Genetic and epigenetic alterations associated with latent liver carcinogenesis due to early-life dichloroacetic acid exposure in mice

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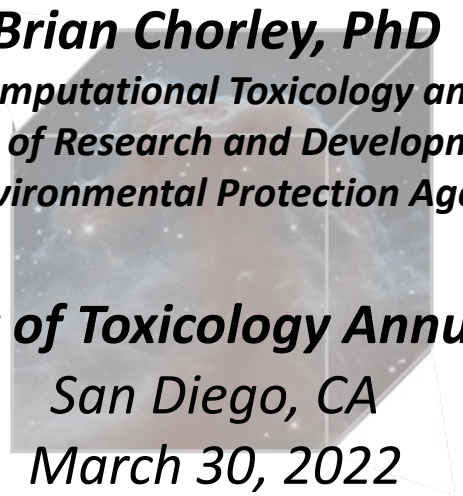
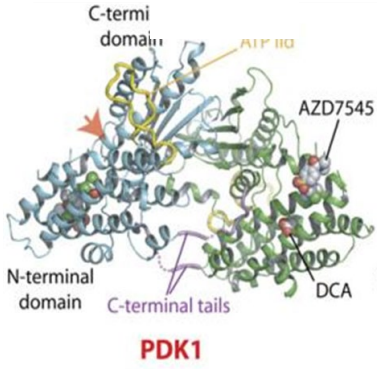


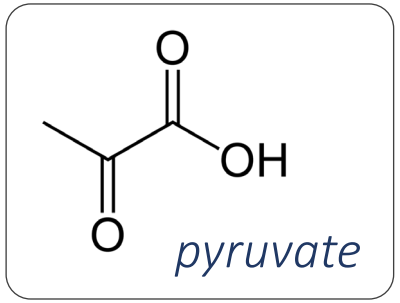
www.nasa.gov: Horsehead nebulae as seen by Hubble (2013)

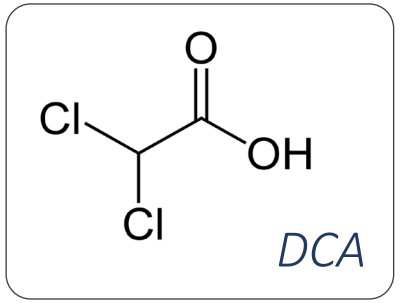
The opinions expressed are those of the speaker and not of the US EPA. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

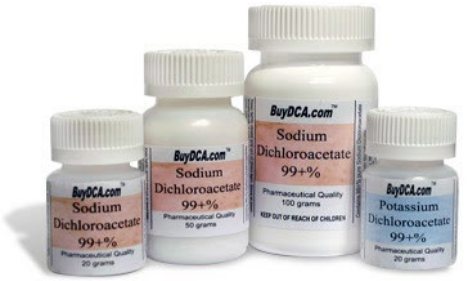
Case study: Dichloroacetic acid

- Common low-level drinking water contaminant
- Distinctive metabolic programming effects
  - pyruvate dehydrogenase kinase (PDK) inhibitor
  - Glutathione S-transferase zeta 1 (GSTZ1) inhibitor
- Liver carcinogen in mice and rats with chronic and **prior** exposure
- Not a direct mutagenic mode-of-action (MOA) but **alternative MOAs unclear**
  - Persistent cell metabolism? Oxidative burden? Mitochondrial/ER stress? Enhanced cell aging?



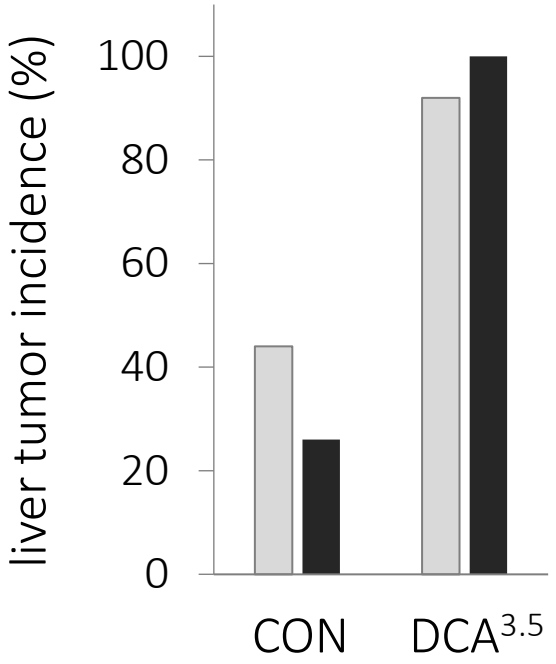


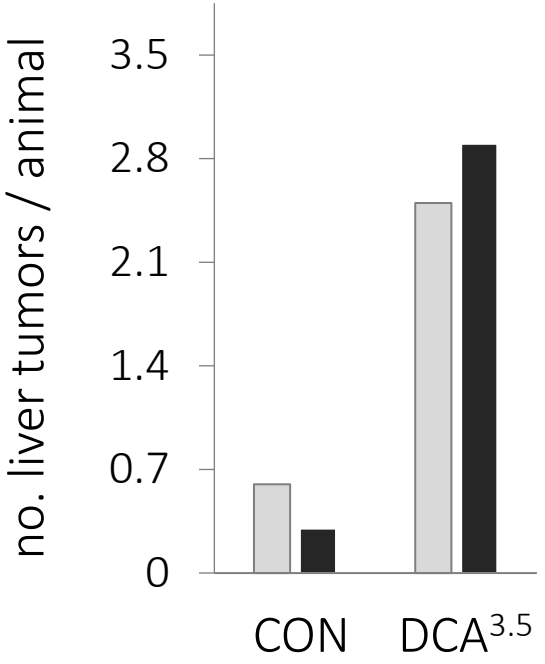




Slide courtesy of Dr. Charles Woods

Early life exposure of DCA increases liver tumor incidence similar to chronic exposure





♂ $B6C3F_1$ mice

Prior DCA (age 4-14 wks)

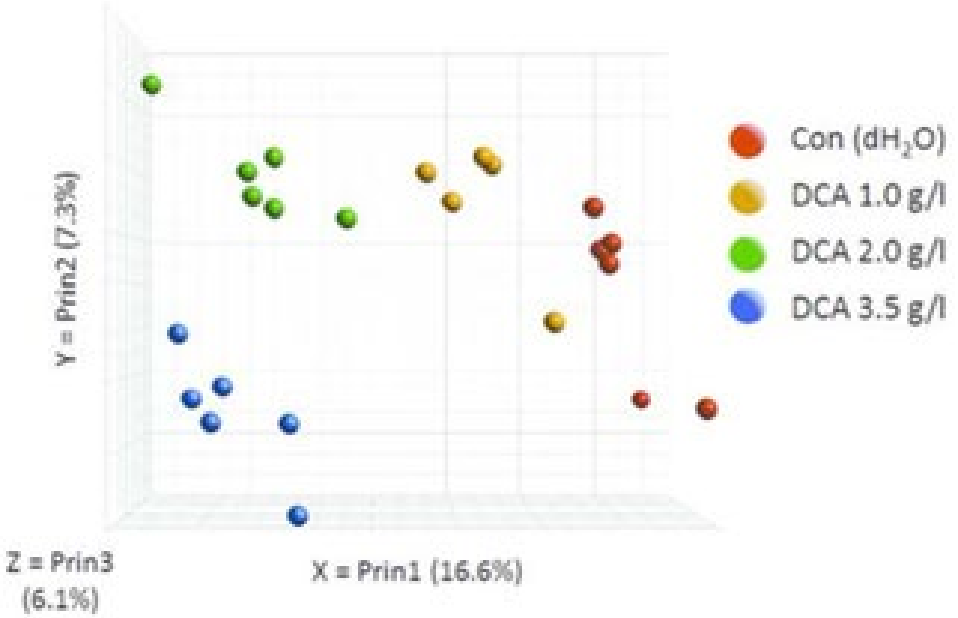
Chronic DCA (age 4-90+ wks)

