

# **A brief summary of data indicating that SREBP activation is a key step in induction of steatosis by PFAS**

**Chris Corton**

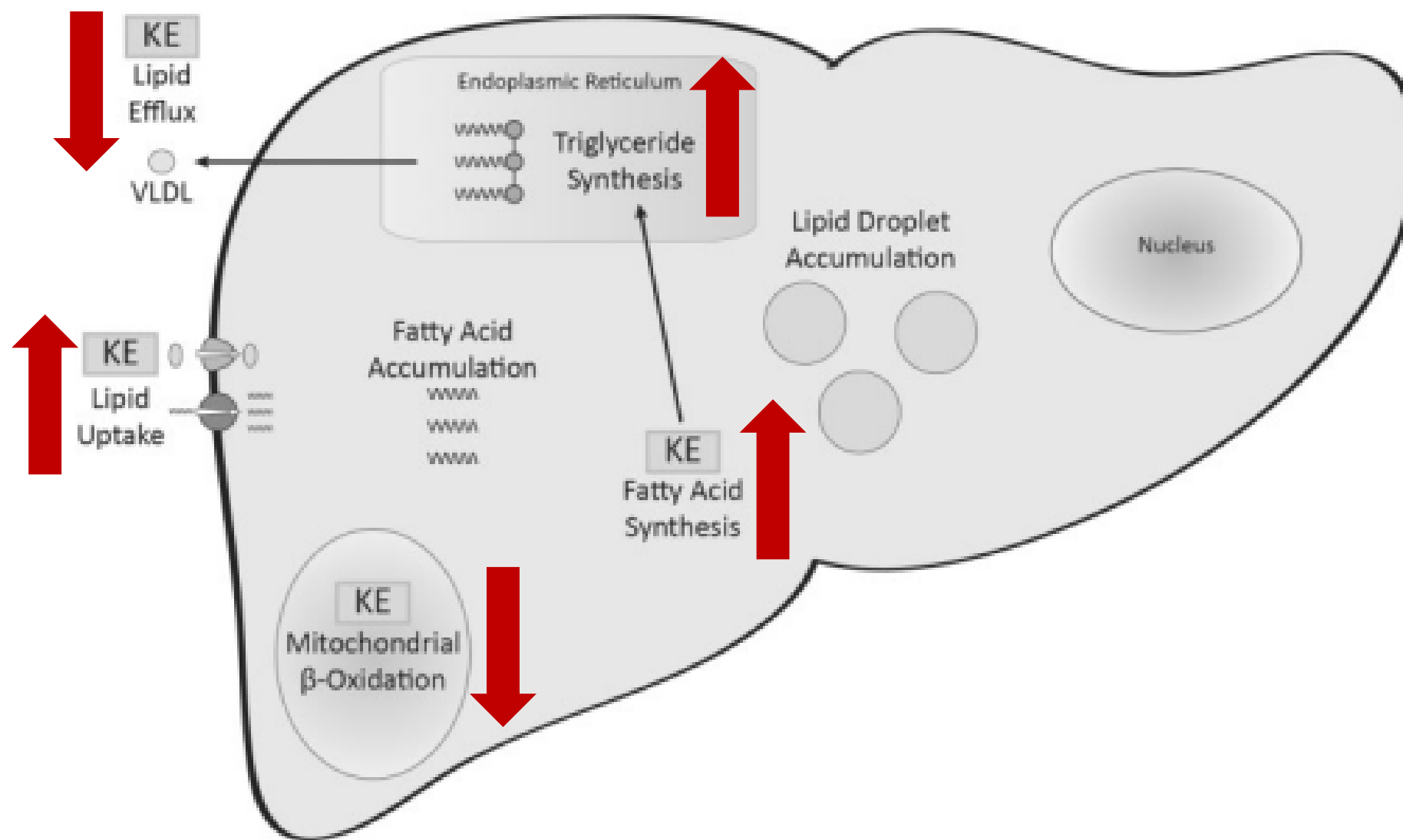


**Center for Computational Toxicology and Exposure  
US-Environmental Protection Agency  
Research Triangle Park, NC**

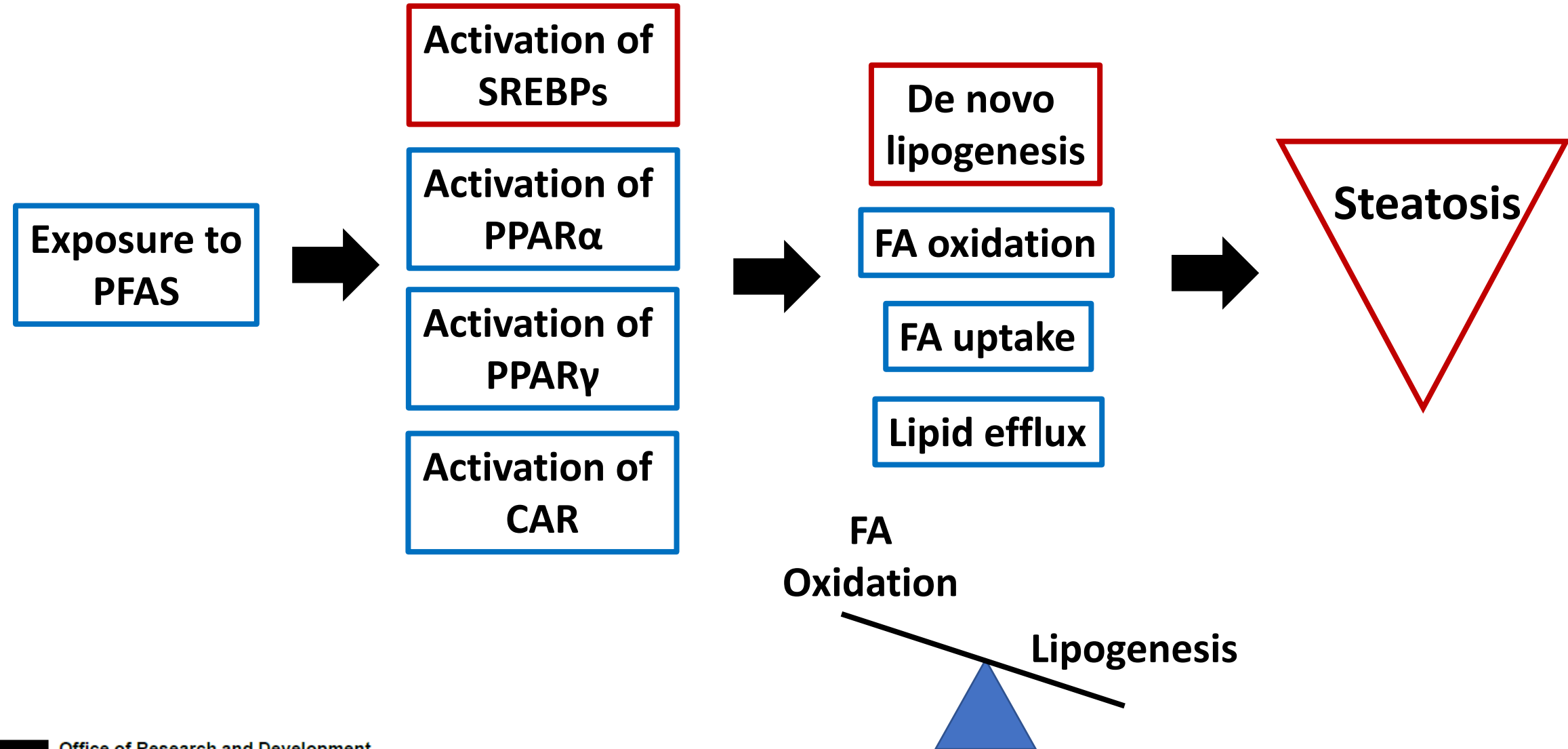
# Disclaimer

- The views expressed are those of Dr. Chris Corton and do not reflect US-EPA policy or product endorsement by the US-EPA.

