

# Genome-wide Studies of Toxic Chemical Impacts on Chromatin State

Thursday March 17<sup>th</sup>, 2016



Ivan Rusyn  
Grace Chappell



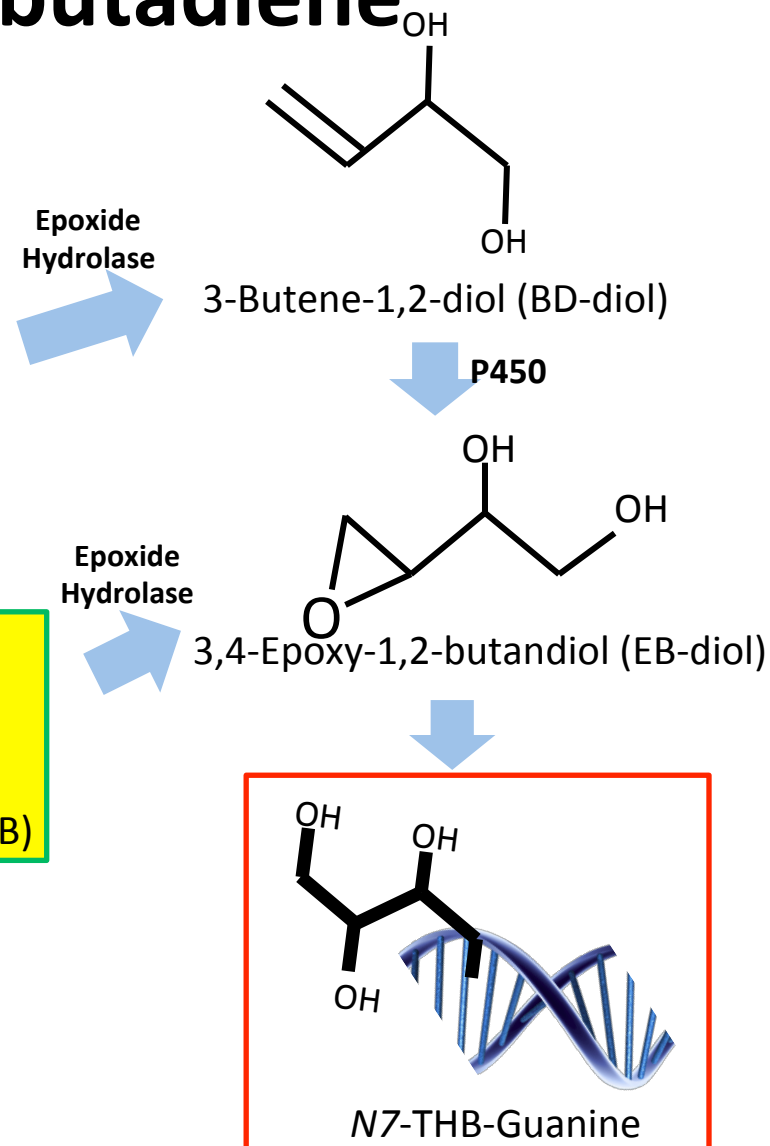
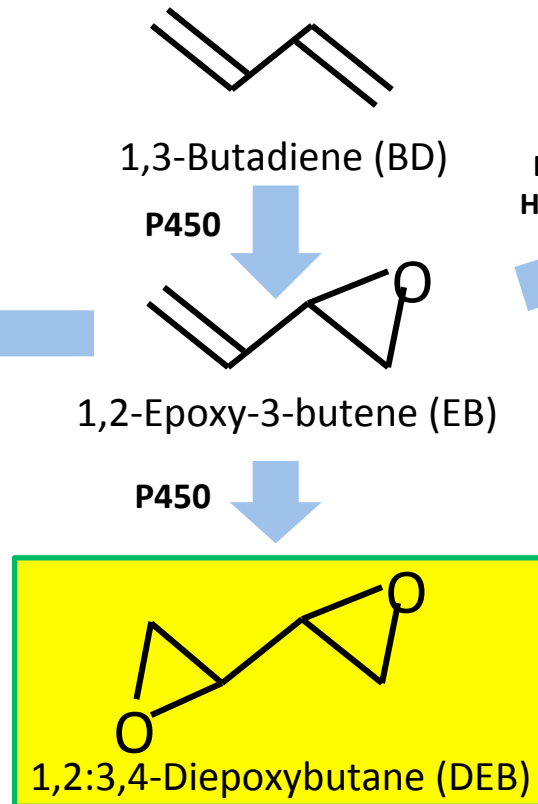
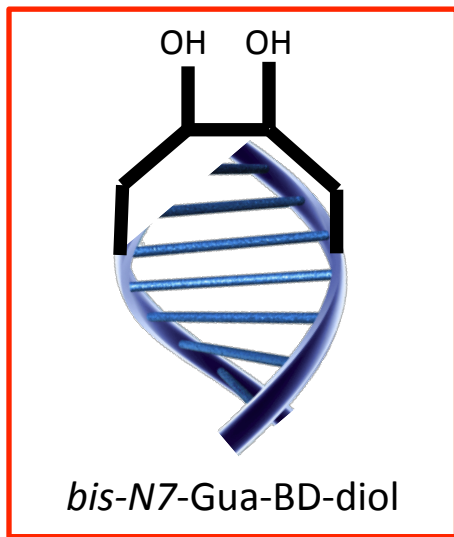
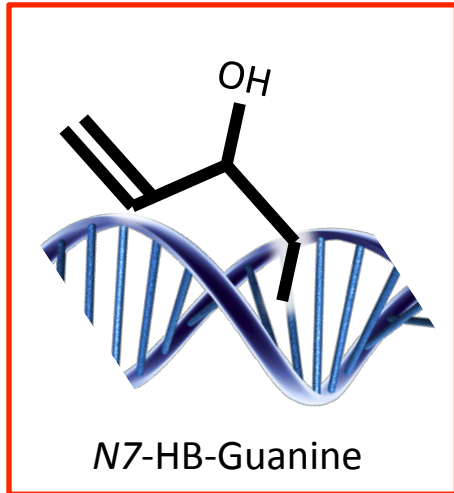
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Jennifer Israel  
Jeremy Simon  
Matt Weiser  
Karl Eklund



