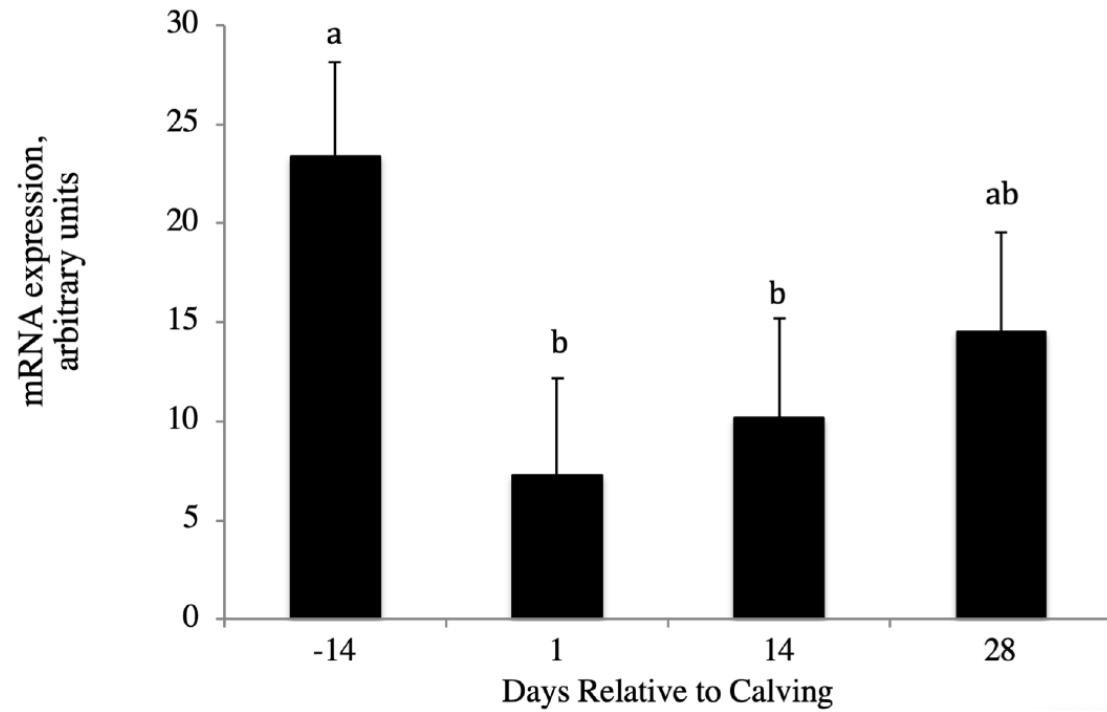
Remobilization of Liver Lipids



Filling in the Details on Hepatic Lipolysis

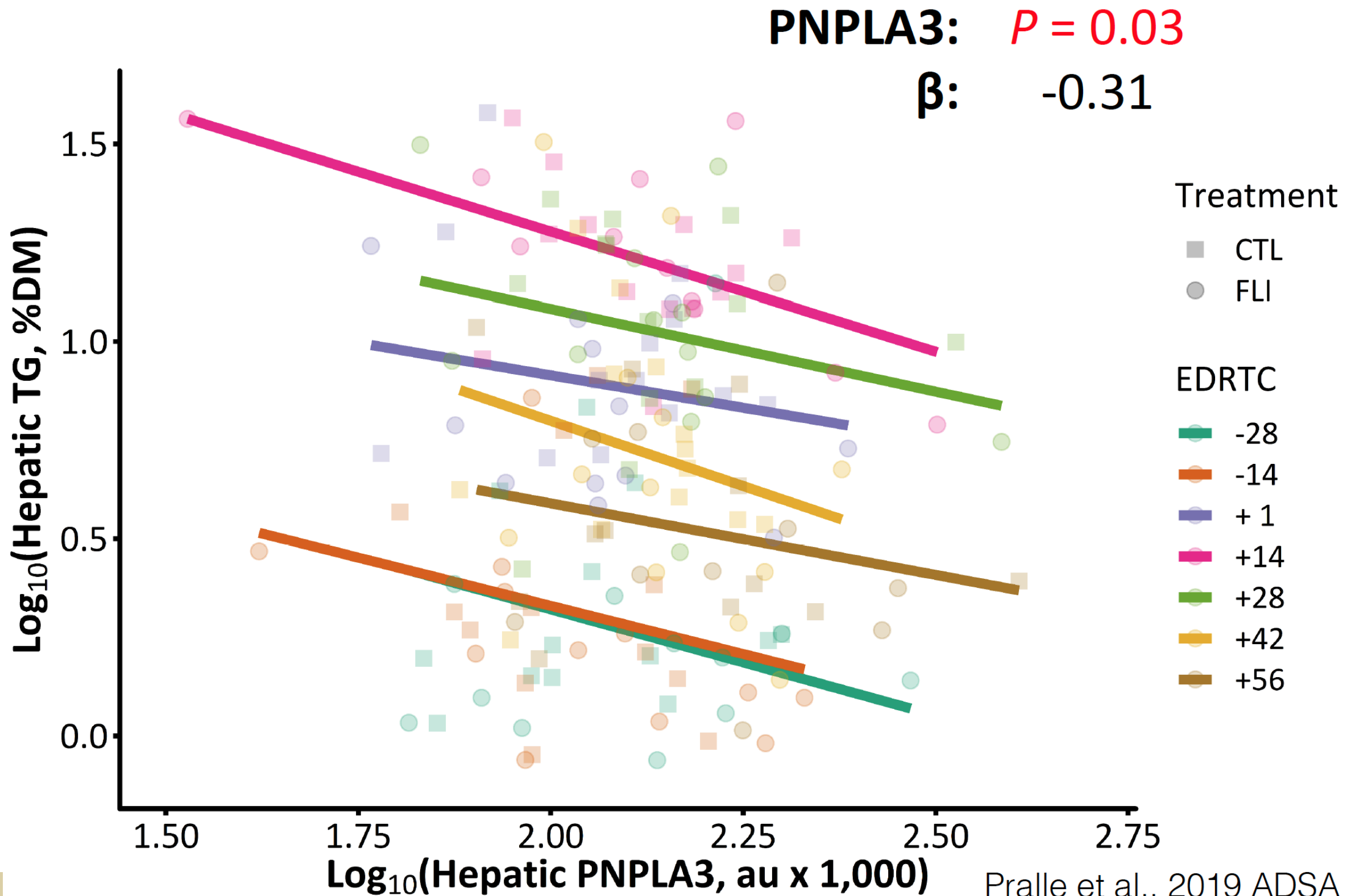


Peripartum PNPLA3



Peripartum PNPLA3

Inversely Related to Liver TG

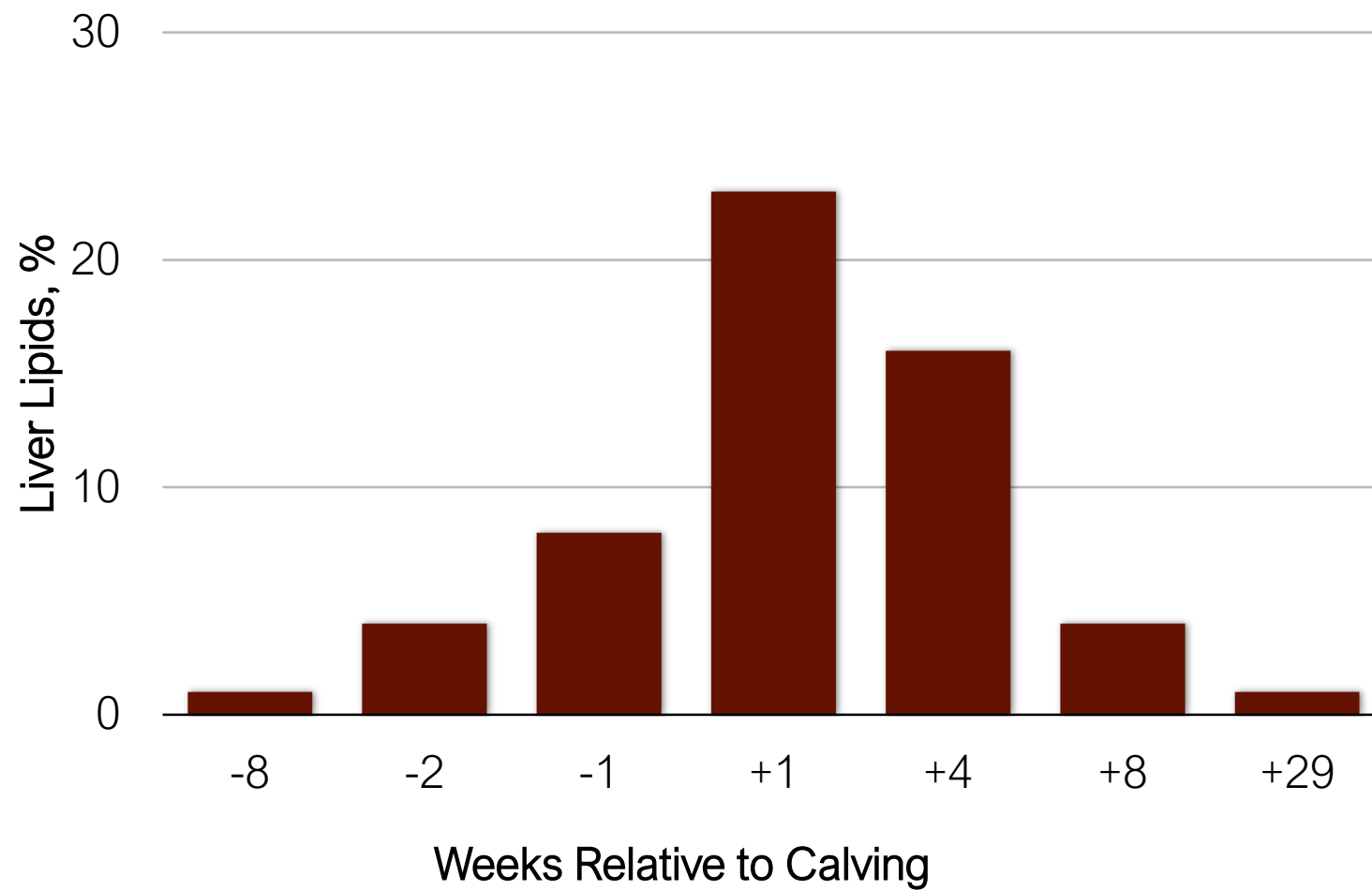