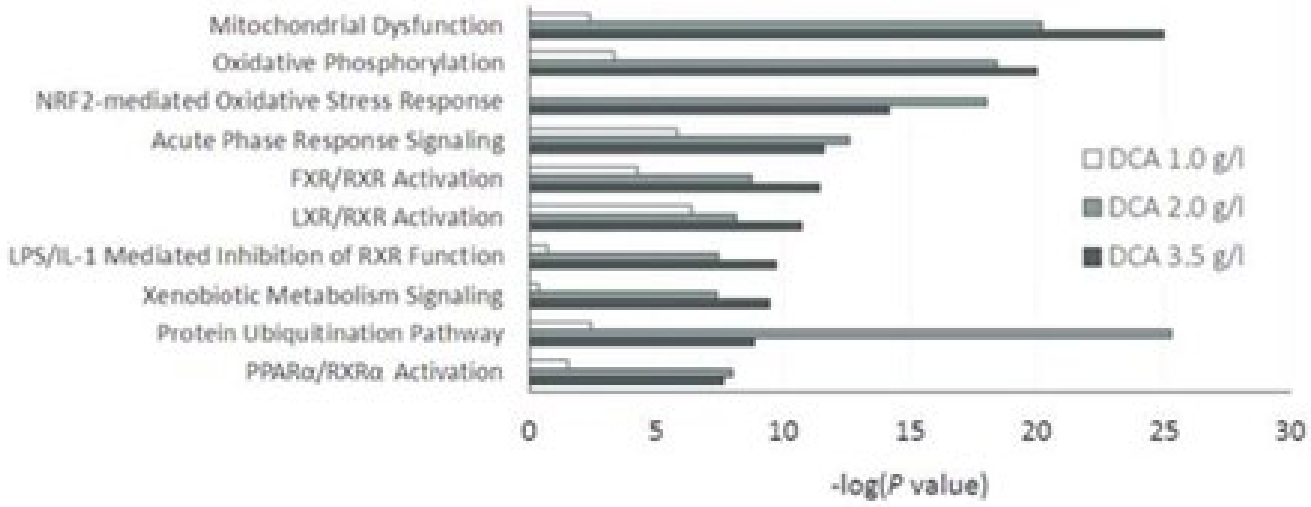
DeAngelo et al. 1998
Wood et al. 2015

Slide courtesy of Dr. Charles Woods

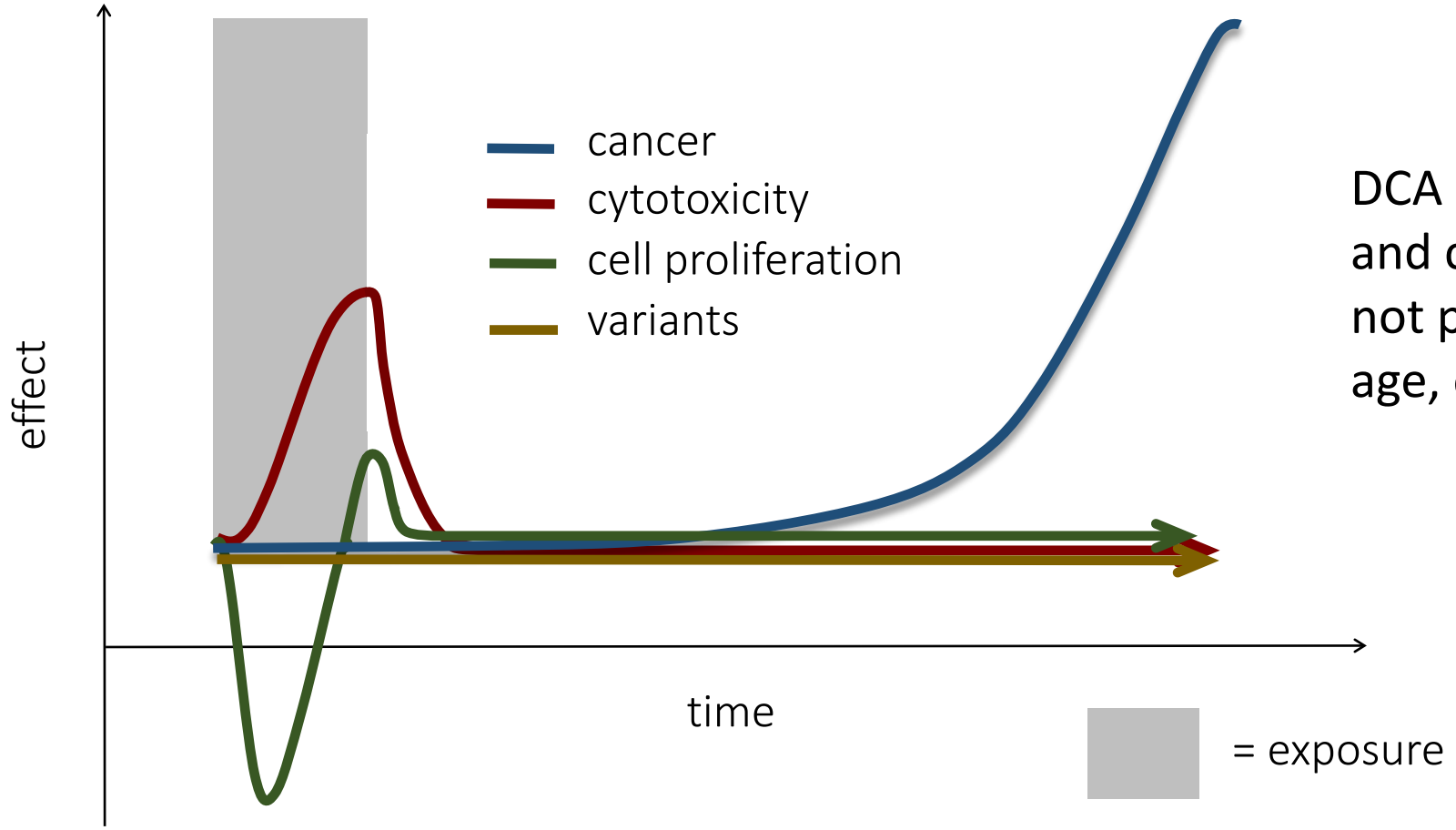
Previous gene expression results with acute 6-day exposure

(Wehmas 2017)





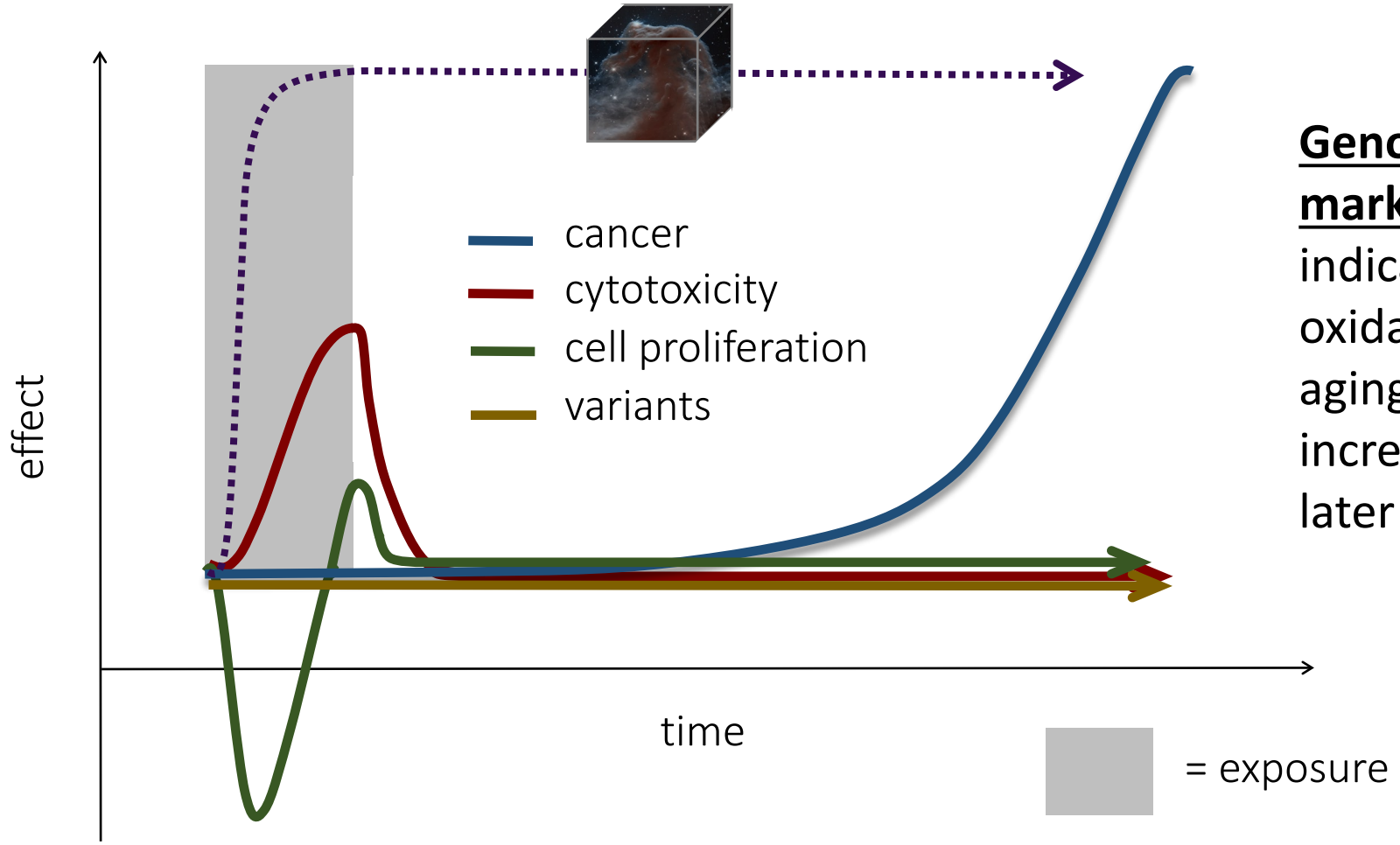
Previous DCA studies summary



DCA effects on cytotoxicity and cell proliferation did not persist, increase with age, or affect variant calls.

DeAngelo et al. 1998
Wood et al. 2015
Wehmas et al. Tox Sci 2017

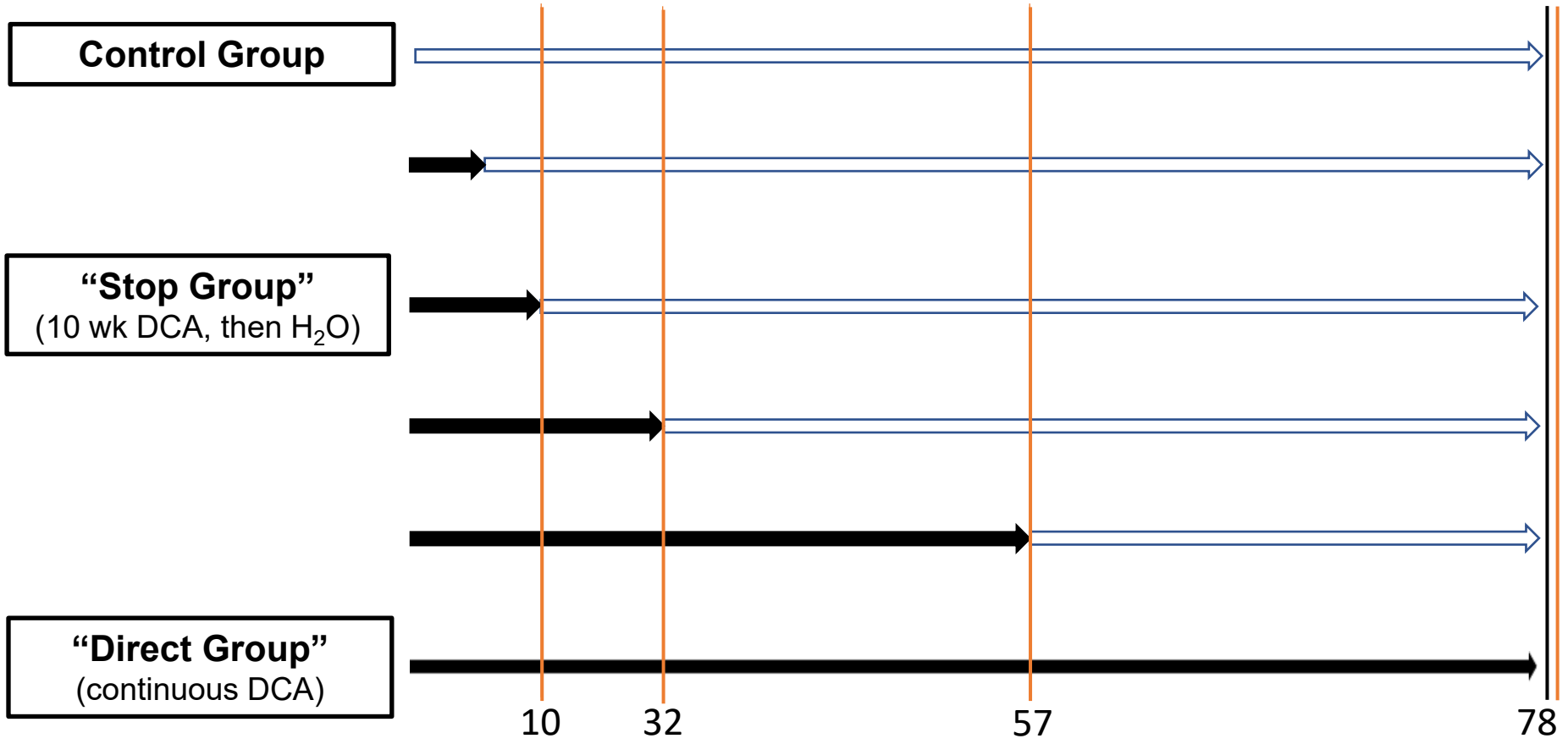
Hypothesis



Genomic or epigenomic markers persist which indicate metabolic/ oxidative stress/advanced aging processes contributing increased liver tumor burden later in life.