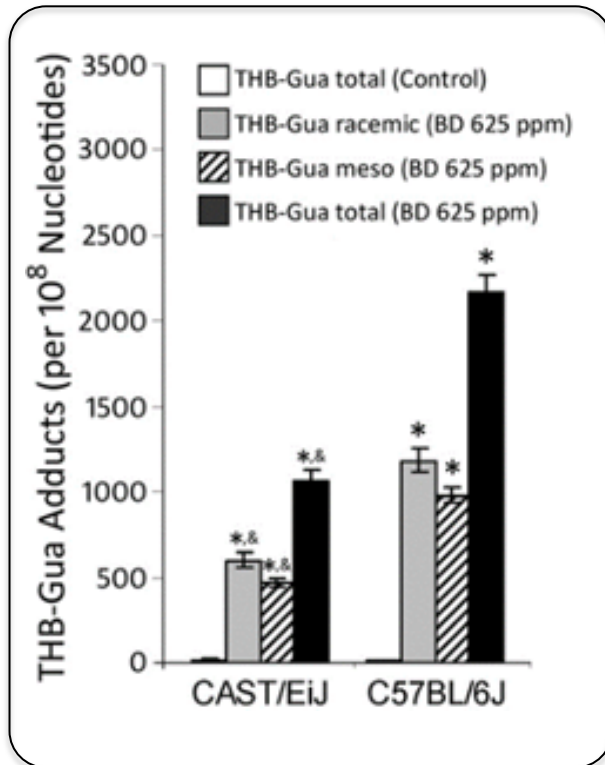
Greg Crawford  
Alexias Safi

# Metabolism of 1,3-butadiene



# BD induces strain-specific epigenetic effects

## DNA Adducts



\* Indicates significantly different from controls, \*\* different between mixtures ( $p < 0.05$ )

Koturbash et al *Tox Sci* (2011)



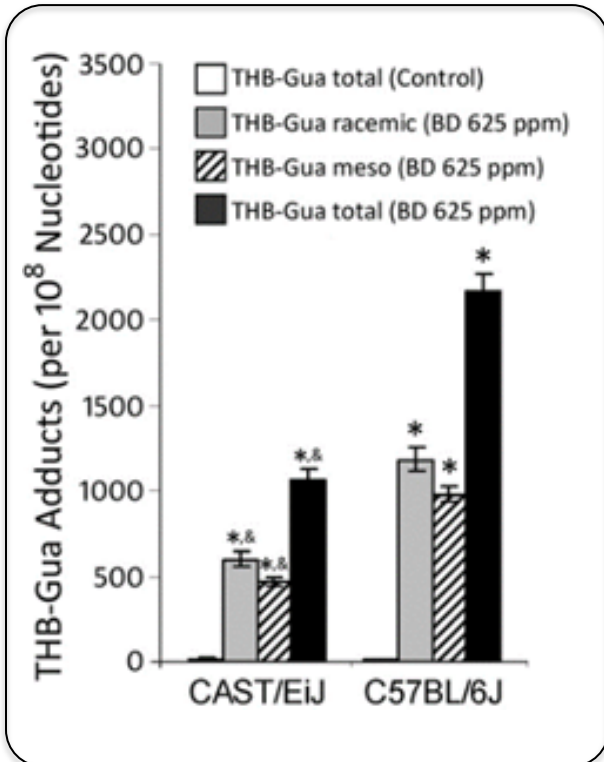
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# BD induces strain-specific epigenetic effects