Hypothesis and Objective

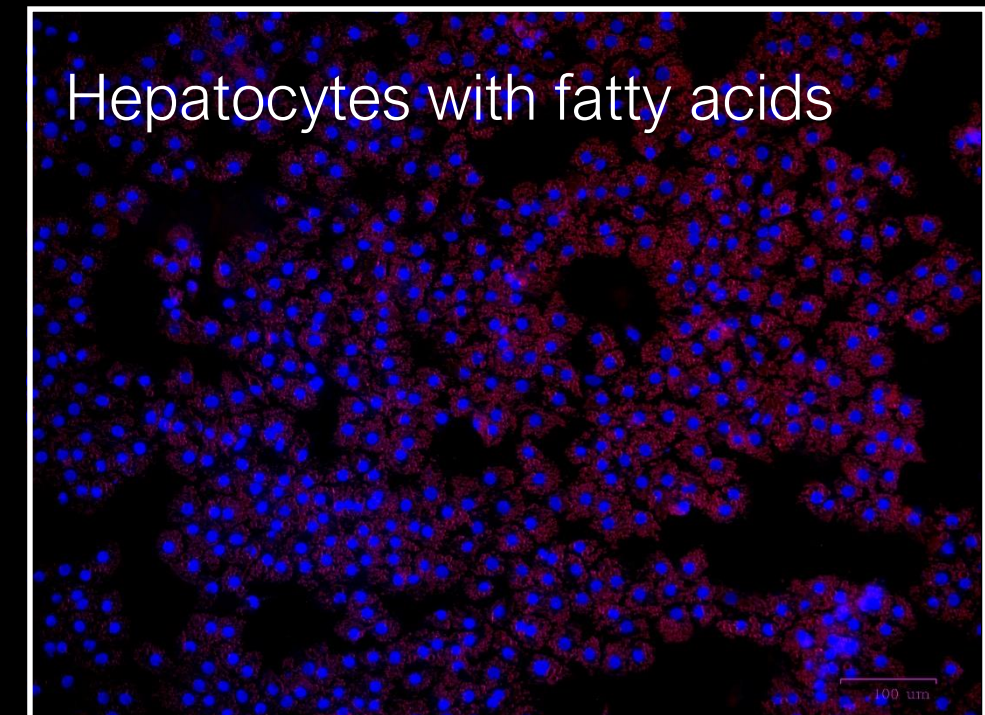
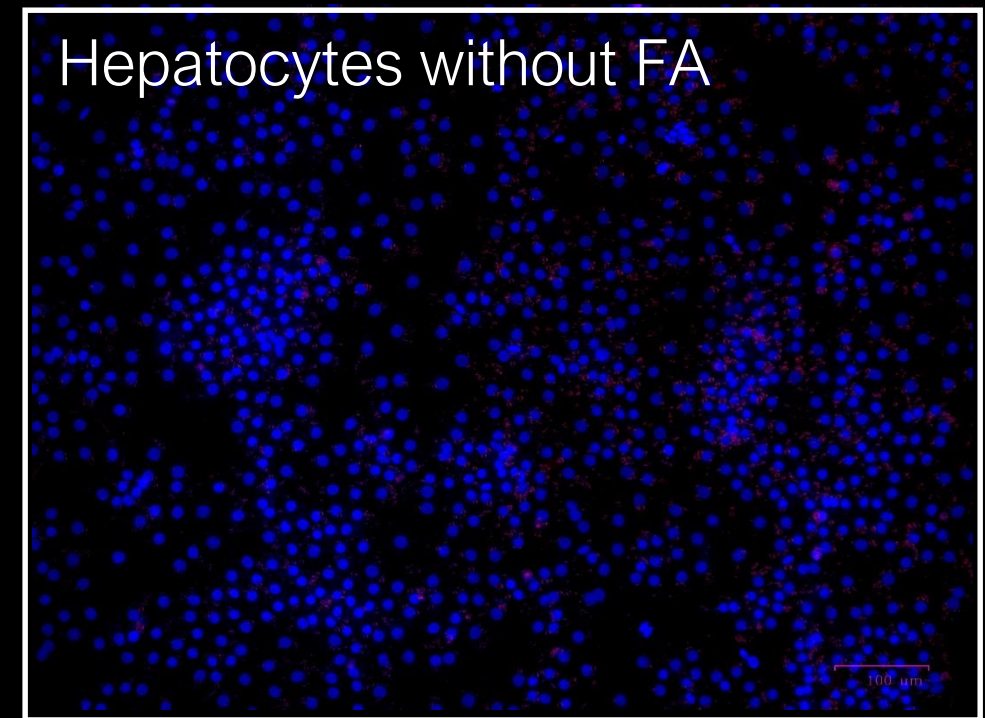


- Working Hypothesis
 - PNPLA3 is involved in liver lipid accumulation in two ways:
 - Decreased basal peripartum abundance is associated with increased liver lipids
 - Increased postpartum abundance allows for “re-mobilization” of stored lipids
- Objective of this research
 - Determine the direct impact of PNPLA3 knockdown
 - Determine if individual fatty acids regulate PNPLA3 abundance