DeAngelo et al. 1998
Wood et al. 2015
Wehmas et al. Tox Sci 2017

DCA stop-exposure time course study



♂ $B6C3F_1$ mice

DCA (3.5 g/l) ➡

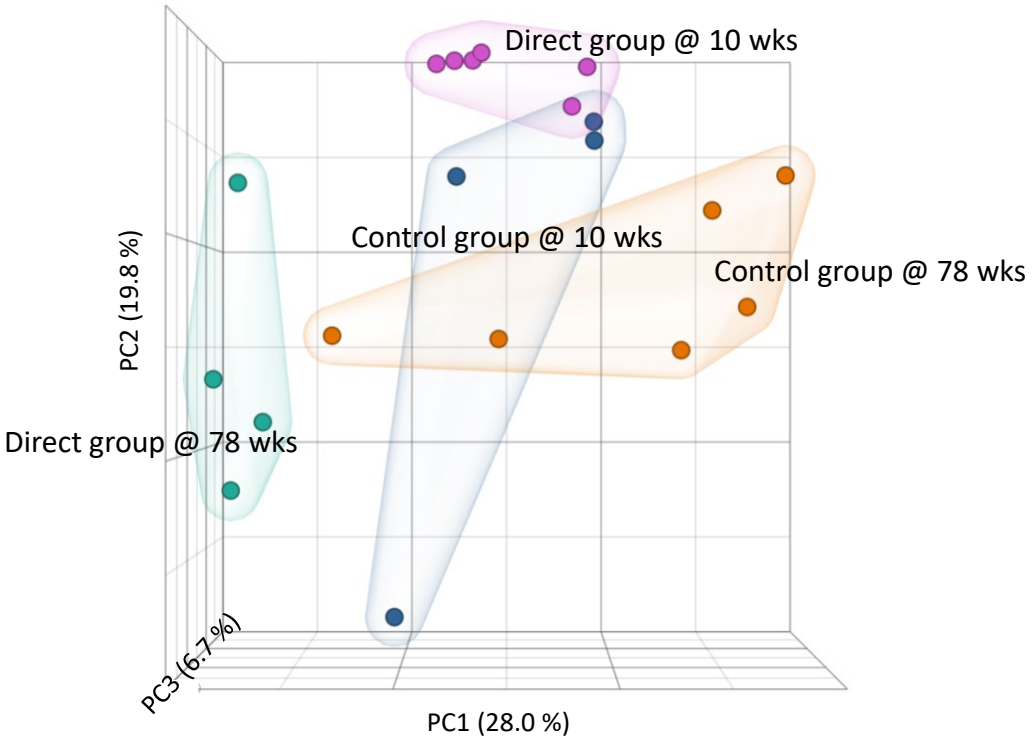
dH_2O ⇨

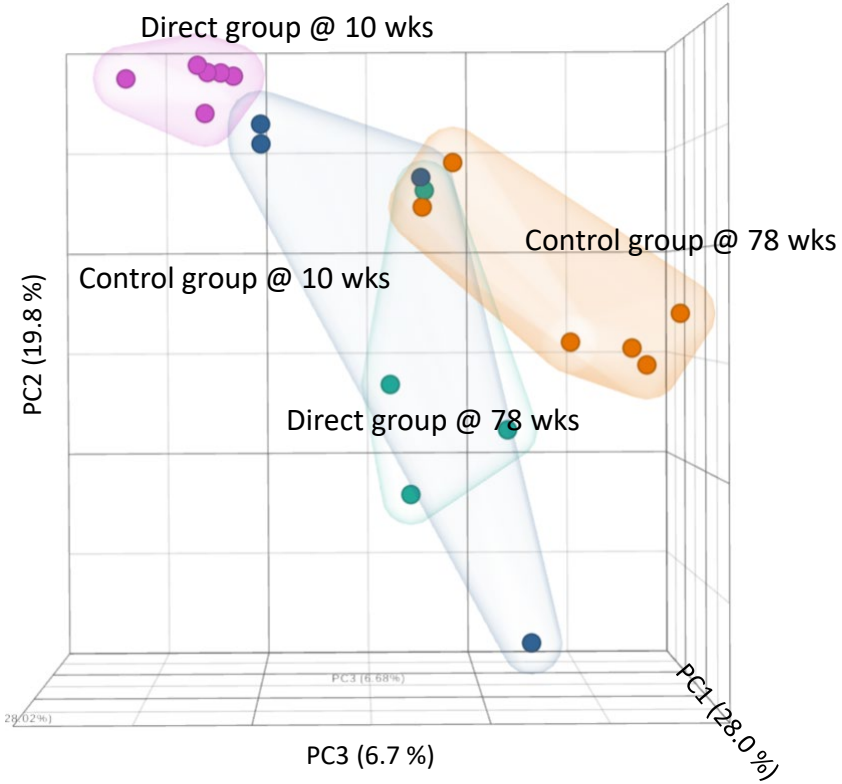
Liver samples available

Frozen	FFPE
Reduced Representation Bisulfite Sequencing	Targeted RNA-seq (1500+ panel, TempO-seq)

Wehmas et al. Tox Sci 2017

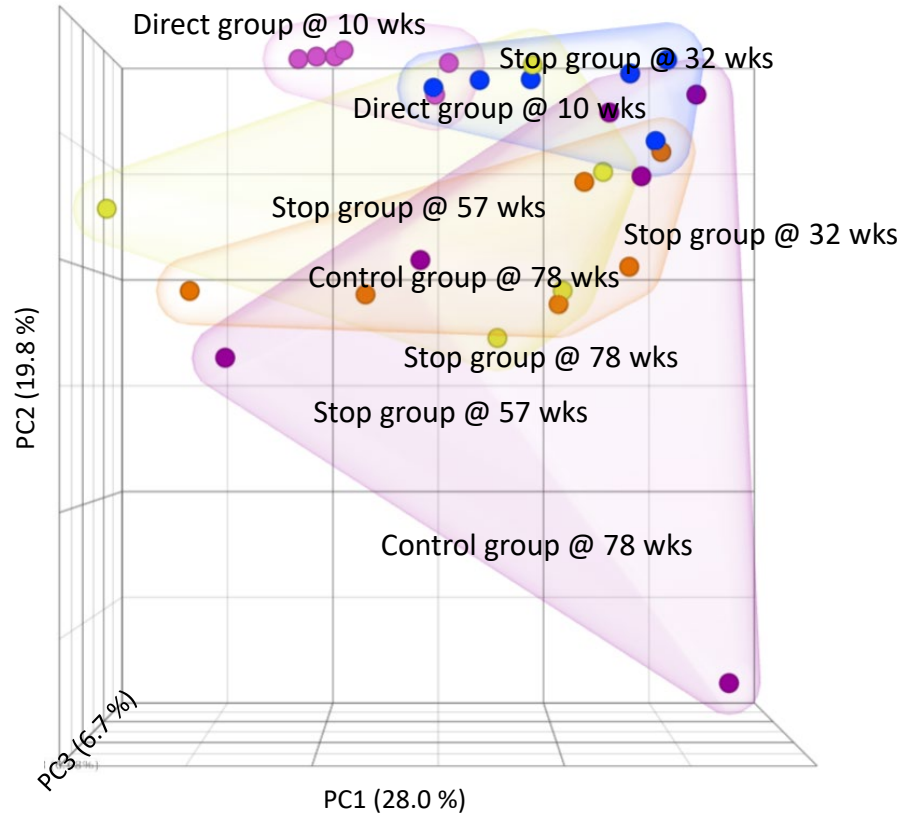
Recent DCA exposure elicits more robust gene expression response



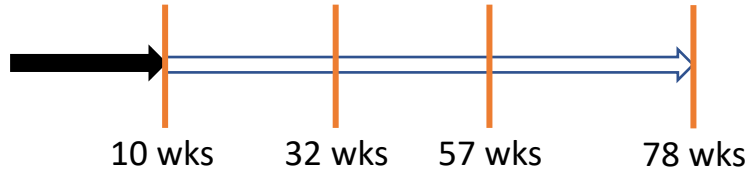


Principal Component Analysis of Gene Expression Data

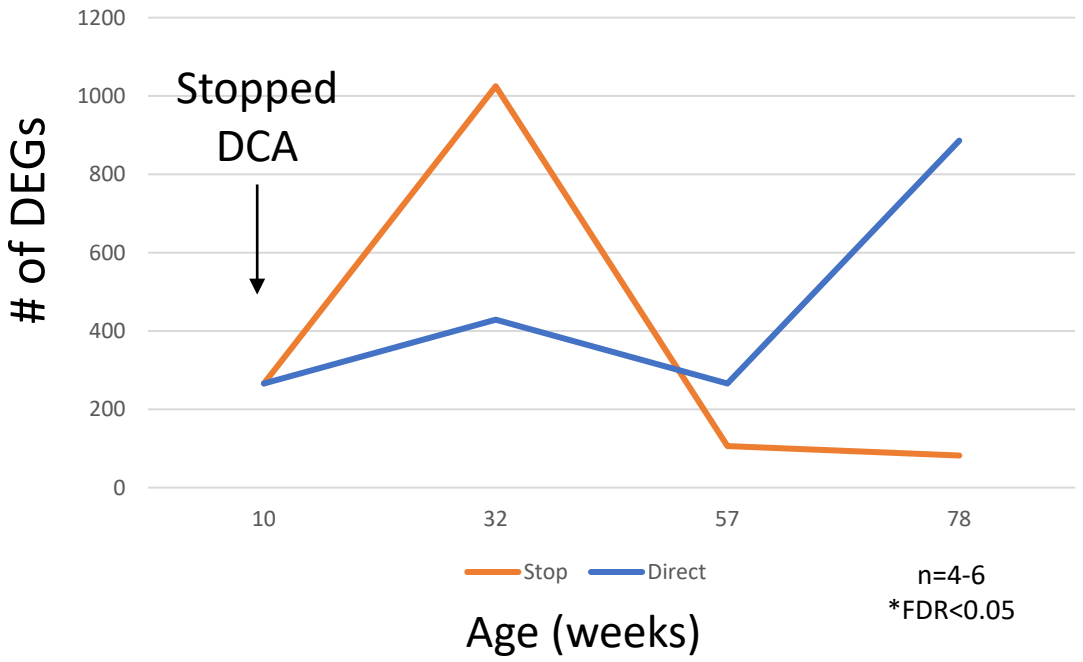
Gene expression drifts toward control levels as time passes after DCA exposure