DNA hypo-methylation Histone modifications

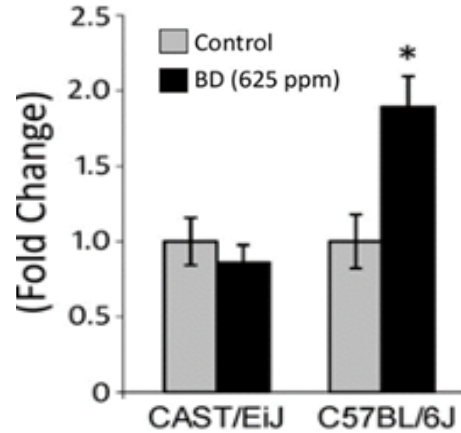
## DNA Adducts



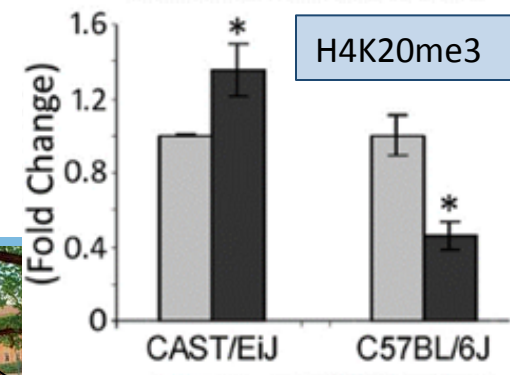
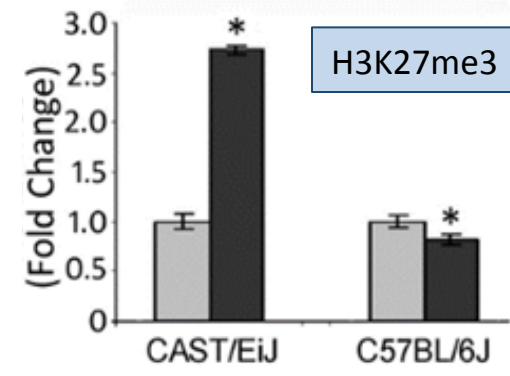
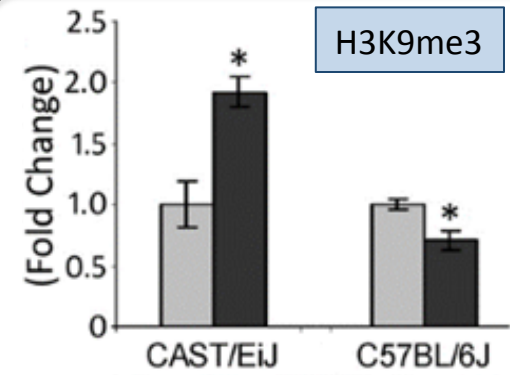
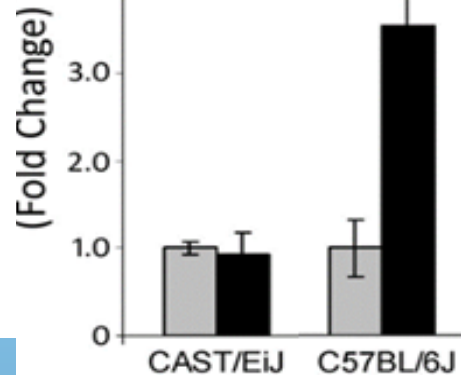
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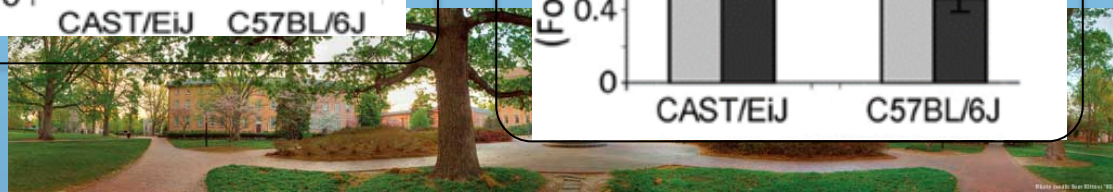
## Global DNA Methylation