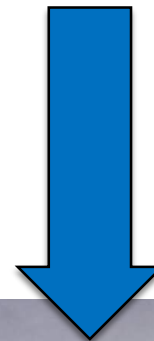
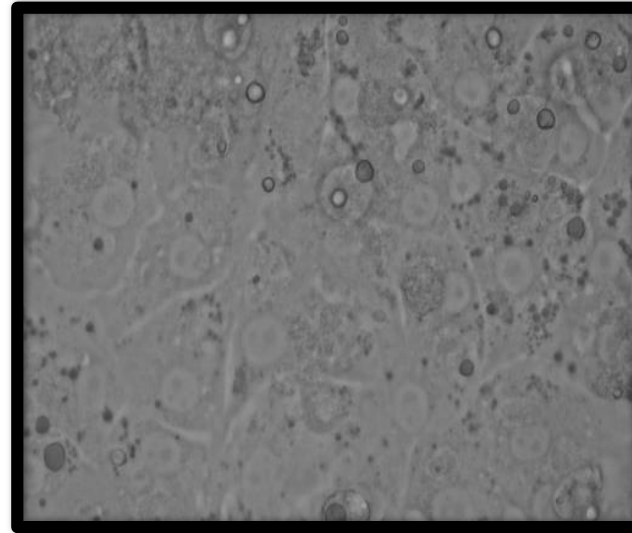
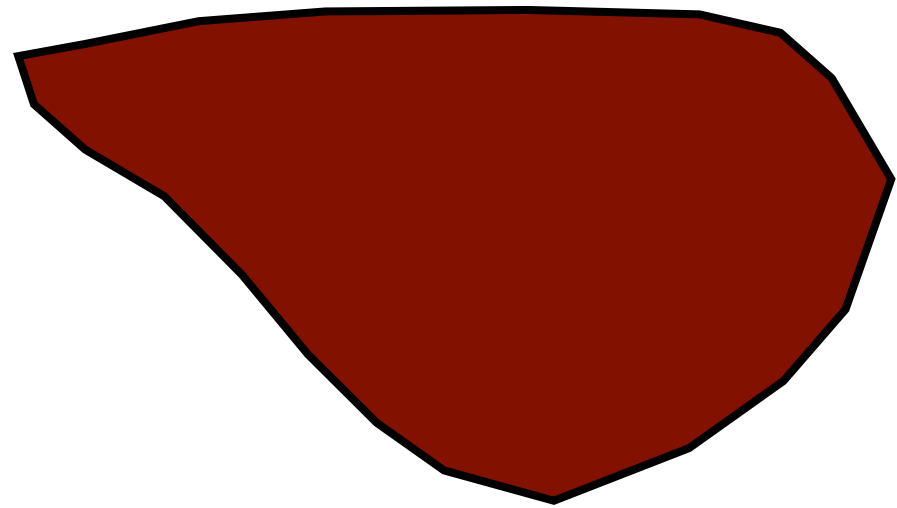
Lipid Accumulation



- Hepatic uptake reflects blood flow and [NEFA]
- Accumulation in vivo peripartum is consistent and can be replicated in primary bovine hepatocytes providing an in vitro model for mechanistic objectives



Primary Hepatocyte Cell Culture

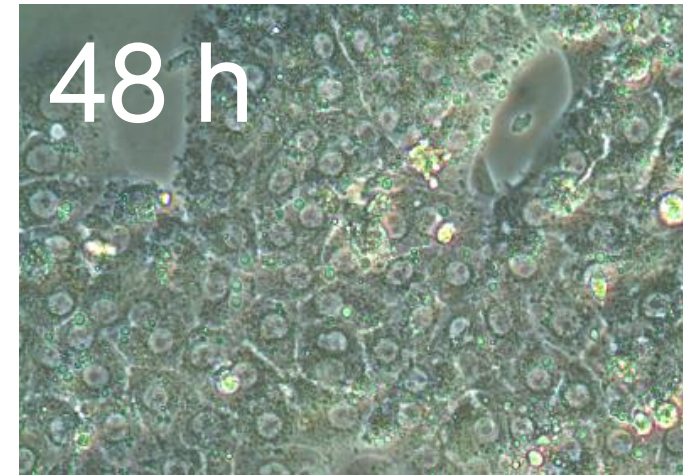
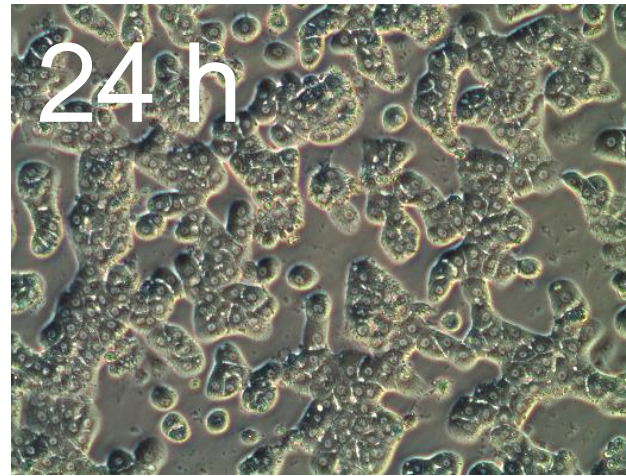
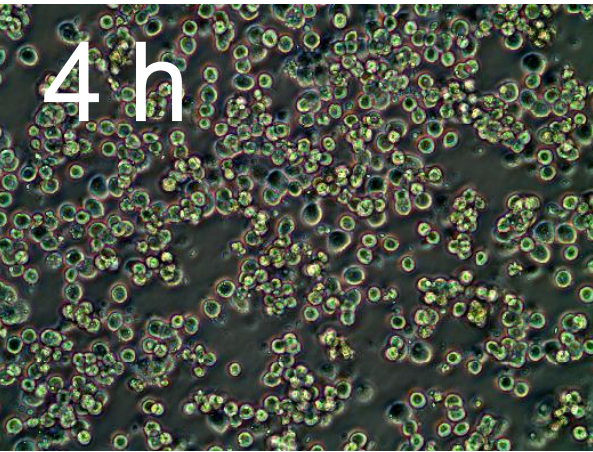


Primary Hepatocyte Cell Culture



- **Use of the in vitro model**
 - Allows for testing several concentrations and combinations
 - Eliminates confounders
 - More direct examination of cause and effect on specific functions
- **Primary bovine hepatocyte culture (Holstein calves < 7 d old)**
 - 3 independent cell isolations (biological replicates)
 - Treatments applied in triplicate (technical replicates)
 - Treatments replicated across blocks for different fates
 - ~2 million cells/35 mm dish are plated in monolayers and do not proliferate in culture
- **Statistical analysis** using Proc Mixed (SAS 9.4) with main effect of treatment and inclusion of calf in the random statement