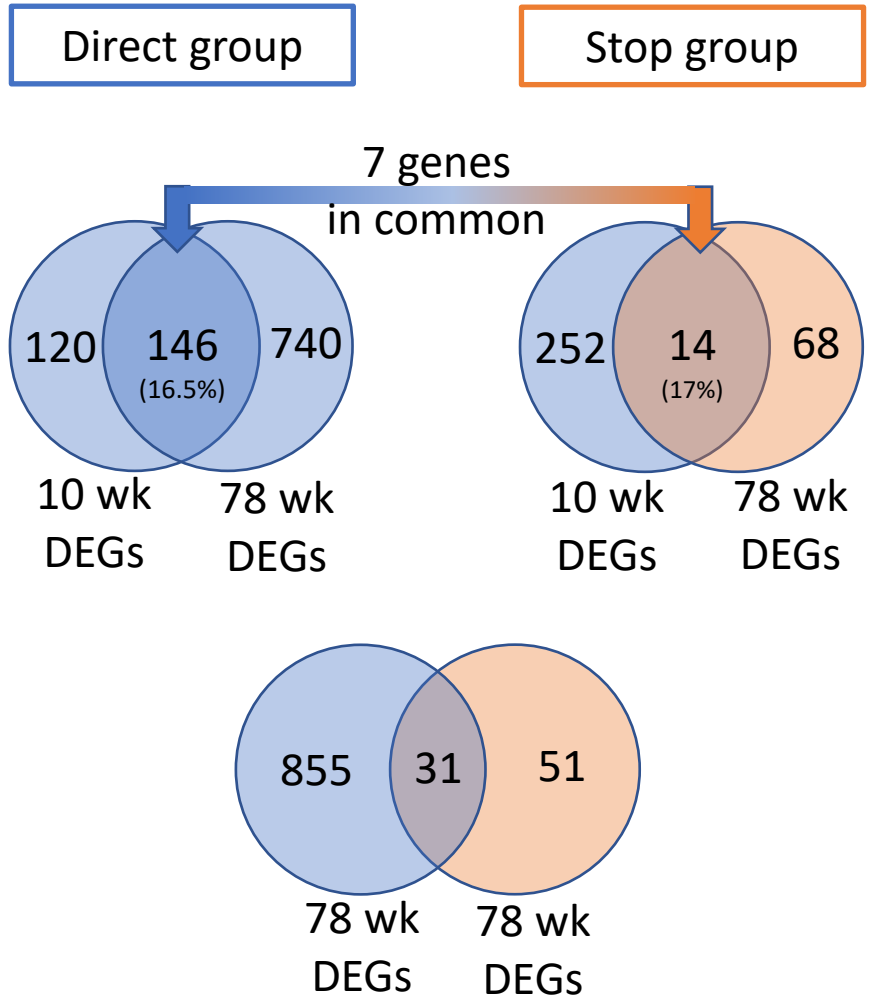


"Stop Group"
(10 wk DCA, then H_2O)

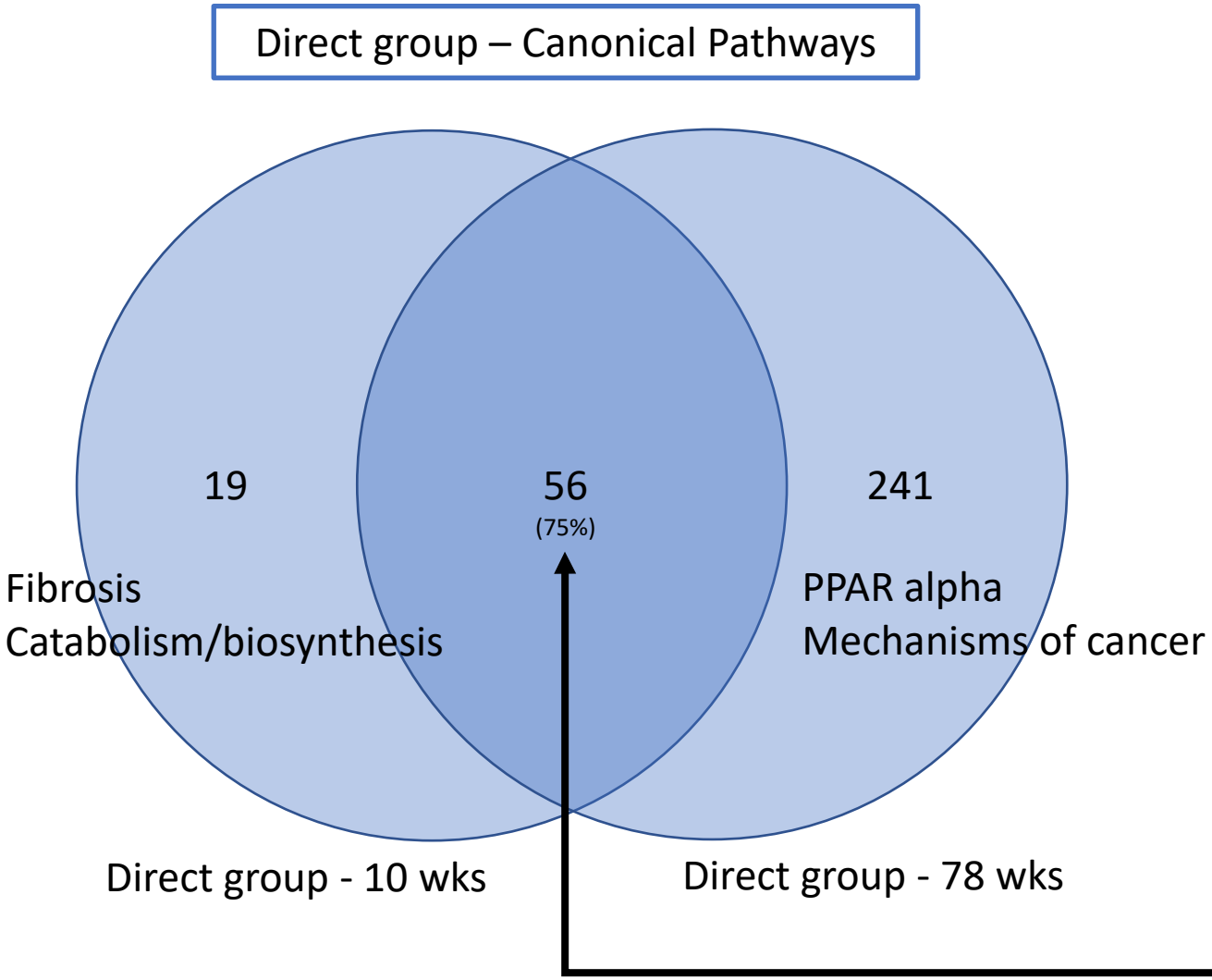


Differentially expressed genes in direct and stop groups





Expected gene pathways altered with direct DCA exposure



Oxidative stress
- NRF2 activation
- AHR activation

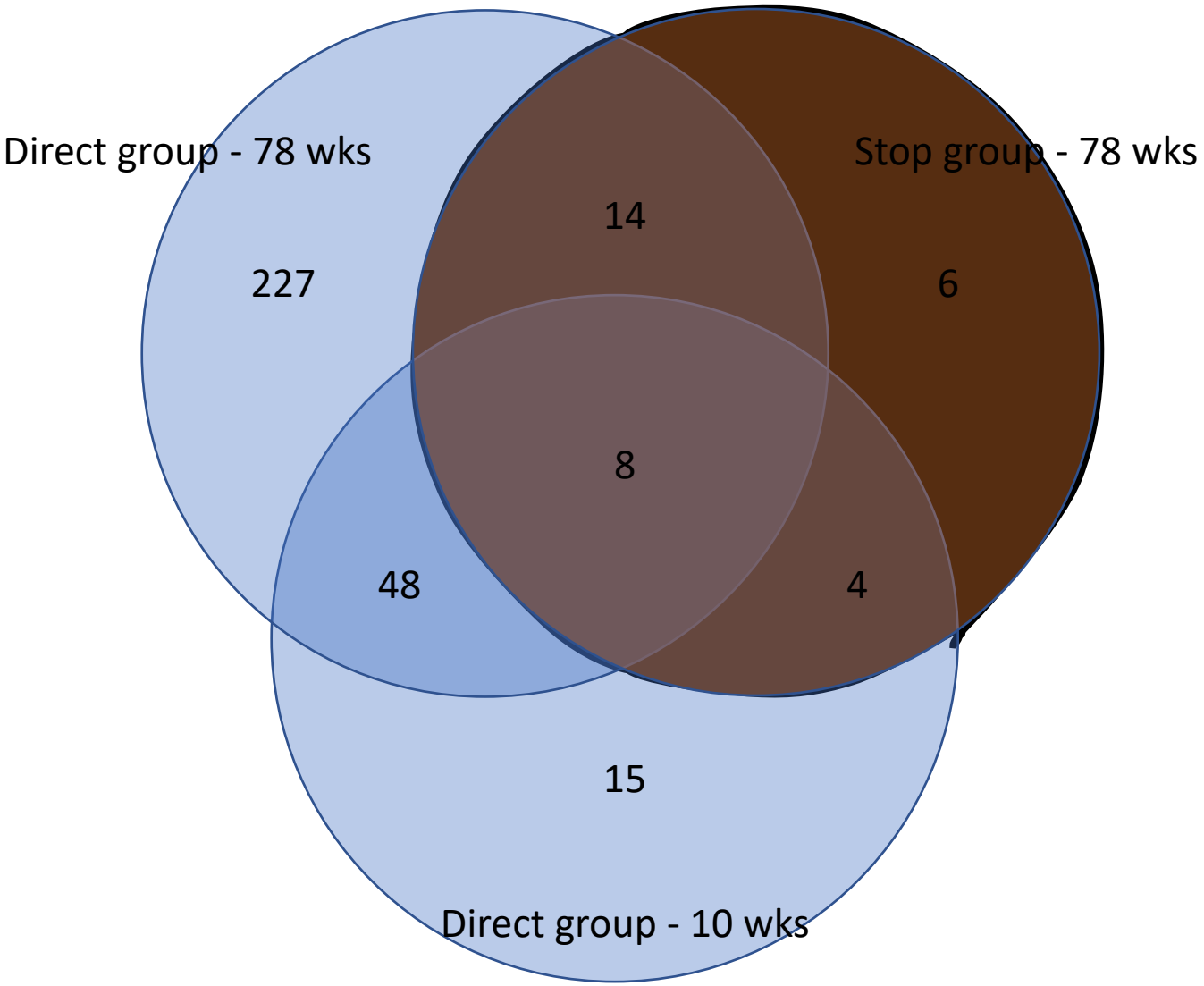
Metabolism/glucose homeostatsis
- Fatty acid B-oxidation
- FXR/RXR; PXR/RXR; GR
- Xenobiotic metabolism
- AMPK signaling