## LINE1 Methylation

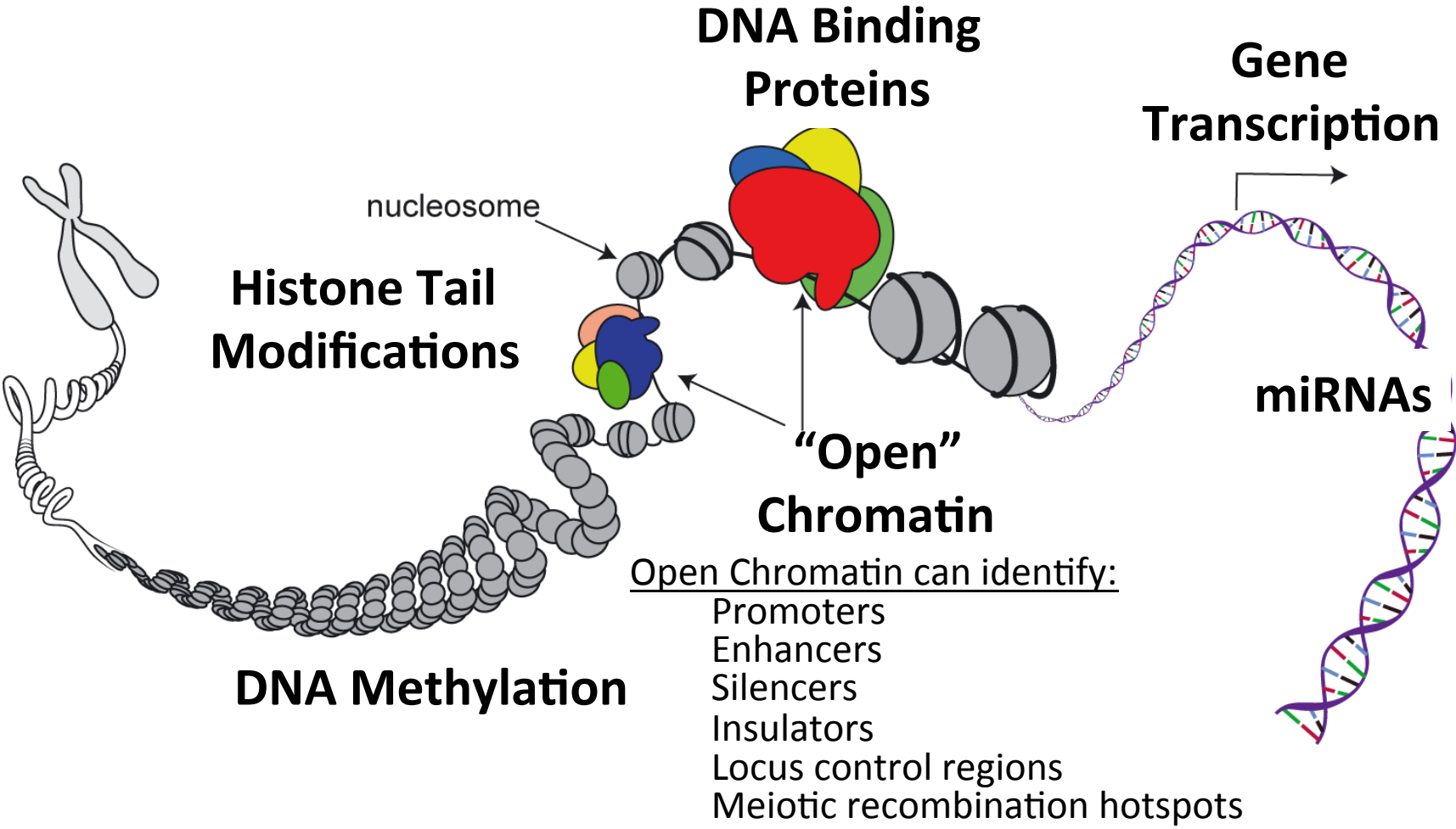


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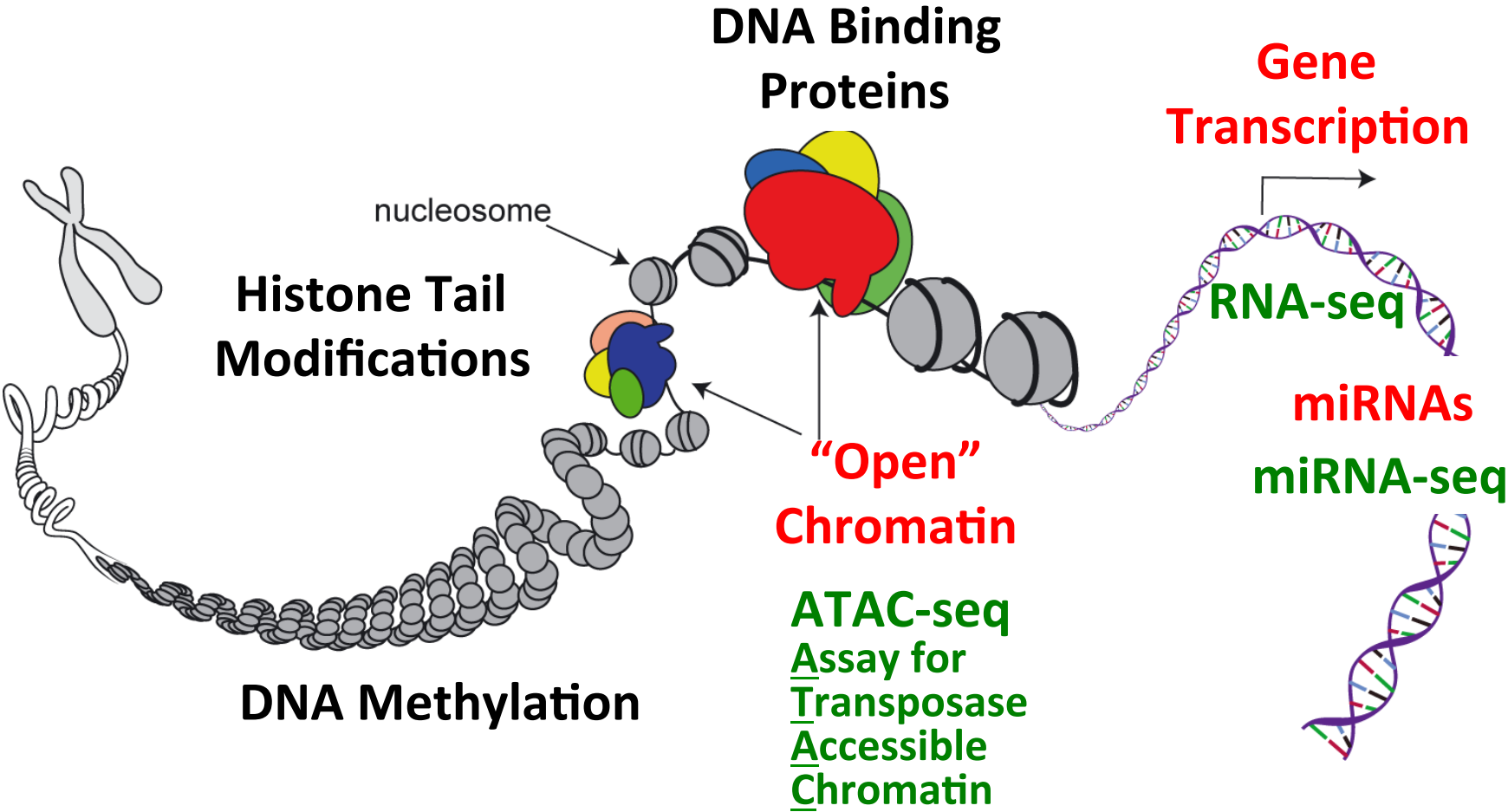