Methodology



Lipid-mediated transfection (
Lipofectamine 2000)
siNON, siKD1, siKD2

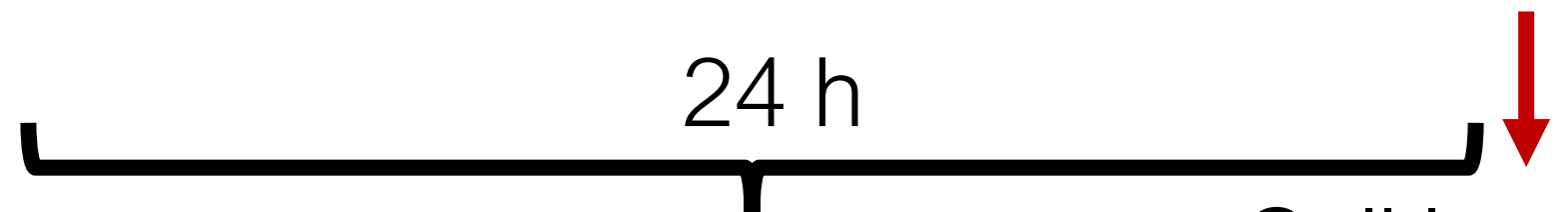


Cell lysate
harvest



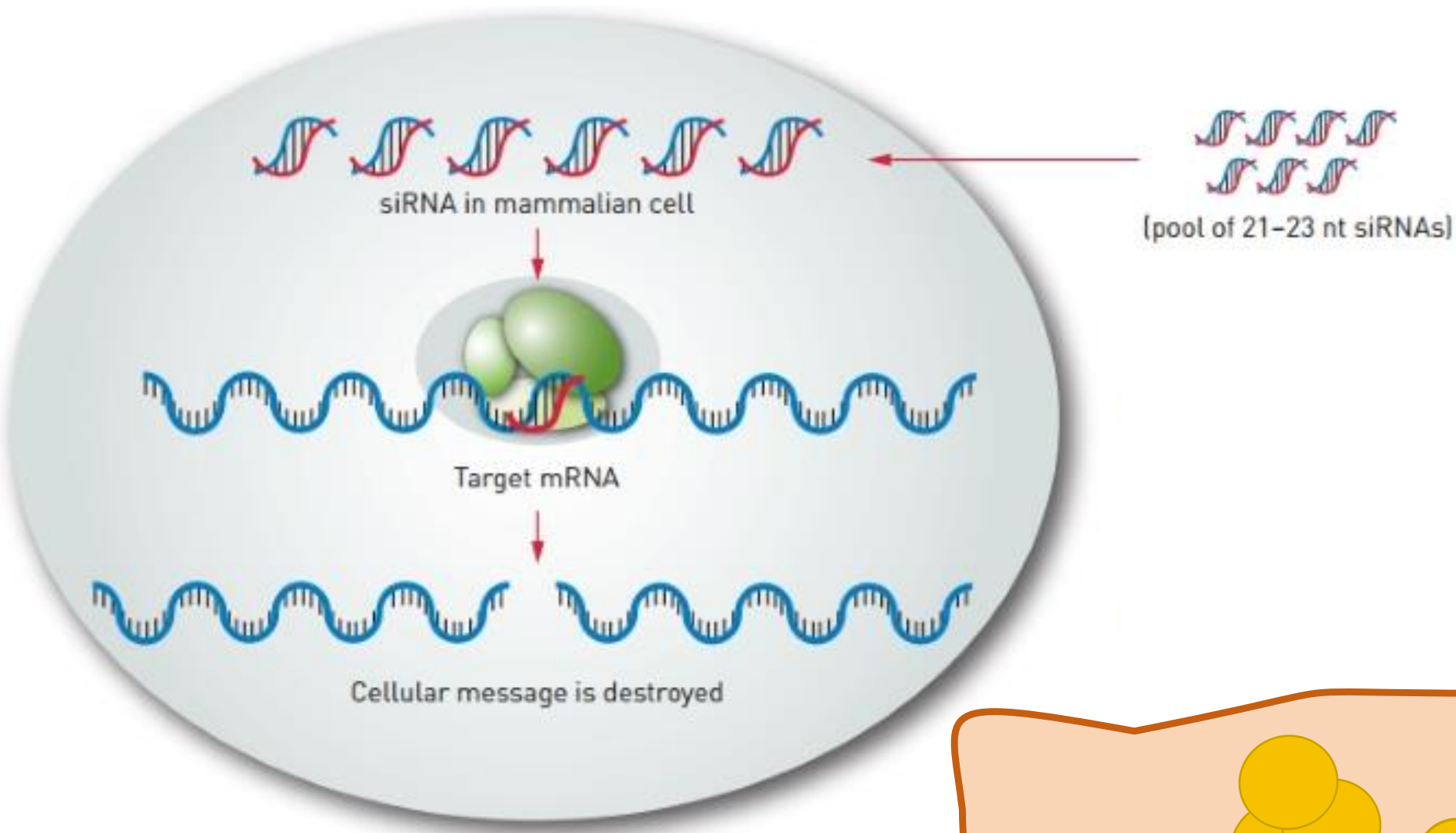
24 h

Fatty acid treatments

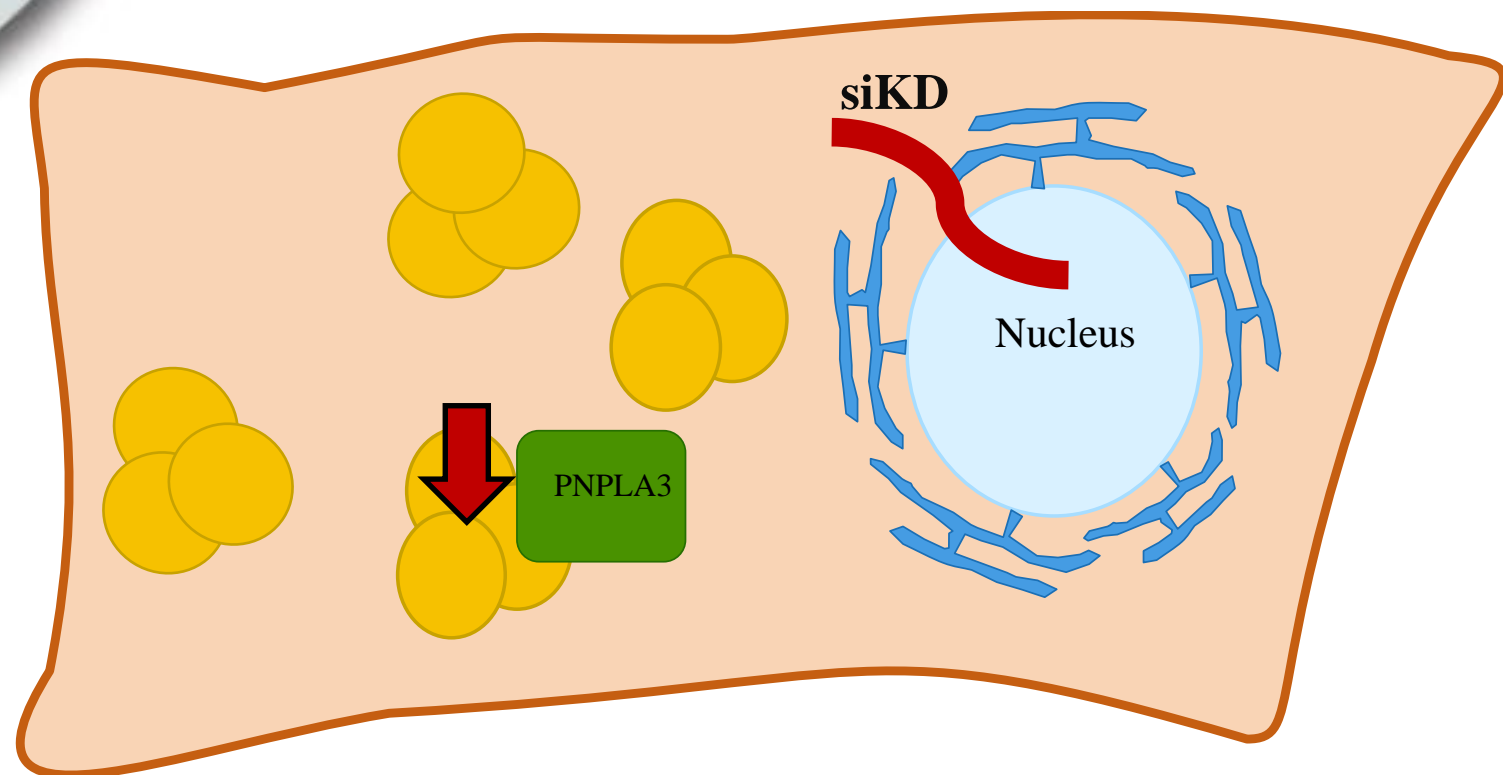


Cell lysate
harvest