# Major molecular events that can contribute to steatosis

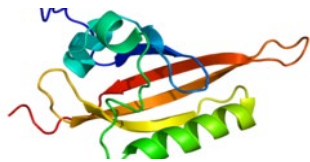


# Hypothesis

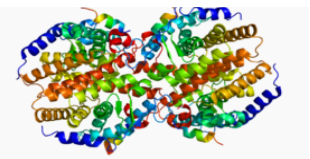


# Biomarkers that predict key events in the livers of mice and rats

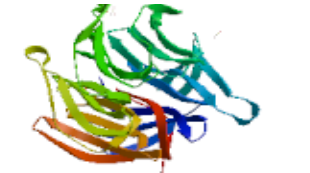
AhR



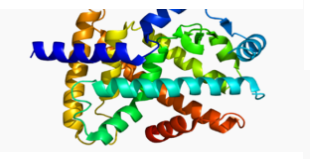
CAR



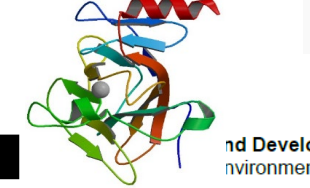
NRF2



PPAR $\alpha$



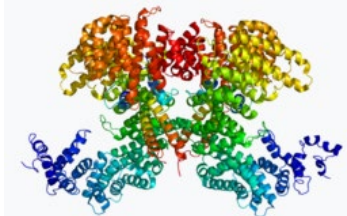
p53



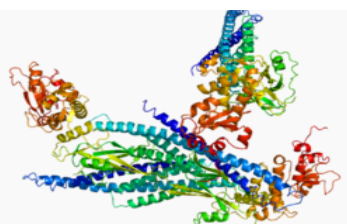
Estrogen  
Receptor  $\alpha$



SREBP



STAT5b

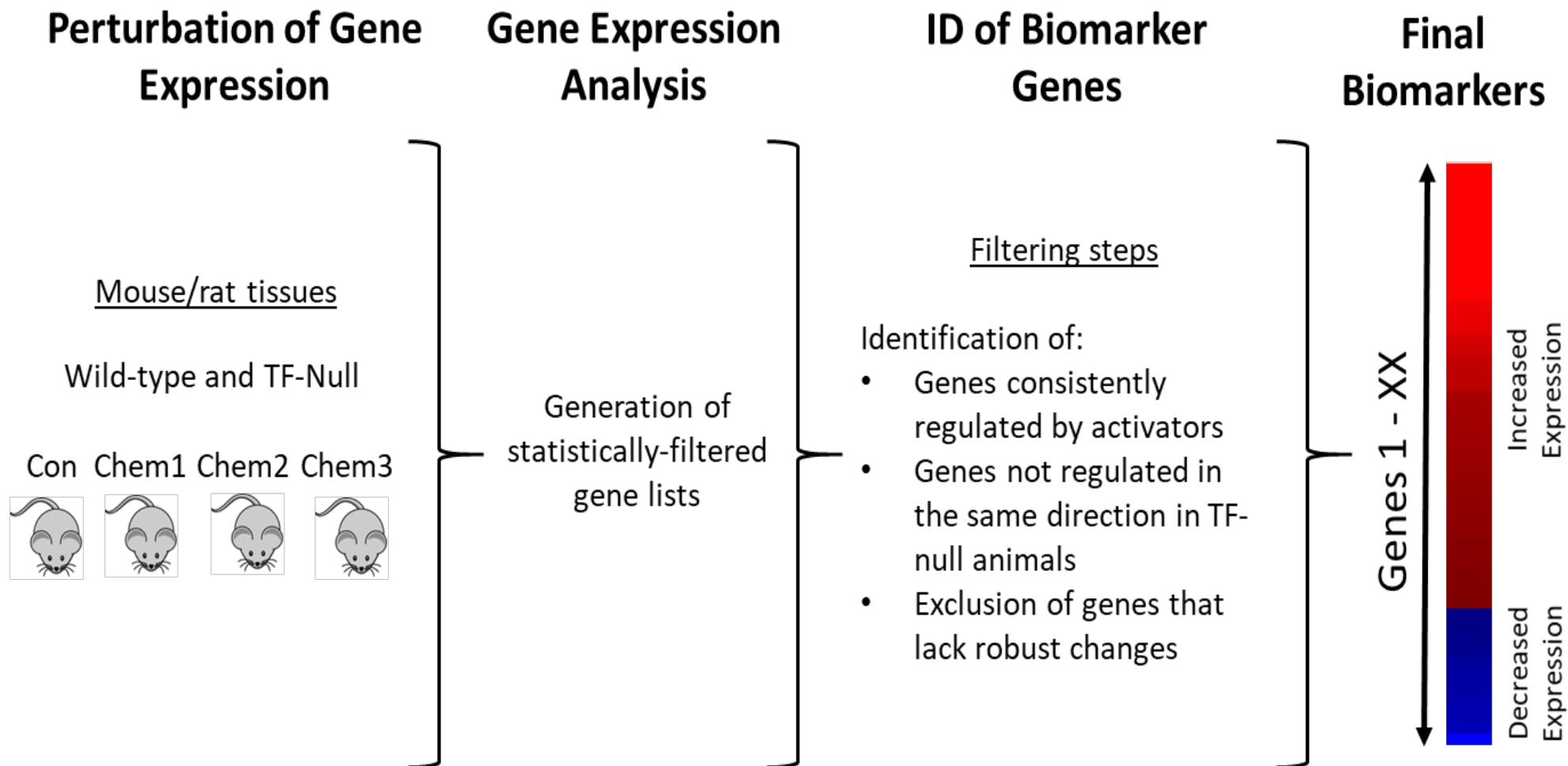


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- Oshida et al. (2015). Identification of Modulators of the Nuclear Receptor Peroxisome Proliferator-Activated Receptor  $\alpha$  (PPAR $\alpha$ ) in a Mouse Liver Gene Expression Compendium. PLoS One. 10(2):e0112655.