# Epigenetics, Regulation and Transcription



# Epigenetics, Regulation and Transcription

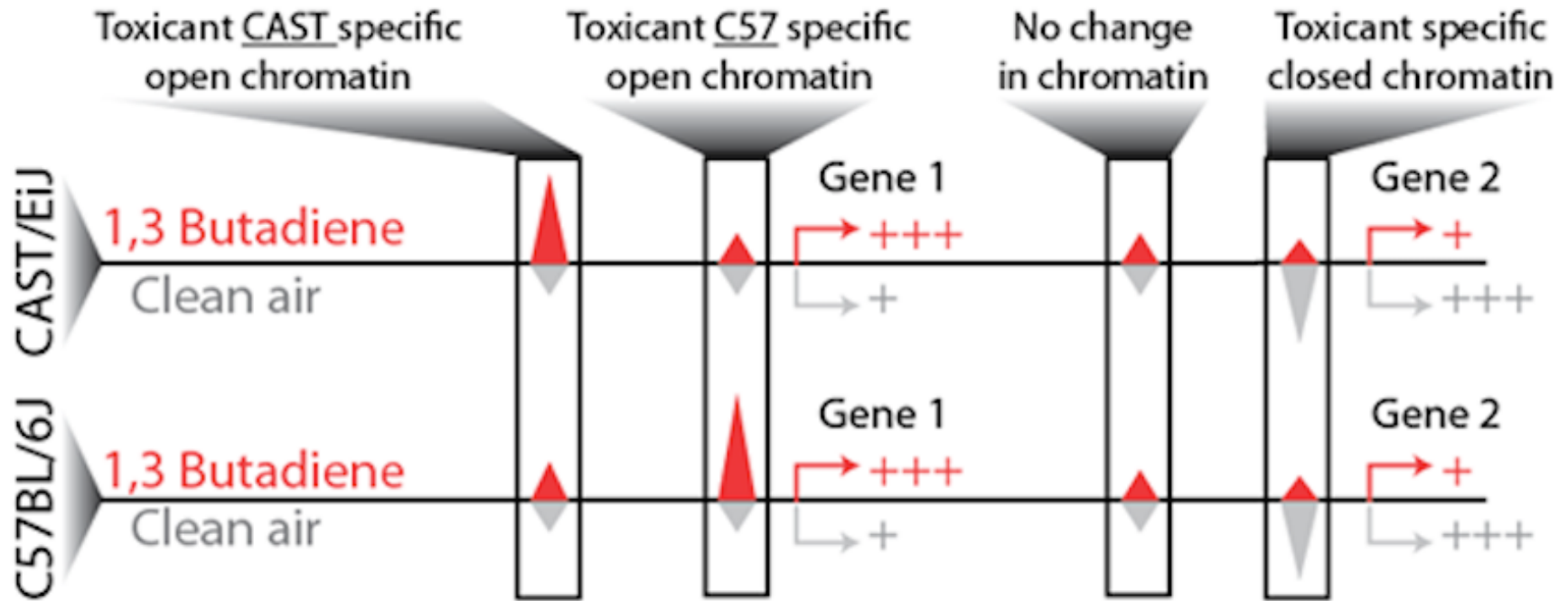


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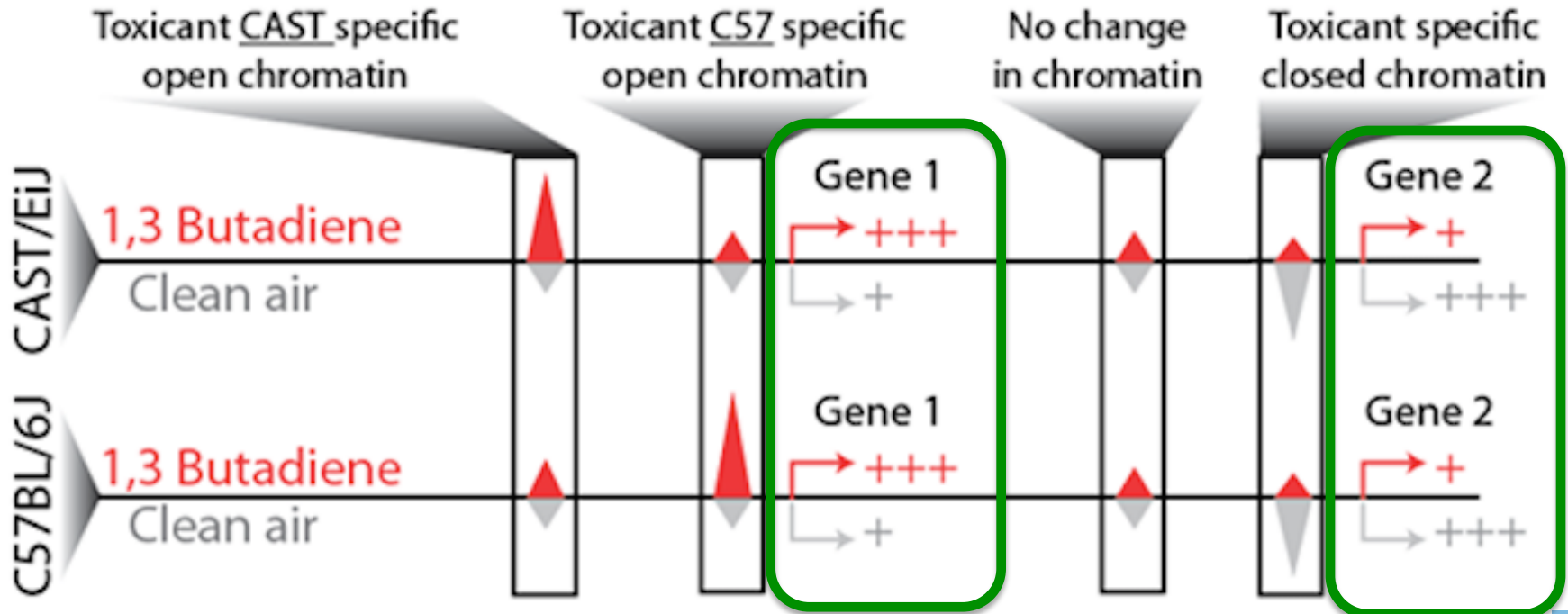
# 1,3 Butadiene Exposure (TaRGET – NIEHS)

<u>Strain</u>	<u>Exposure</u>		<u>Tissue</u>	<u>Assays</u>
C57BL/6 (sensitive)	0 PPM (clean air)	2 week exposure →	Lung	RNA
CAST/EiU (resistant)	625 PPM 1,3 Butadiene		Liver	miRNA
			Kidney	ATAC