Cell cycle control/apoptosis
- p53 signaling
- PI3K/AKT signaling
- Wnt/B-catenin
- G1/S Checkpoint regulation
- mTOR signaling

Insulin signaling
- PI3K/AKT signaling
- Sirtuin pathway

Coagulation/collagen deposition
- Coagulation system
- Intrinsic/extrinsic prothrombin
- GP6 pathway
- Complement system

Stop vs. Direct group – Canonical Pathways



Stop in common with direct @ 78 weeks

Oxidative stress

NRF2 activation

AHR activation

Cell regulation/proliferation

ERK-MAPK Signaling

PI3K Signaling

HOTAIR Regulation

Stop in common with direct @ 10 weeks

Cell regulation/proliferation/cancer

Mitotic Roles of Polo-Like Kinase

Specific for stop group @ 78 weeks

VDR/RXR Activation

Growth Hormone Signaling

Mitochondrial/Oxidative Phosphorylation

Apelin Liver Pathway

RAR Activation

Ketolysis (OXCT1)

Inflammation

Calcium Transport (ATP2A1)

Insulin Signaling

IGF-1 Signaling

B Cell Receptor Signaling

Coagulation/collagen/ECM deposition

IL-6 Signaling

Hepatic Fibrosis / Stellate Cell Activation

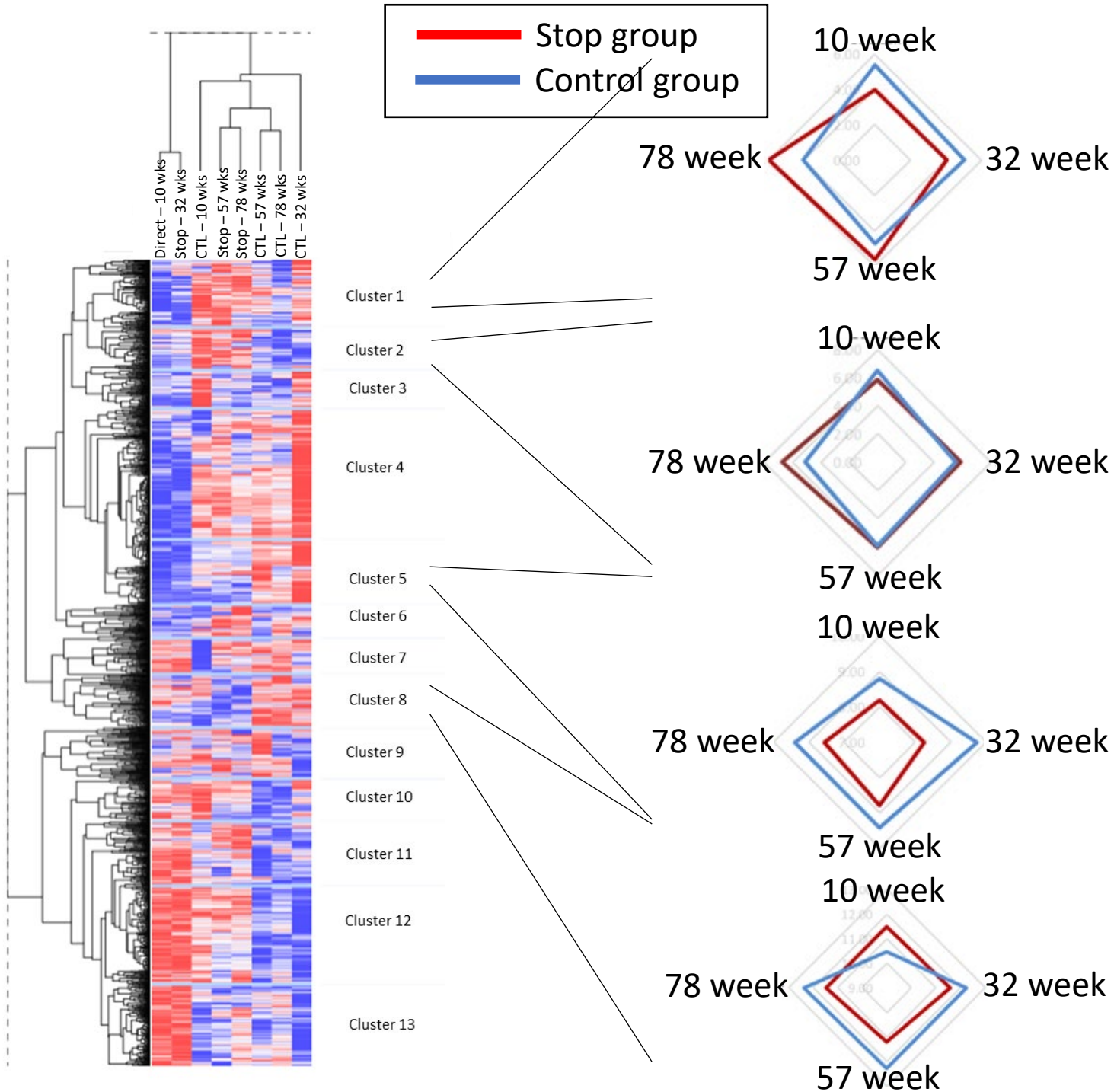
Dendritic Cell Maturation

Coagulation/collagen/ECM deposition

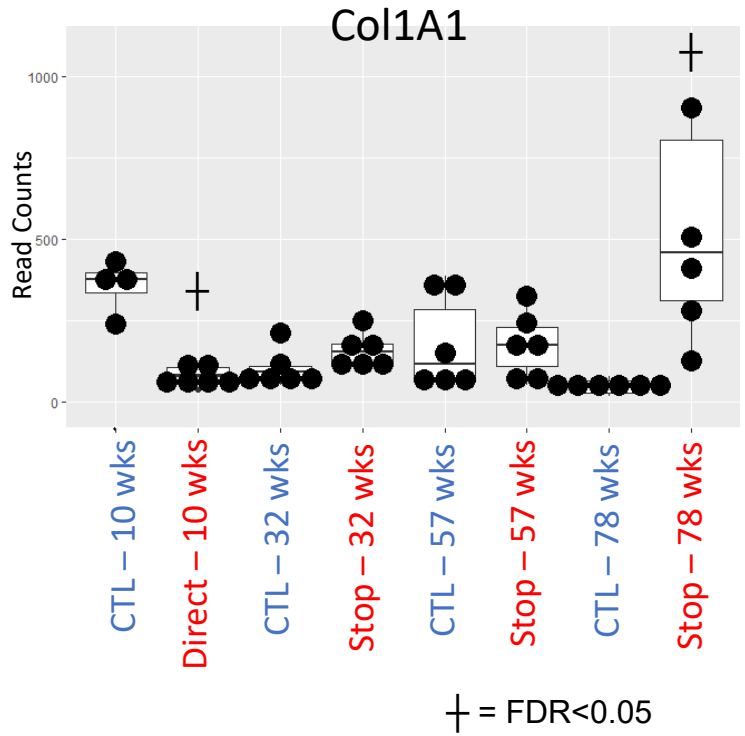
GP6 Signaling

Intrinsic Prothrombin Activation

ILK Signaling



The Stop Group "Flips" Over Time

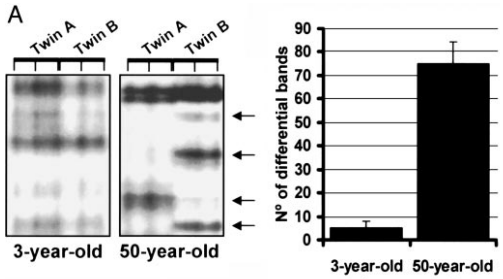


Why epigenetics?

It tells us something we cannot (and need to) know otherwise

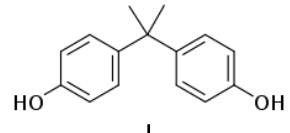
Environmental exposures can alter the epigenome in quantitative way

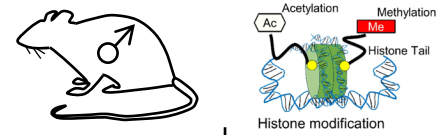
Wolff et al. *FASEB* 1998



Fraga et al. *PNAS* 2005

Alterations occur early after exposure and persistent epigenetic marks may serve as a “footprint” of environmental exposure



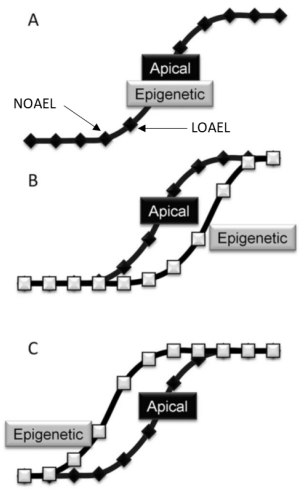


The alterations may be mechanistically linked to adverse outcomes, susceptibility, or even multi/transgenerational effects