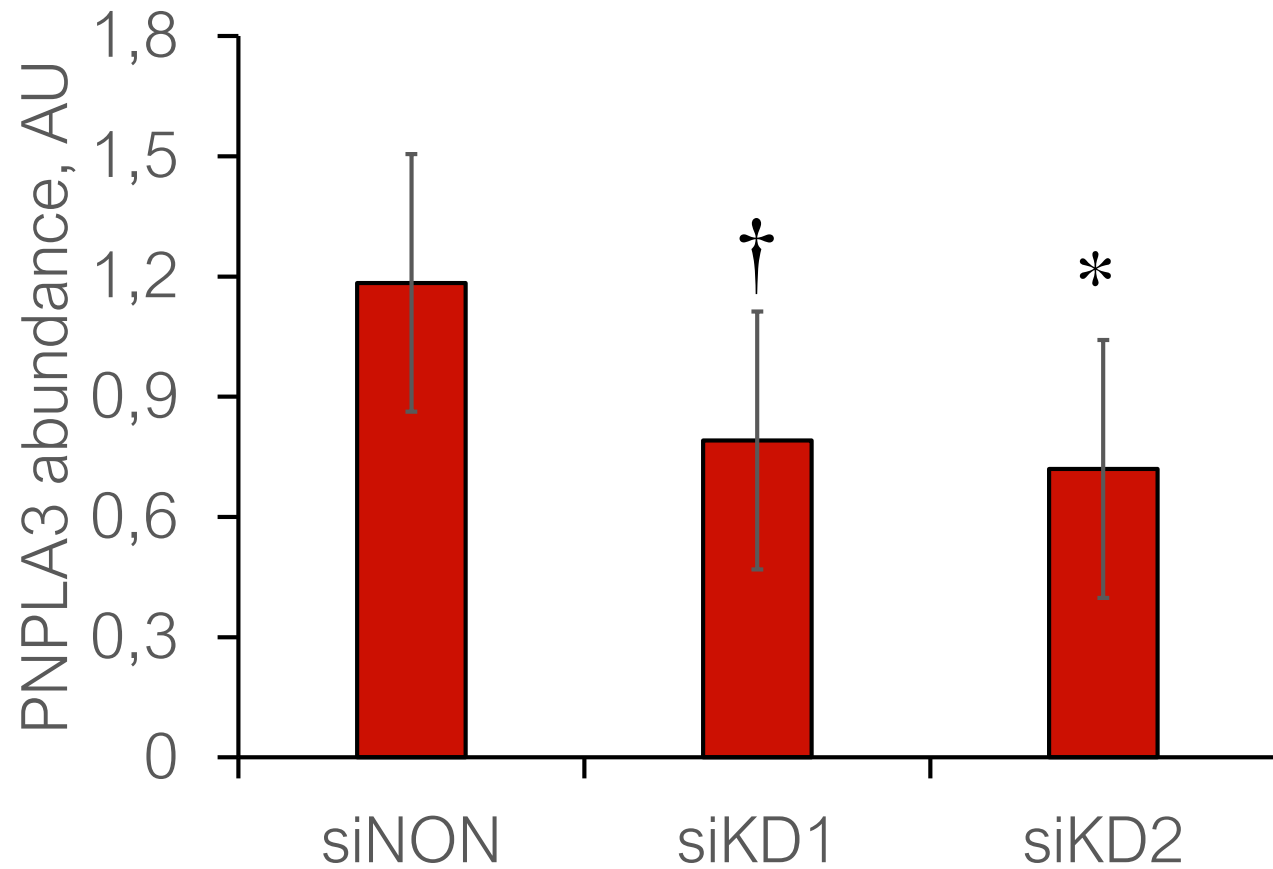
siRNA Knockdown



Adapted from ThermoFisher

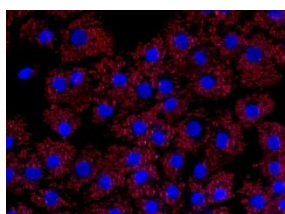
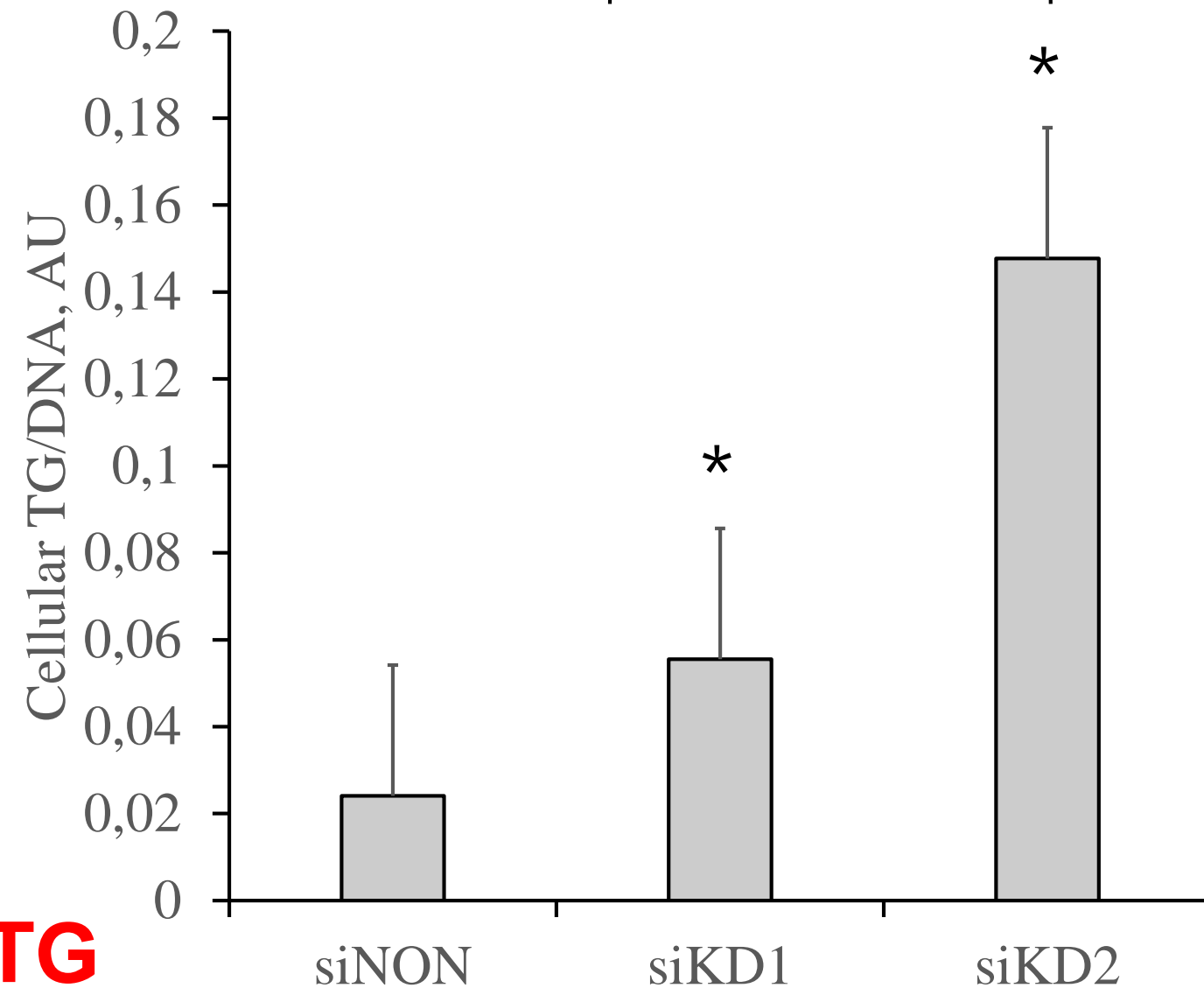