# 1,3 Butadiene Exposure (TaRGET – NIEHS)

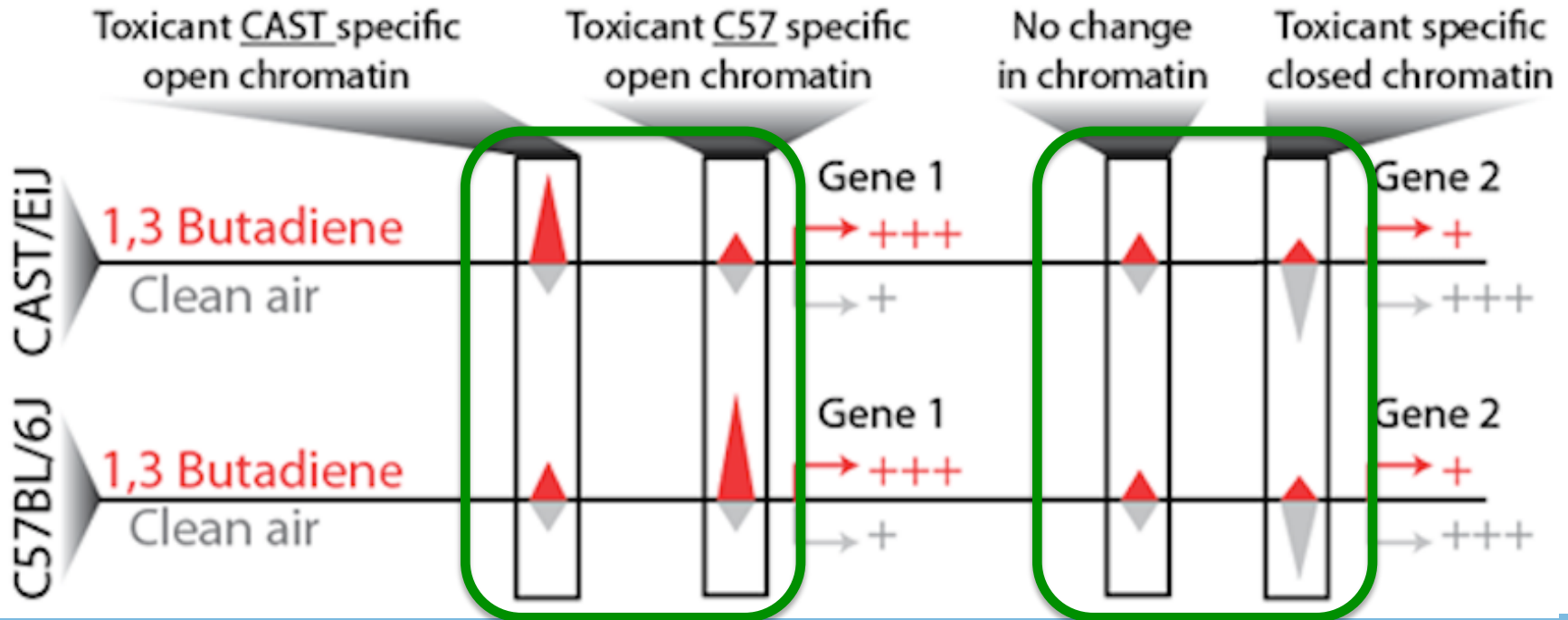
<u>Strain</u>	<u>Exposure</u>		<u>Tissue</u>	<u>Assays</u>
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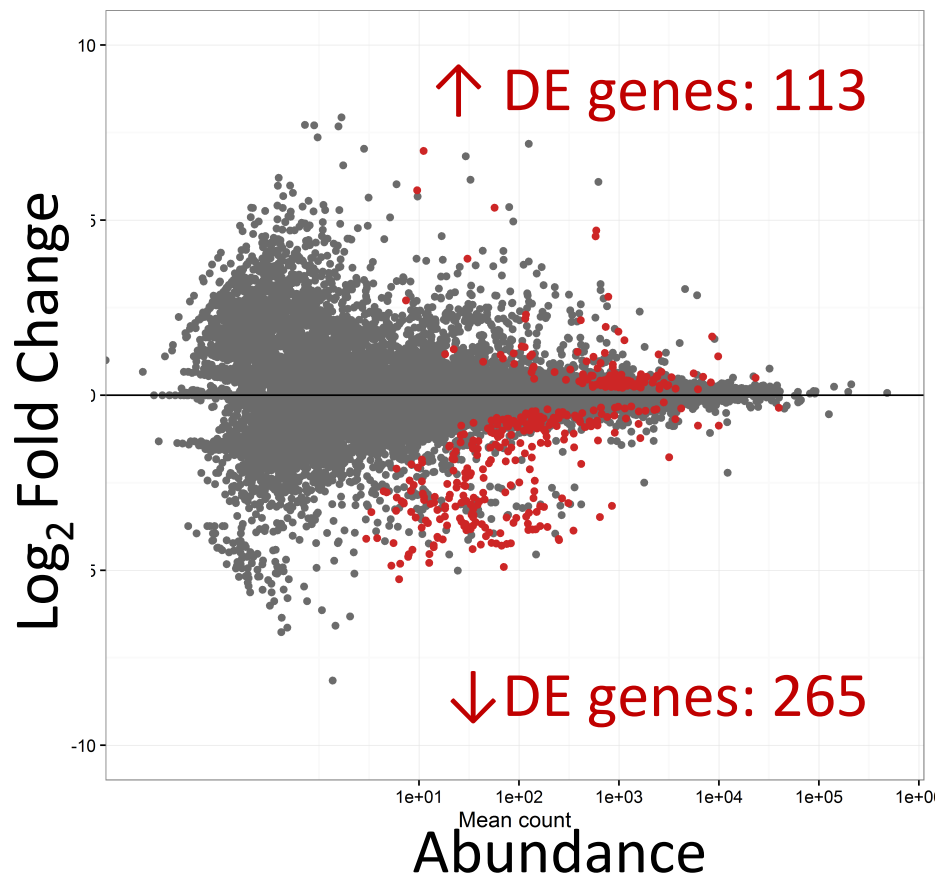