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- Rooney et al. (2018). Activation of CAR leads to activation of the oxidant-induced Nrf2. Toxicol Sci. 167:172-189.
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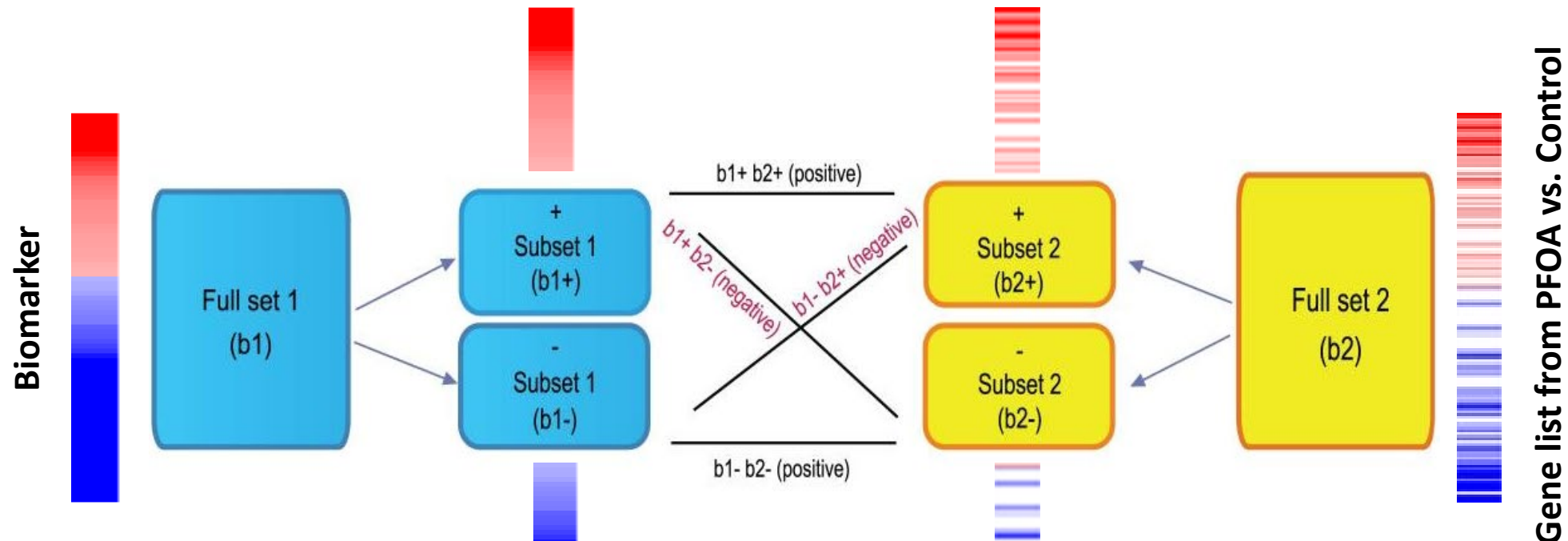
**PPAR $\alpha$ : Balanced accuracy = 98%**

**SREBP: Balanced accuracy = 95%**

# Construction of biomarkers from microarray data generated in animal tissues

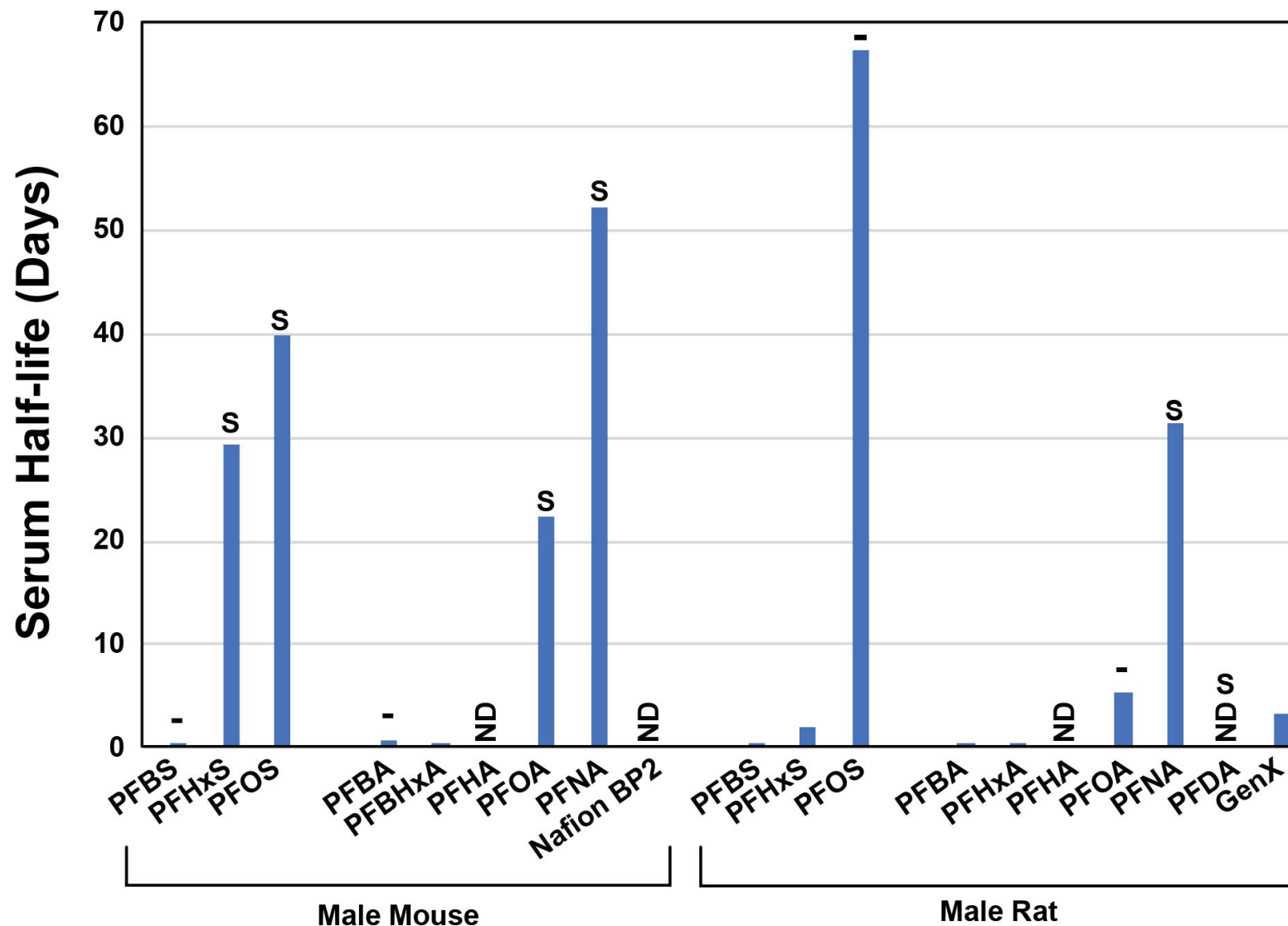


# Computing directionality and final correlation scores between two gene lists



- $\text{Score}(b1, b2) = \text{sum}(b1+b2+, b1+b2-, b1-b2+, b1-b2-)$
- Running Fisher Test p-value
- Direction of the correlation