Western diet challenge

Liver metabolic dysfunction

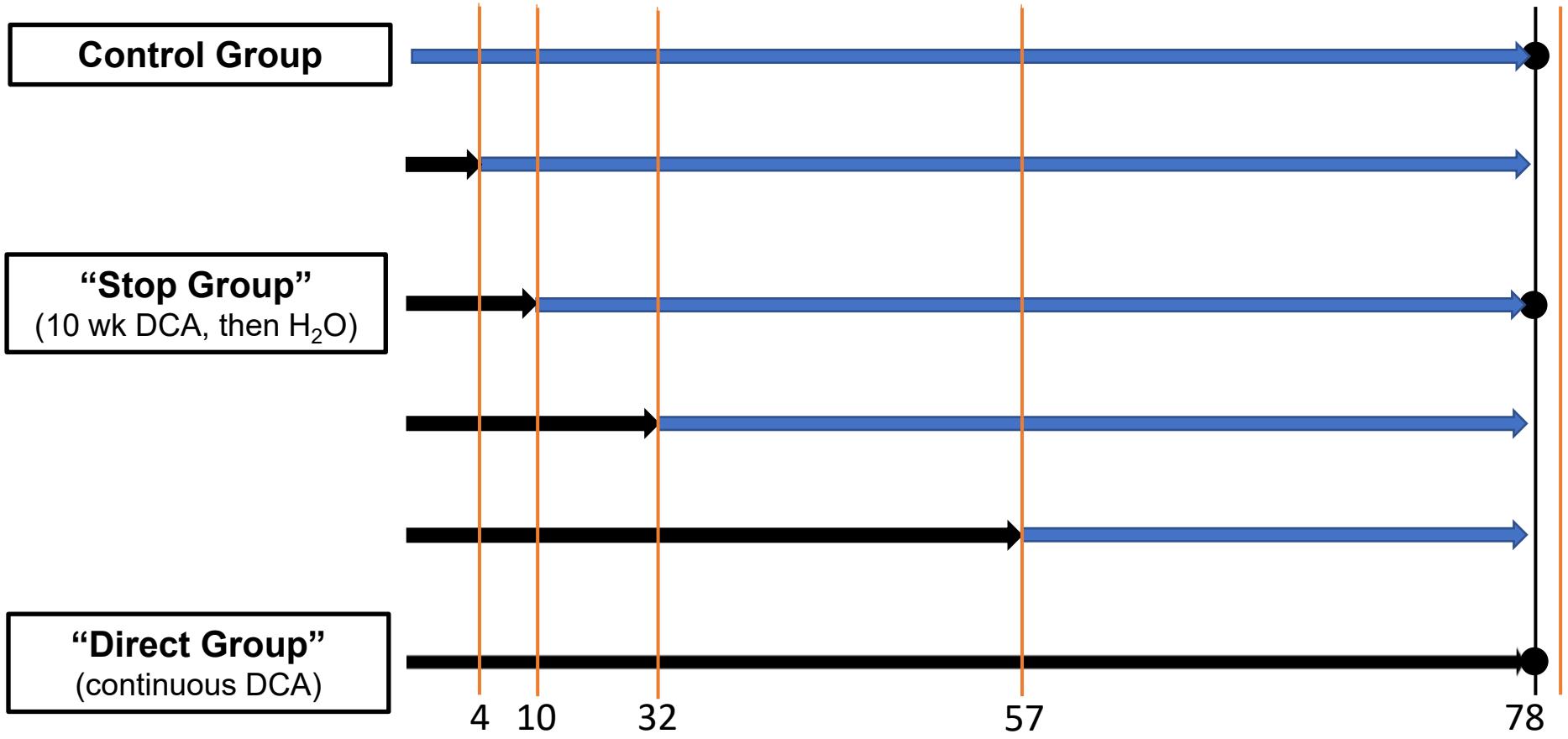
Trevino *et al. Nat Comm* 2020



Alyea et al. *Env Mol Mutagenesis* 2014

Risk evaluation for a chemical substance must account for additional risks of exposed or susceptible subpopulations and epigenetic alterations could be testable/measurable endpoints to inform these issues

DCA stop-exposure time course study



♂ $B6C3F_1$ mice

DCA (3.5 g/l) →

dH_2O →

Samples available

Frozen	FFPE
RRBS	Focused RNA-seq

Wehmas et al. Tox Sci 2017

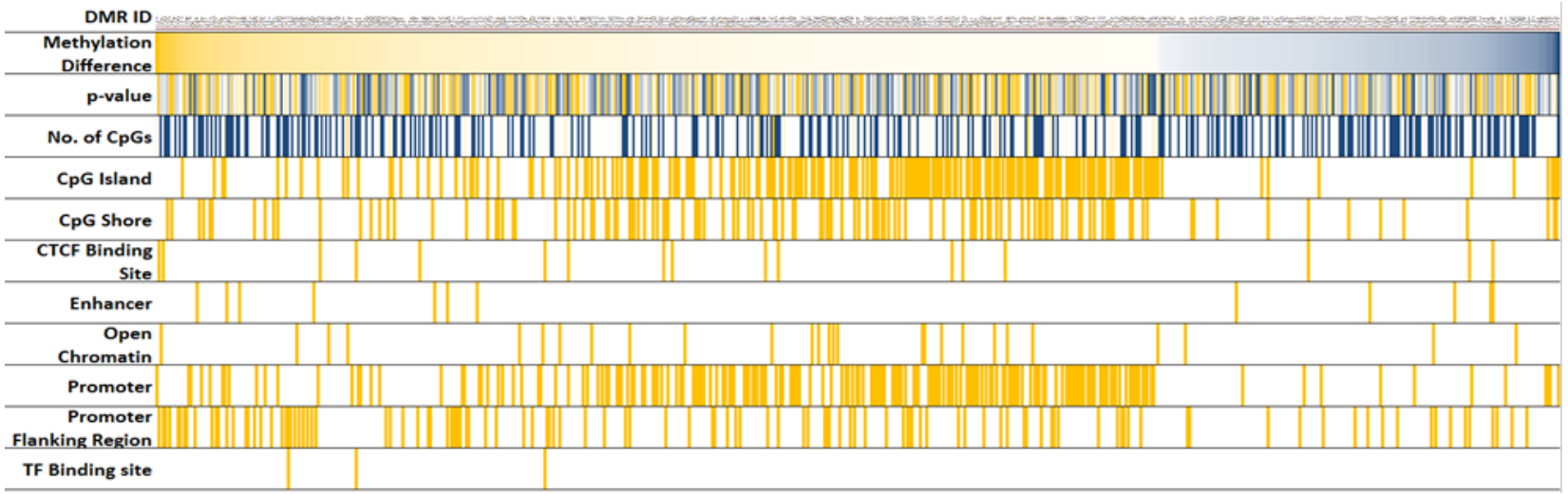
EPA United States Environmental Protection Agency

Differentiated Methylated Regions (DMRs) in Direct and Stop Groups @ 78 weeks

Feature	# in Direct DMR	% in Direct DMR		# in Stop DMR	% in Stop DMR
5'UTR	478	12%		93	14%
3'UTR	86	2%		14	2%
Exon	642	15%		141	21%
Intron	844	20%		122	18%
Promoter	213	5%		66	10%
Intergenic	1909	46%		226	34%
Total	4172			662	

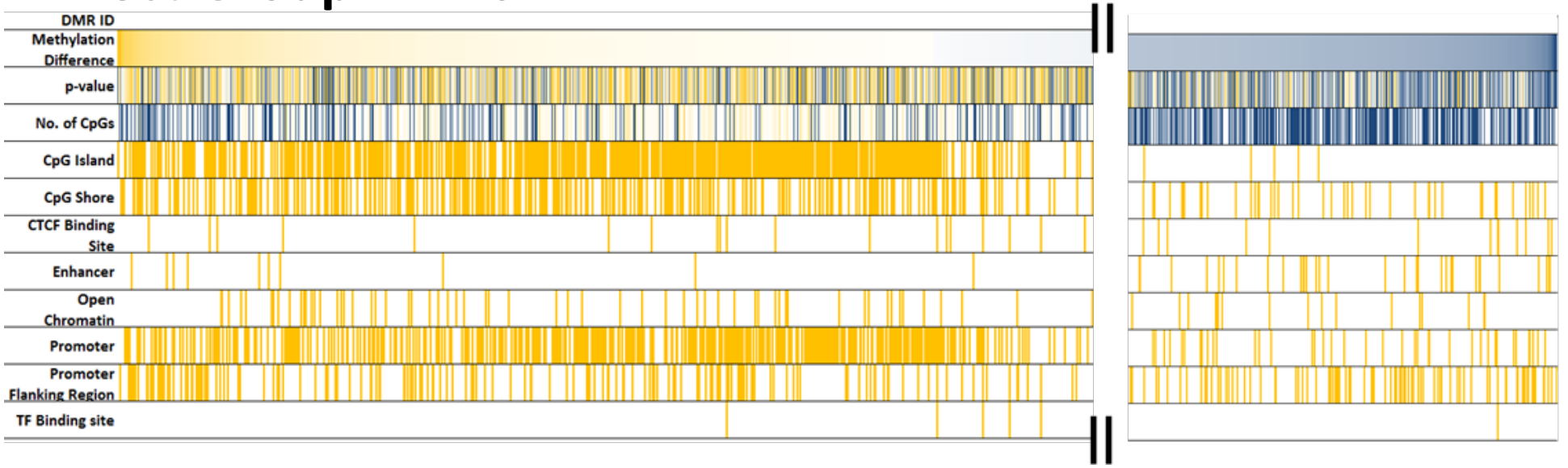
Overview of the Direct and Stop Regulatory-Linked DMRs

Stop Group DMRs



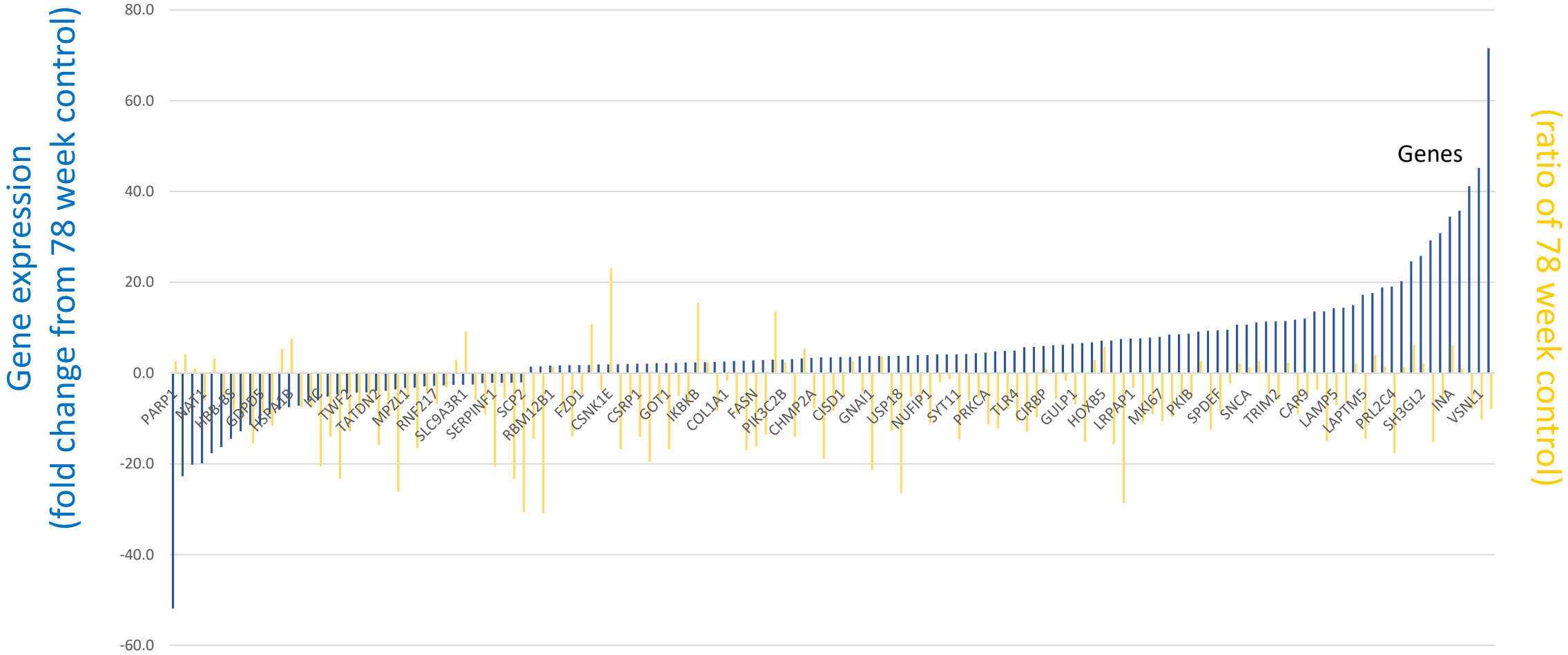
422 linked to regulatory regions **64% of all Stop DMRs**