# BD exposure-induced changes in lung mRNAs

C57BL6 (sensitive)



CAST (resistant)



● Differentially expressed ( $q < 0.1$ ; DESeq)

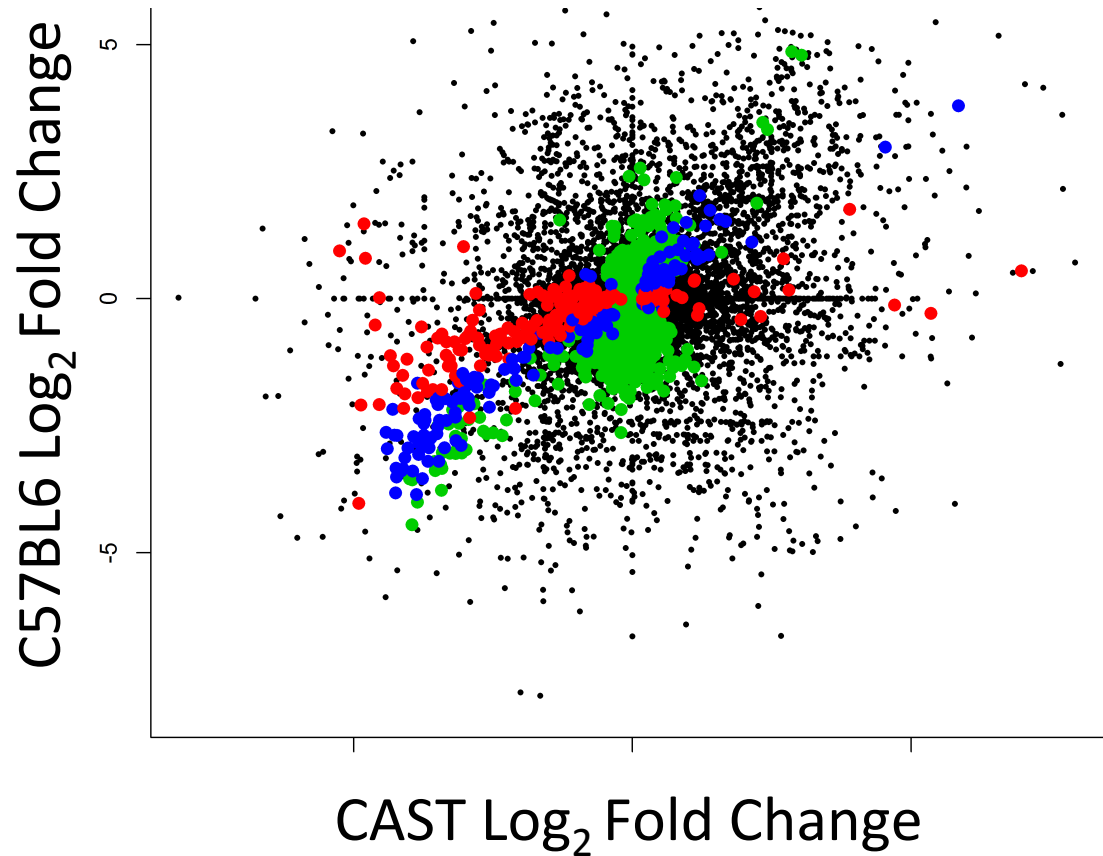
● Not significant



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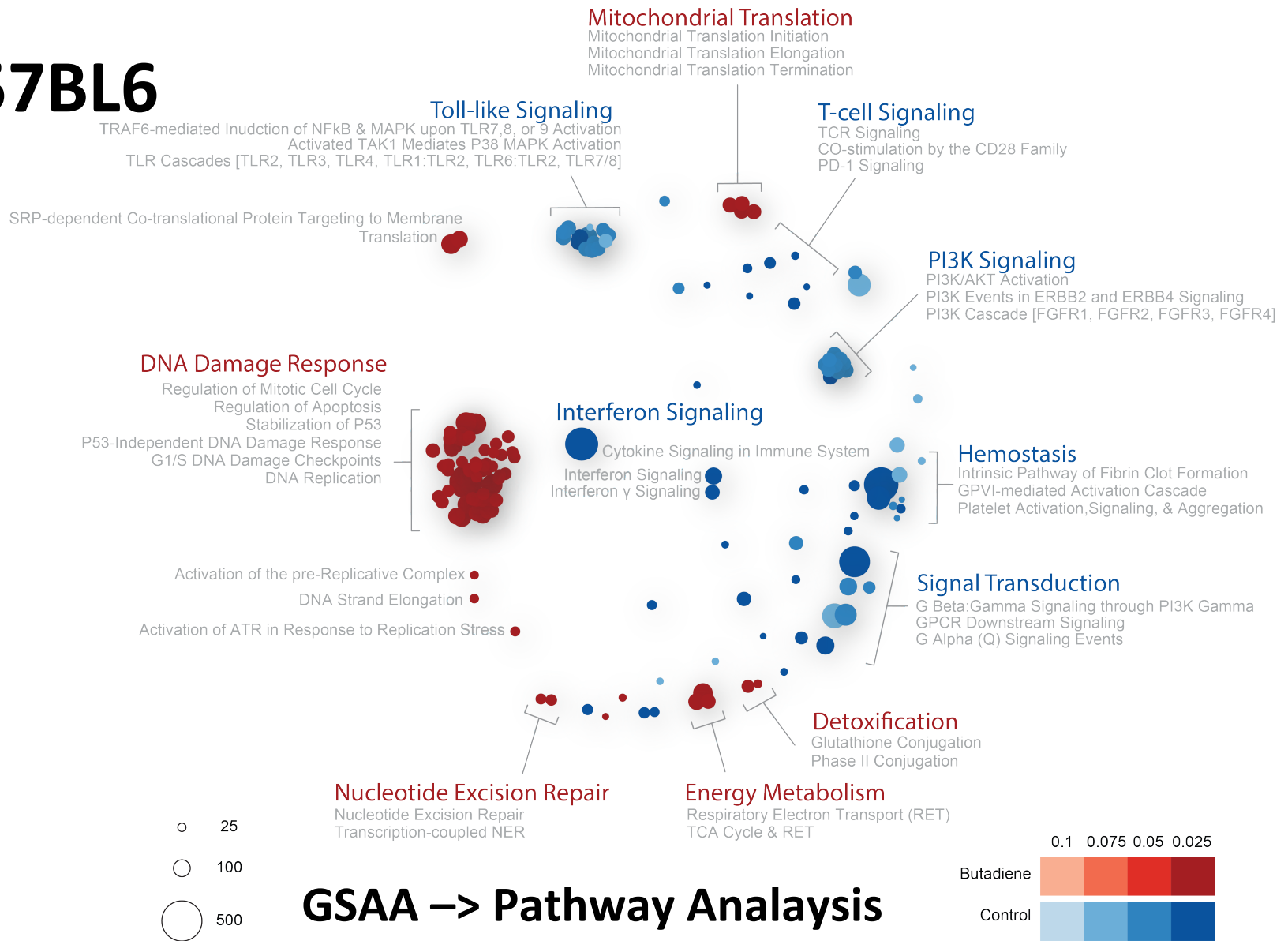
# C57BL6 vs CAST mRNA changes