PNPLA3 Impacts Lipid Accumulation



33 and 39% Knockdown

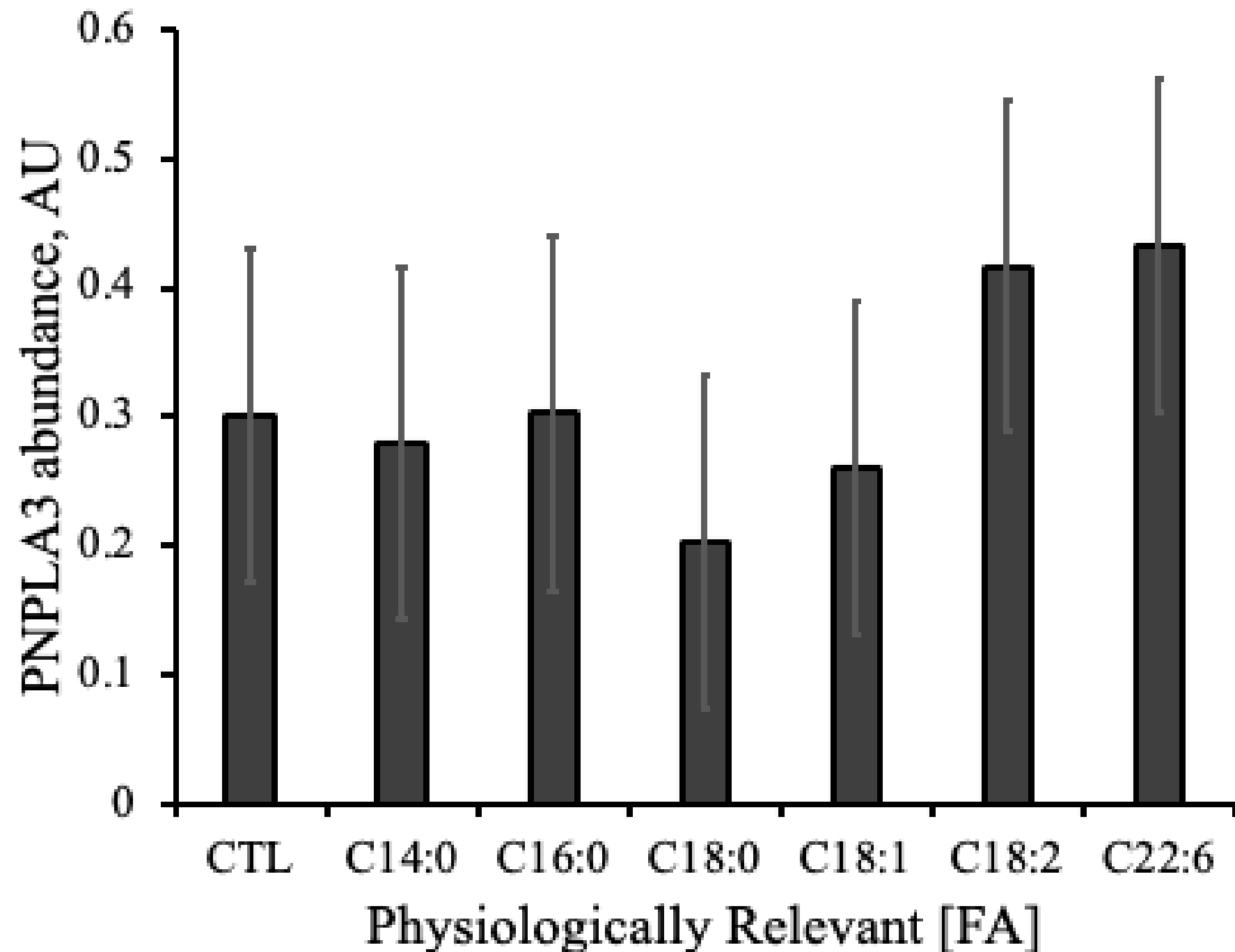
2.3 and 6.2 times as much TG



How is PNPLA3 Regulated?

FA	Conc, mM
Myristic C14:0	0.009
Palmitic C16:0	0.175
Stearic C18:0	0.279
Oleic C18:1	0.021
Linoleic C18:2	0.005
DHA ⁴ C22:6	0.0113

Protein Abundance

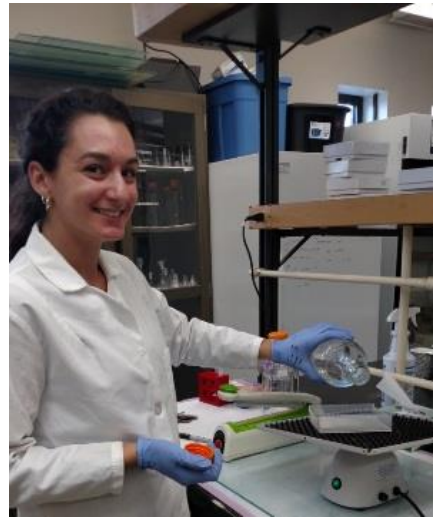


Conclusions

- PNPLA3 likely plays a key role in the extent and recovery of fatty liver in dairy cows
- Knockdown of PNPLA3 causes an increase in cellular lipids
- Treatment with physiologically relevant concentrations of individual fatty acids did not significantly alter PNPLA3 protein abundance
 - Further examination of PNPLA3 regulation, by fatty acid combinations relevant to in vivo conditions, is needed

Acknowledgments

See posters for more details



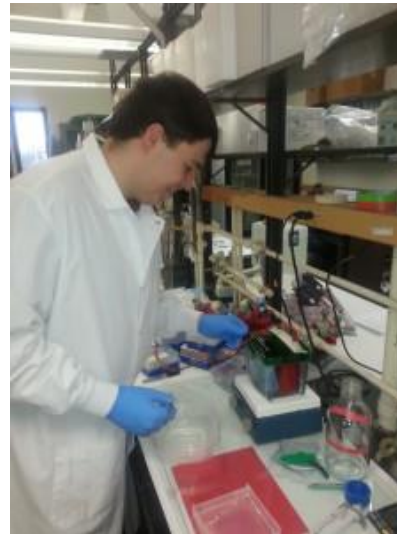
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Questions?