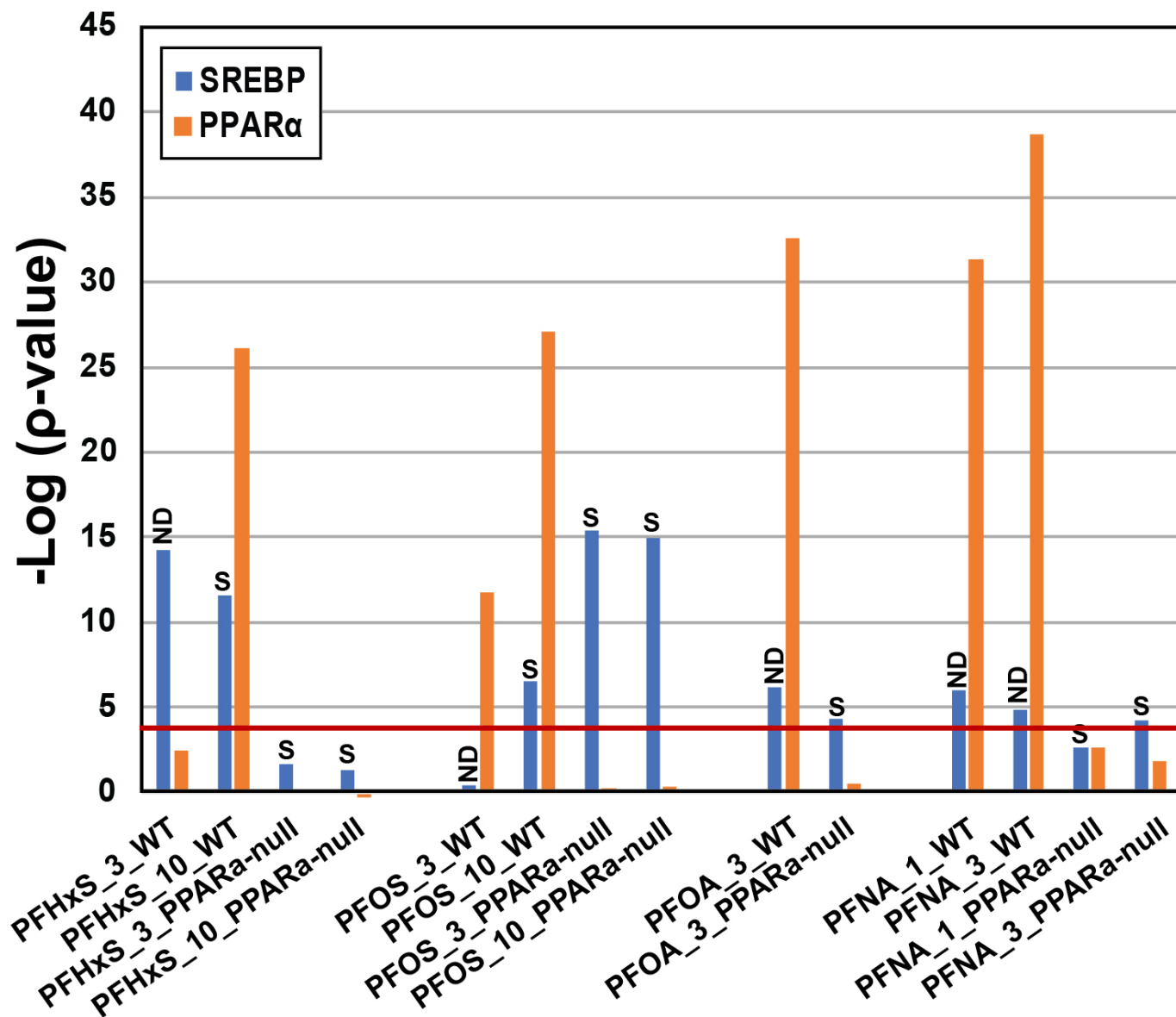
# PFAS With Longer Serum Half Lives Cause Steatosis



Discrepancy with PFOS in male rats could be due to differences in exposure conditions between