Direct Group DMRs

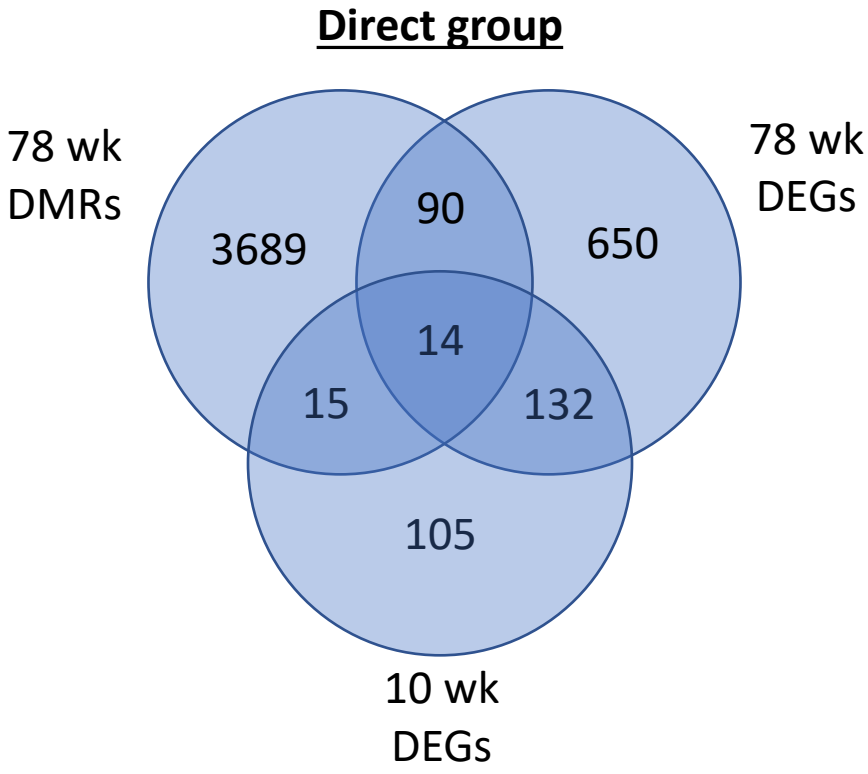


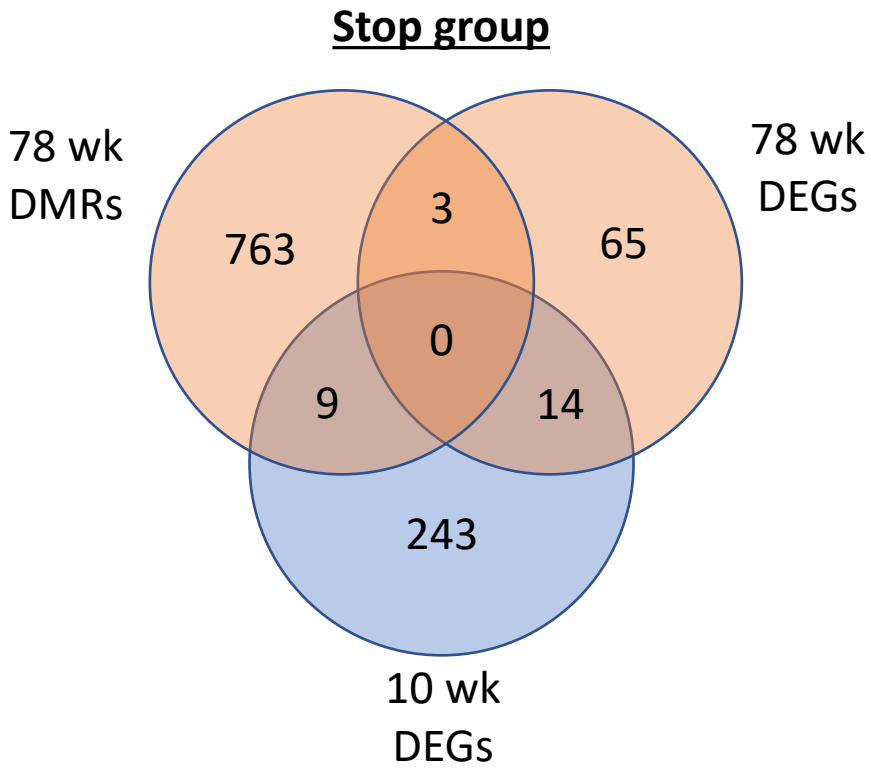
1536 linked to regulatory regions **37% of all Direct DMRs**

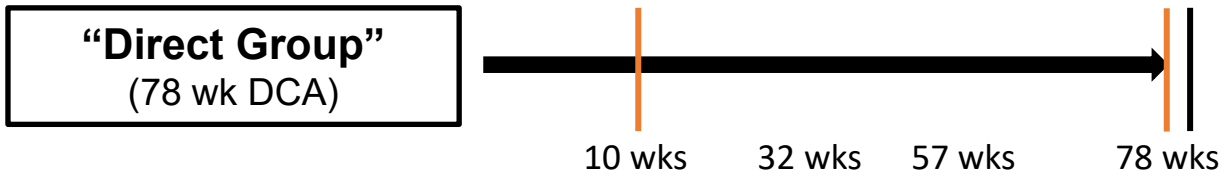
Correlation of Gene Expression and Direct Group DNA Methylation @ 78 weeks

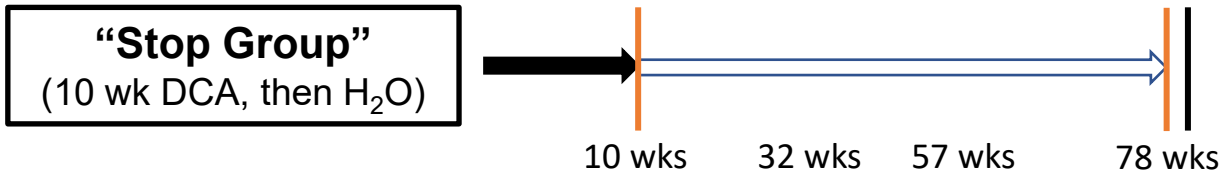


Differentially expressed genes in direct and stop groups

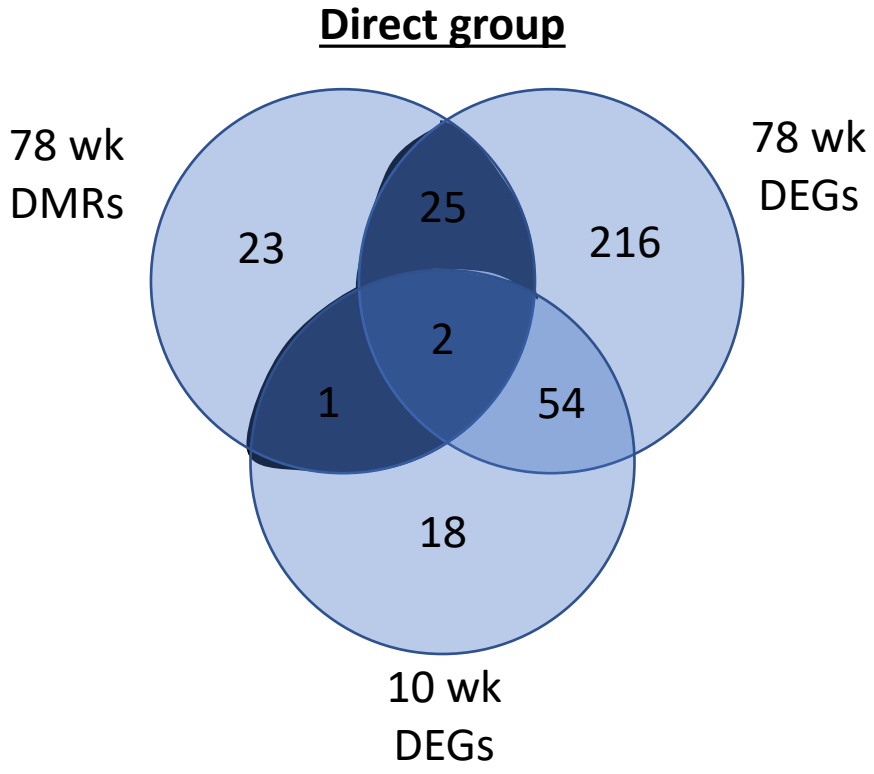








Gene pathway comparisons in direct groups



Cell regulation/proliferation/cancer

PPARα/RXRα Activation
Growth Hormone
Signaling by Rho Family GTPases
RhoGDI Signaling