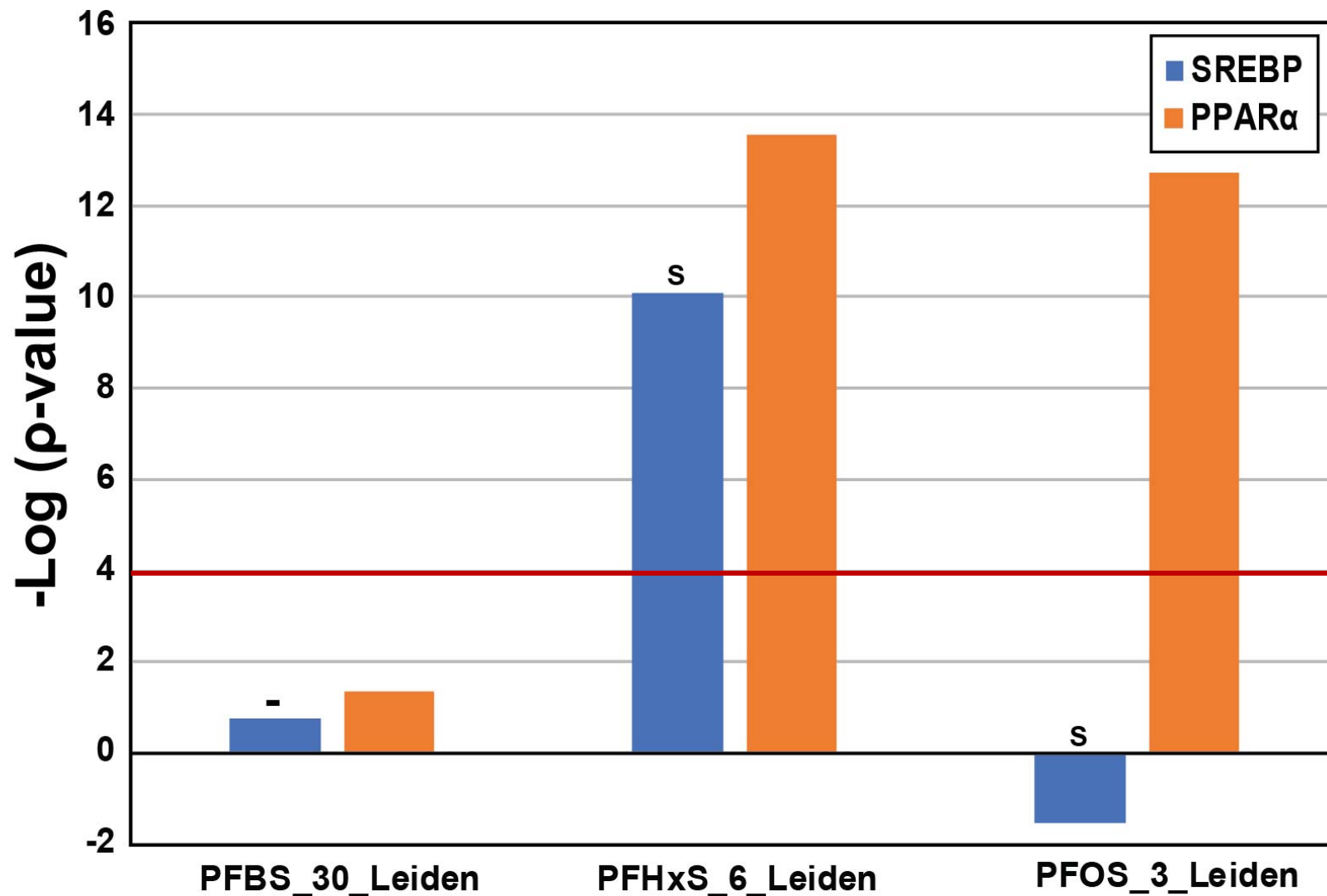


# SREBP is activated when steatosis is observed



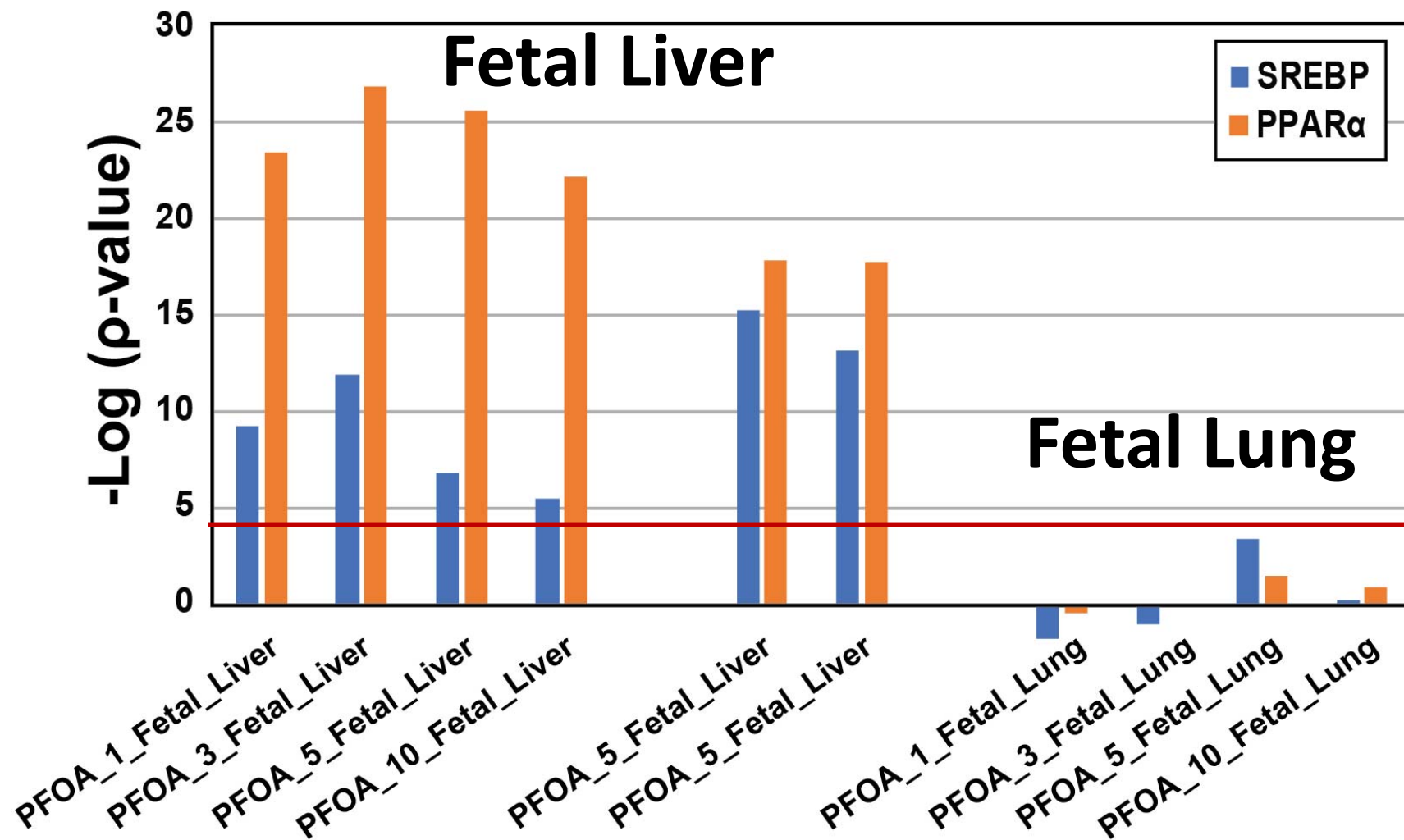
- Studies in wild-type and PPARa-null mice
- PPARa-null mice routinely have steatosis with or without chemical exposure
- Wild-type mice treated with PFHxS, PFOS, PFOA, PFNA

# SREBP is activated when steatosis is observed

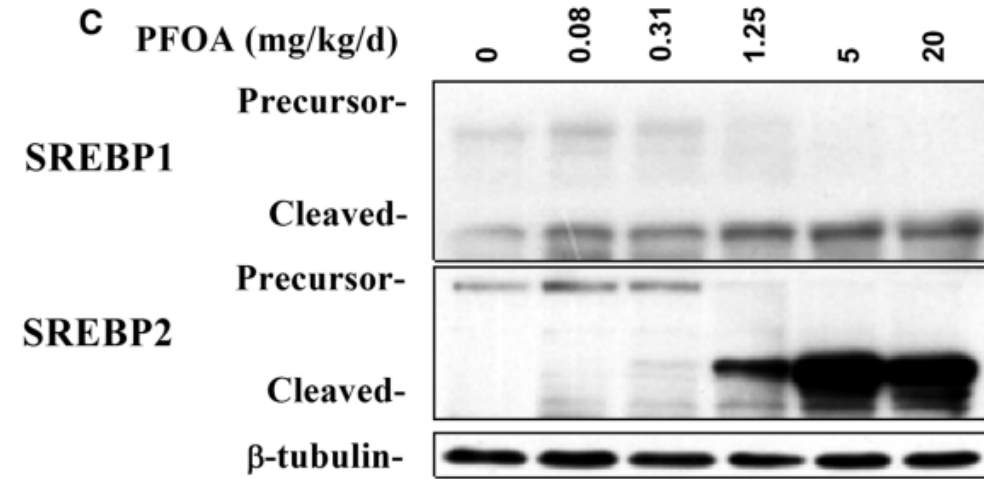
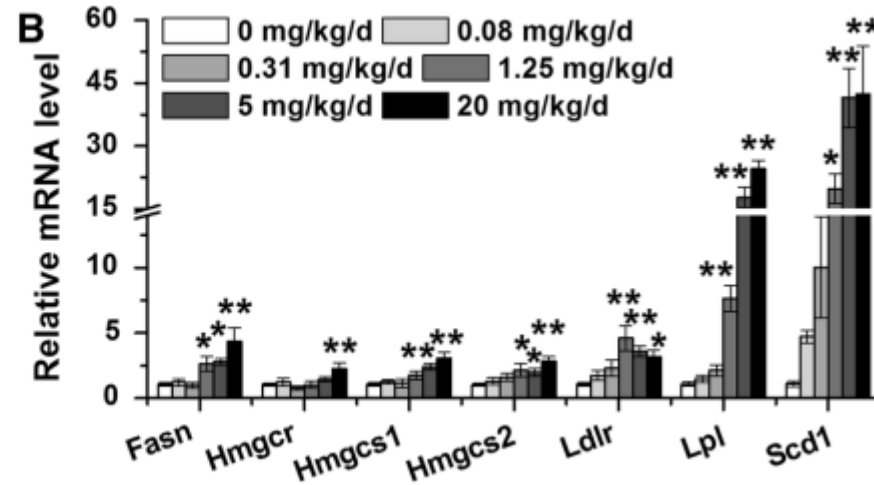
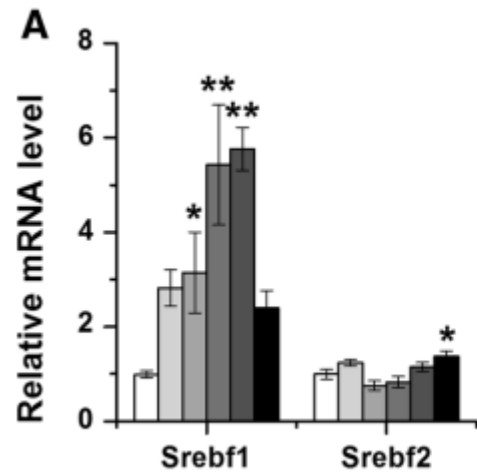


- APOE\*3-Leiden.CETP mouse study; 4-6 week exposures
- Microarray data from Bijland et al. 2011 ToxSci 123:290

# SREBP is activated in fetal liver but not fetal lung



# SREBP protein processing and activation of regulated genes after 28-day exposure to PFOA



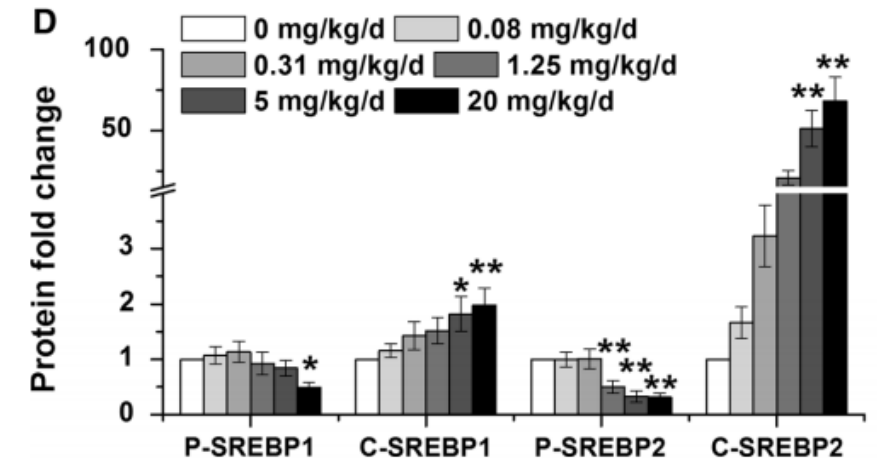
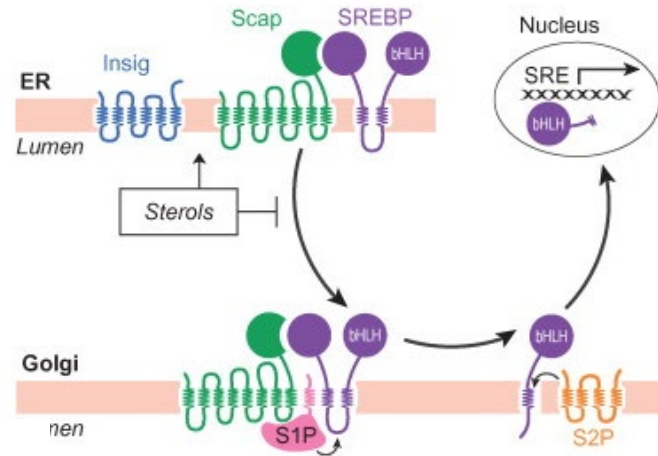
SREBP1a