Insulin Signaling

Type II Diabetes Mellitus Signaling

Insulin Signaling

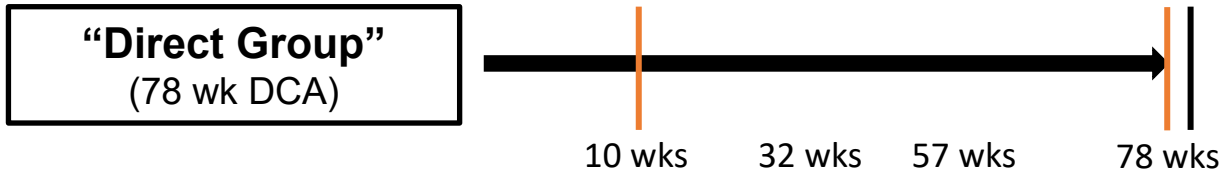
cAMP-mediated signaling/PKA

Coagulation/collagen/ECM deposition

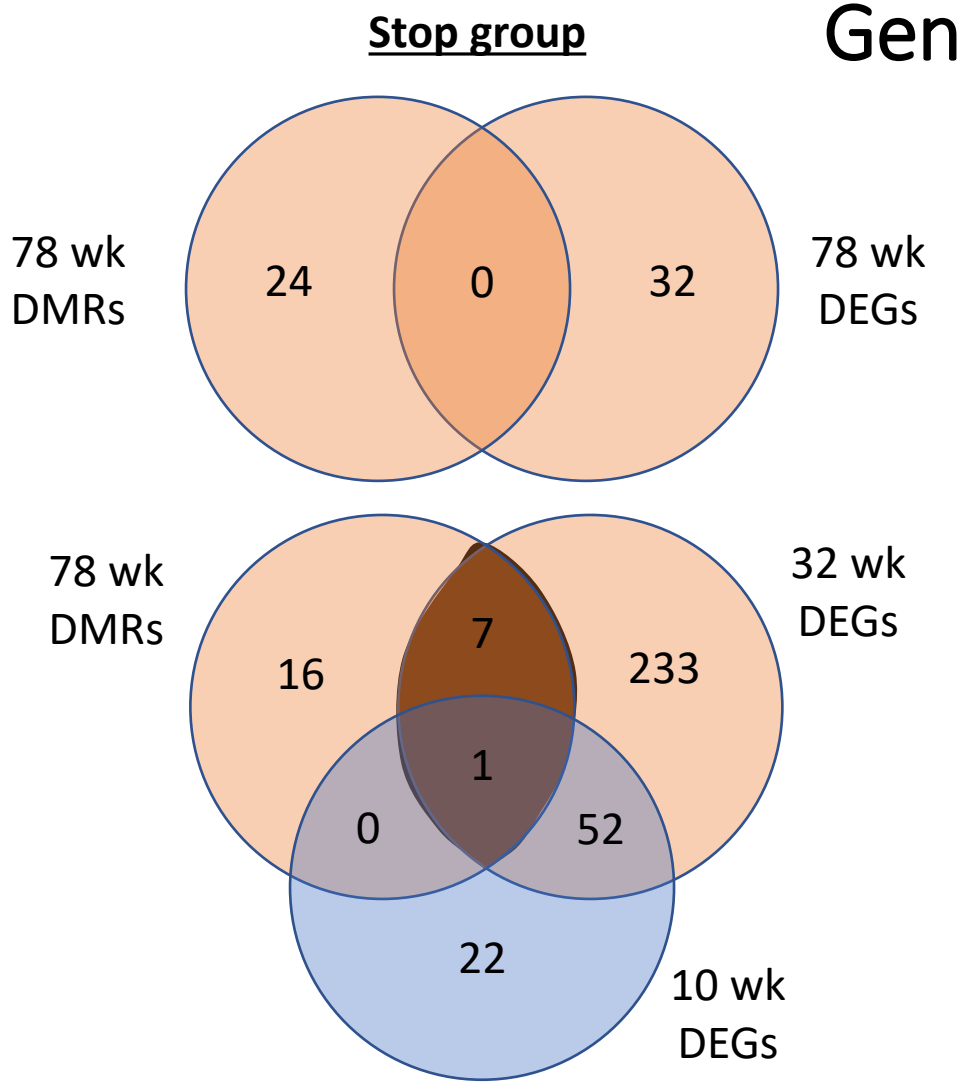
G-Protein Coupled Receptor Signaling
Hepatic Fibrosis / Hepatic Stellate Cell Activation
Leptin Signaling in Obesity
Gα12/13 Signaling
Corticotropin Releasing Hormone Signaling

Coagulation/collagen/ECM deposition

Signaling by Rho Family GTPases
Gαs Signaling
Endothelin-1 Signaling



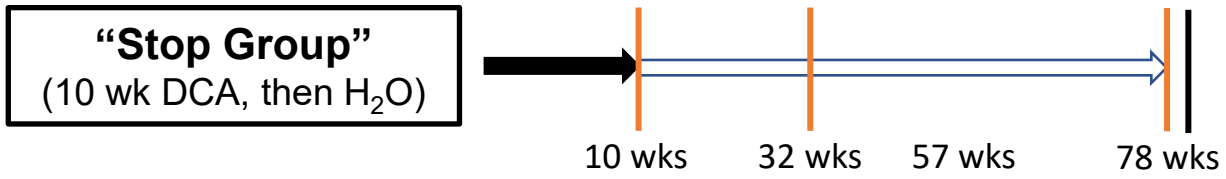
Gene pathway comparisons in stop groups



Cell regulation/proliferation/cancer
- Wnt/β-catenin Signaling *

Insulin Signaling
- Insulin Receptor Signaling

Coagulation/collagen/ECM deposition
- Inhibition of Angiogenesis by TSP1
- Relaxin Signaling
- Axonal Guidance Signaling
- IL-1 Signaling



What do the ‘omics indicate about our DCA stop-exposure condition?

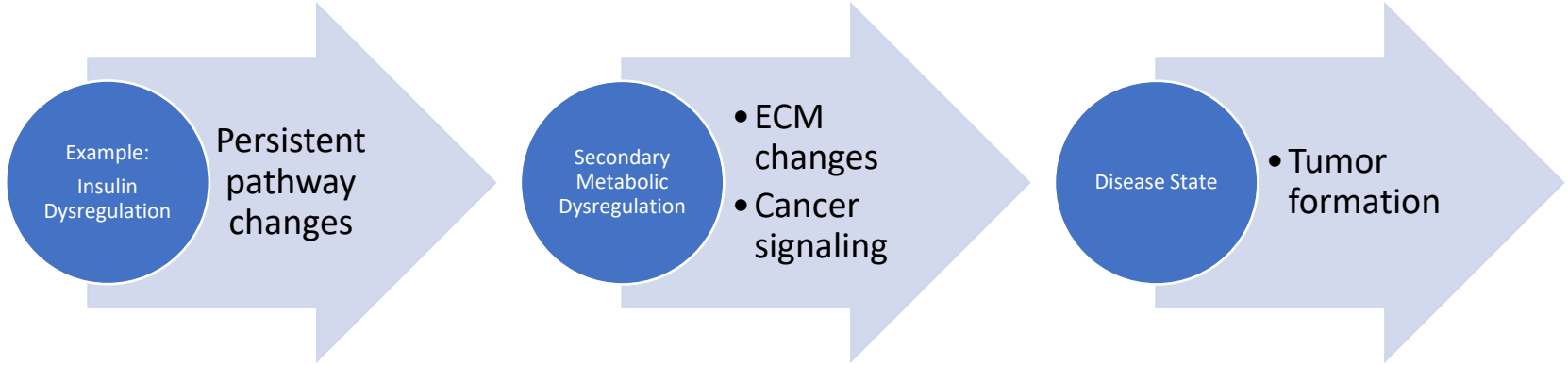
Direct DCA Effects

Mitochondria/OXPHOS

ROS generation

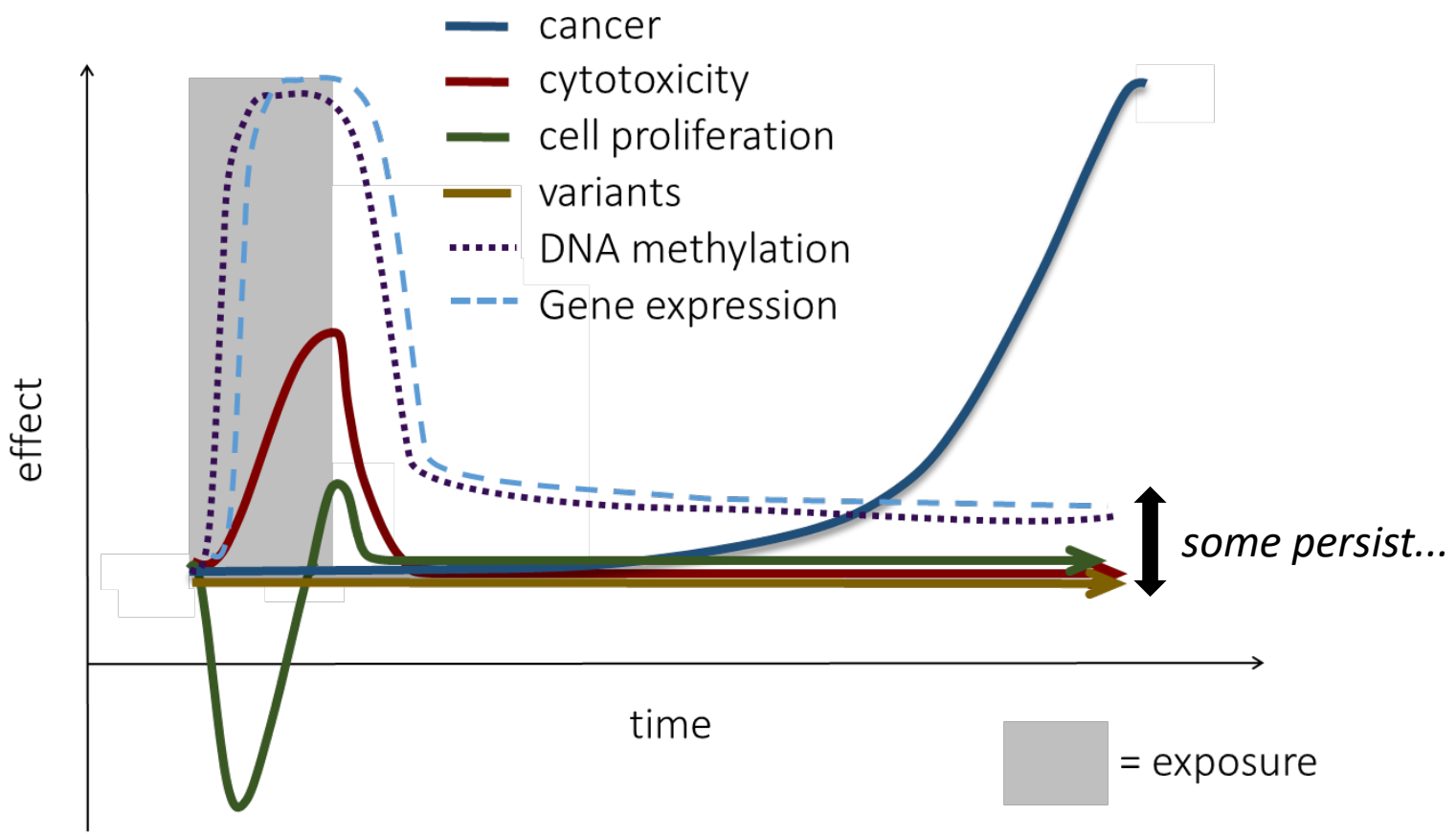
Inflammatory Response

Initial reduction in ECM related gene expression



“Stop Group”
(10 wk DCA, then H_2O)

Working Hypothesis for Stop Group



Summary

A distinct methylation profile is induced by constant exposure to DCA

- Matched known mechanisms of action for DCA
- Transcriptomic profile matched methylation

Gene pathway analysis of stop group reflects similar perturbations seen with early and late direct DCA exposure

- Gene patterns suggest a “flip” compared to matched time point controls during early exposure and later-in-life effects

There is little direct intersection of the Stop DEGs and DMRs at 78 weeks

- Stop DMRs reflect perturbations earlier in life due to DCA
- Indication of pathways that later demark gene and pathway alterations that are likely contributing to carcinogenicity

Acknowledgements

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NHEERL Research Core Units

John Chamberlin (now at U. of Utah)

National Institute of Environmental Health Sciences

Biostatistics & Computational Biology Branch

Brian Bennett

Pierre Bushel (now at BlueRock Therapeutics)

Environmental Epigenomics and Disease Group

Douglas Bell

Neal Englert (now at LabCorp)

Emory University

Benjamin Barwick