SREBP1c

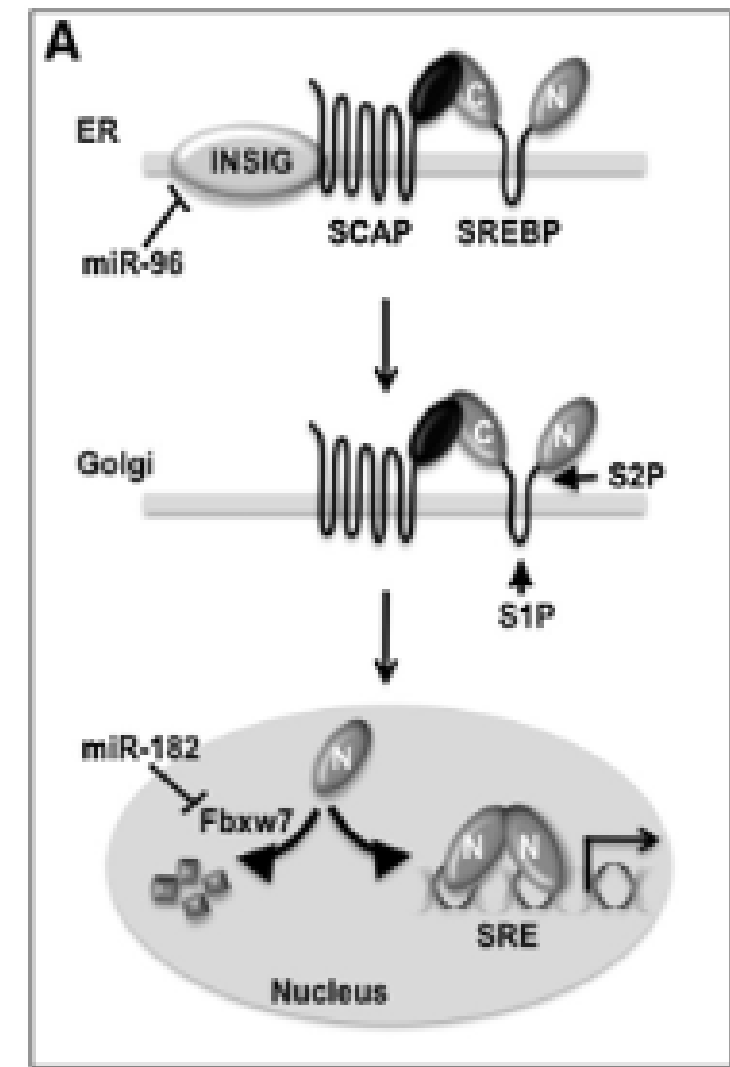
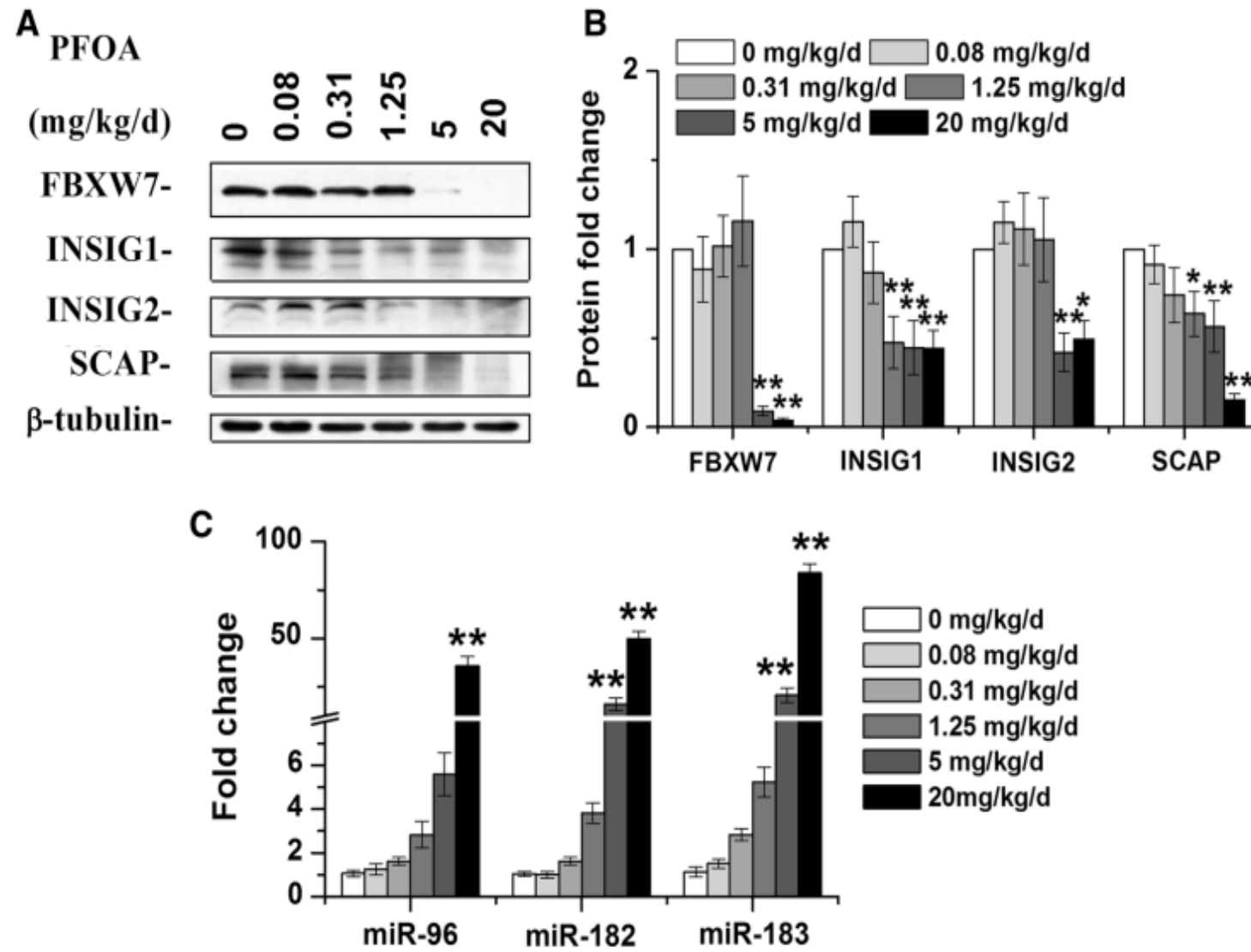
SREBP2

Lipogenic genes

Cholesterogenic  
genes



# Protein expression of key factors in the SREBP maturation pathway and miR183-96-182 cluster expression



# ACKNOWLEDGEMENTS



Environmental Protection Agency

**John Rooney**

Natalia Ryan

Brian Chorley

Thomas Hill



PamGene

Rinie van Beuningen

Rene Houtman



City of Hope Medical Center, Duarte  
Shiuan Chen



NIEHS

Nicole Kleinstreuer



Health  
Canada

Health Canada

Carole Yauk

Andrew Williams



Merck

Frank Sistare

Chunhua Qin



Universiteit  
Leiden

University of Leiden

Bob van de Water

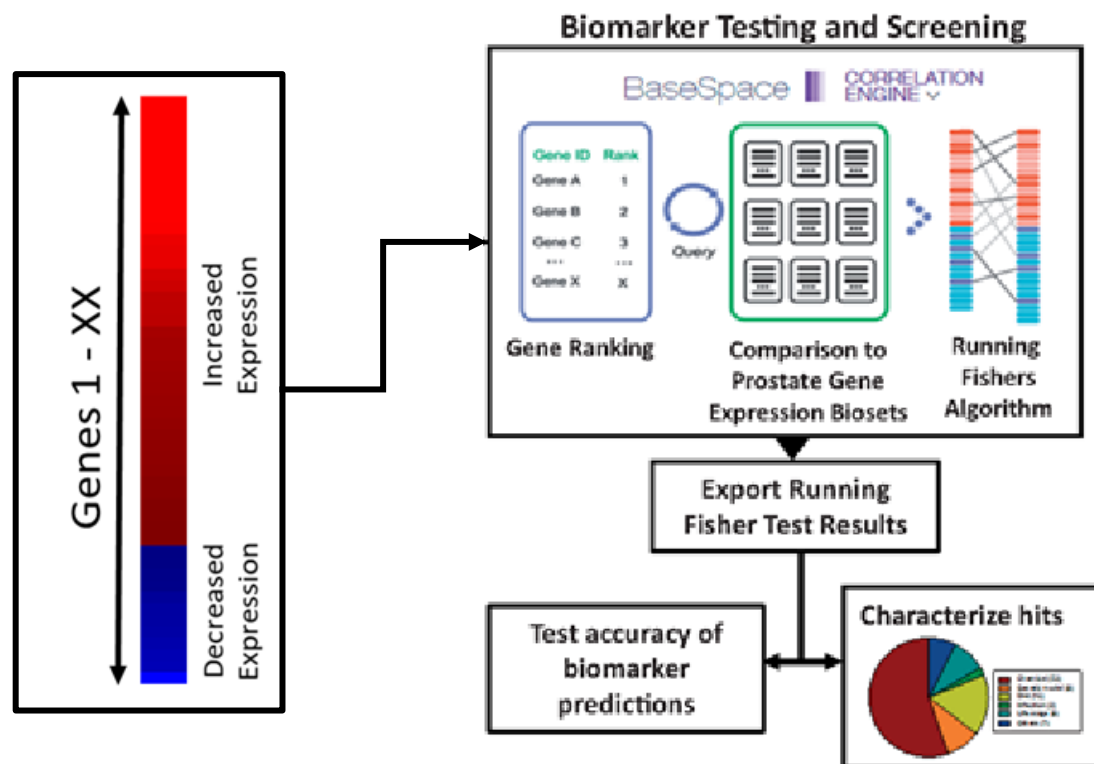
Steve Hiemstra



**Support from EPA Chemical Safety for  
Sustainability Research Program**

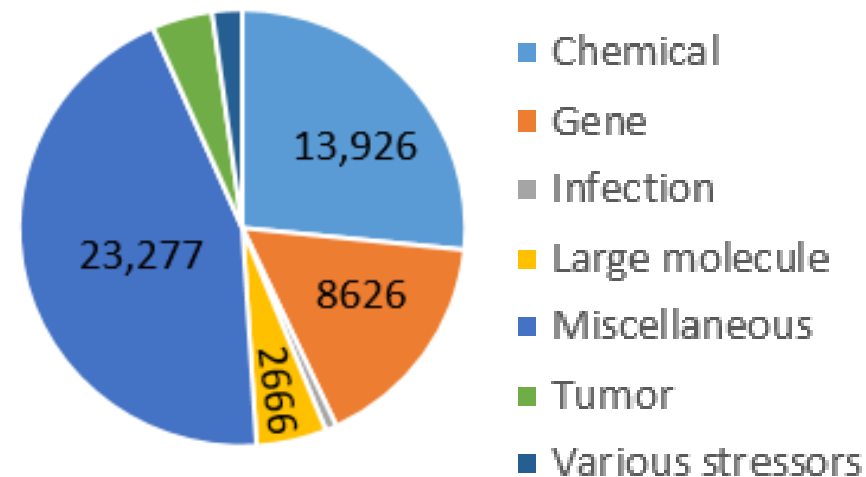
# Extras

# Comparing gene lists in BSCE



- Utilize Illumina's BaseSpace Correlation Engine
- Contains ~130,000 microarray comparisons of statistically significant genes
- Valuable computational tools
- Compares all microarray comparisons to each other in a pairwise fashion using a Running Fisher test
- For each pair-wise comparison: generates the number of overlapping genes, correlation direction and p-value

- ~51,600 microarray comparisons in human database



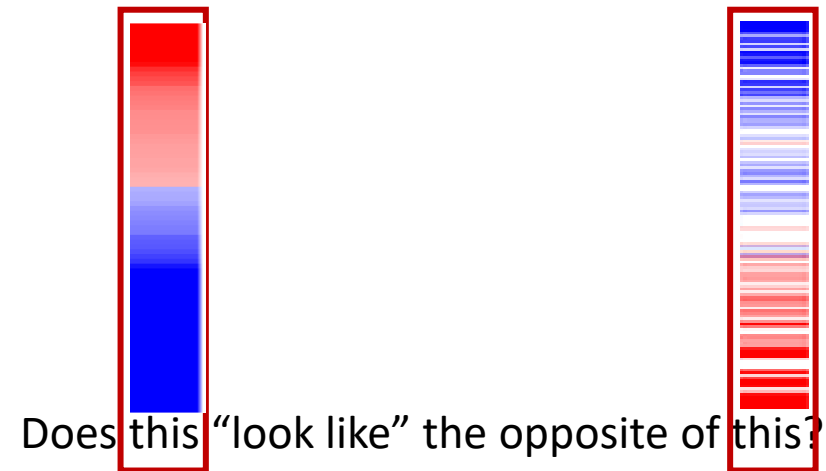
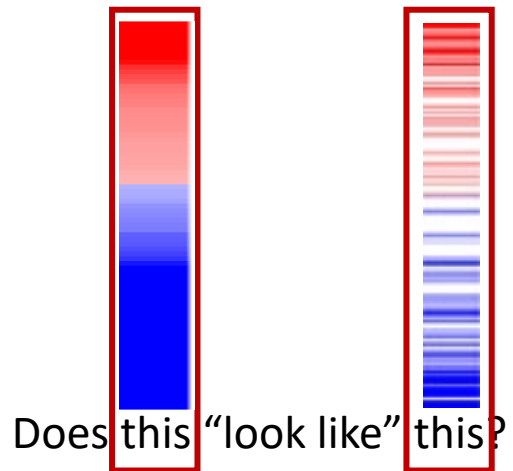
- Includes ~1950 chemicals
- ~8600 perturbations of ~1700 genes (knockdowns, overexpression, mutants)
- Greatly accelerated construction and analysis of biomarkers

Derived from Rooney et al. Toxicol Sci. 166:146-162



# Correlation analysis using the Running Fisher Test

- Identification of factors (chemicals, hormones, diets, genes, etc.) that “look” like your gene list



- Correlation can be determined computationally using the Running Fisher test in BSCE