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# C57BL6

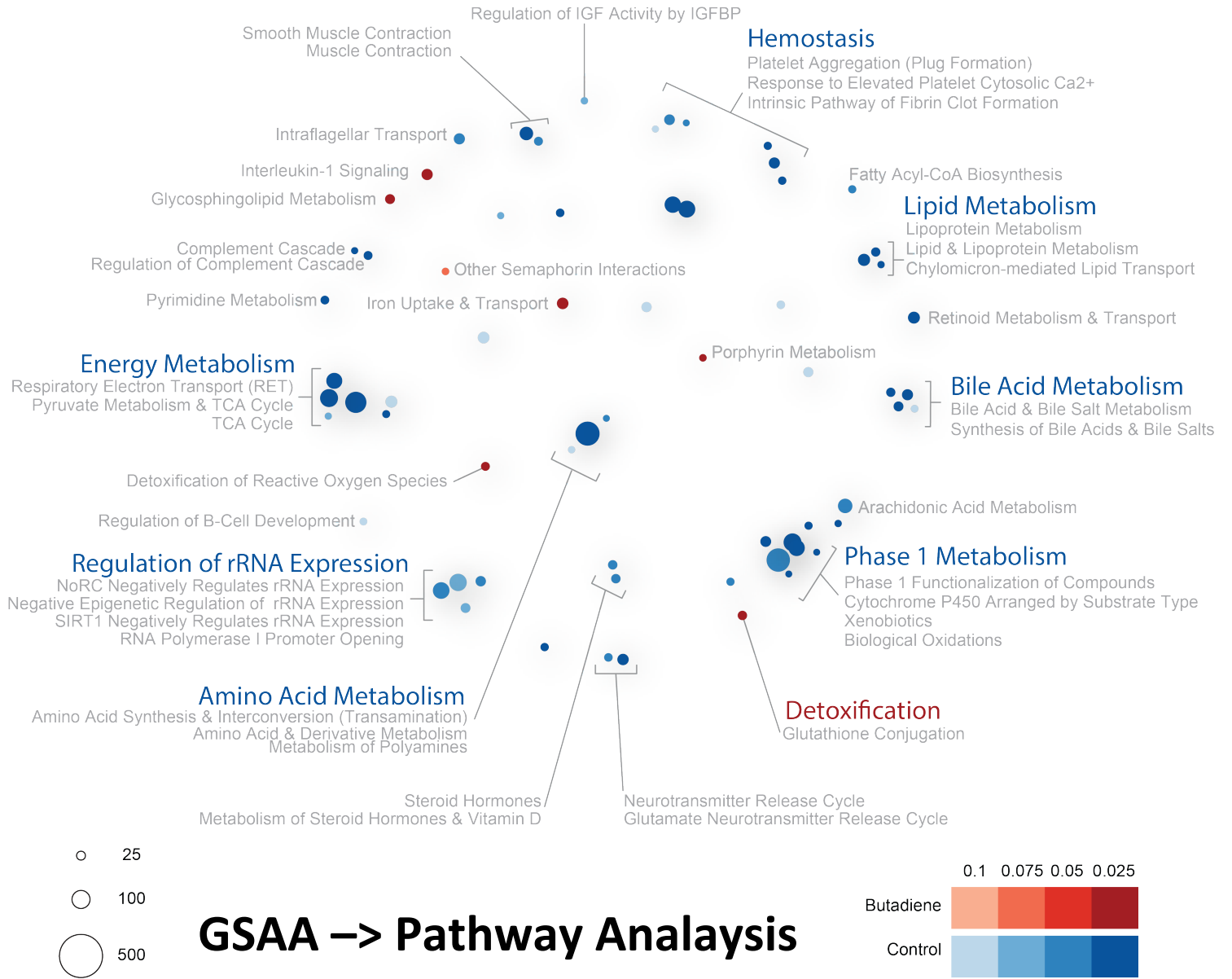


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# CAST



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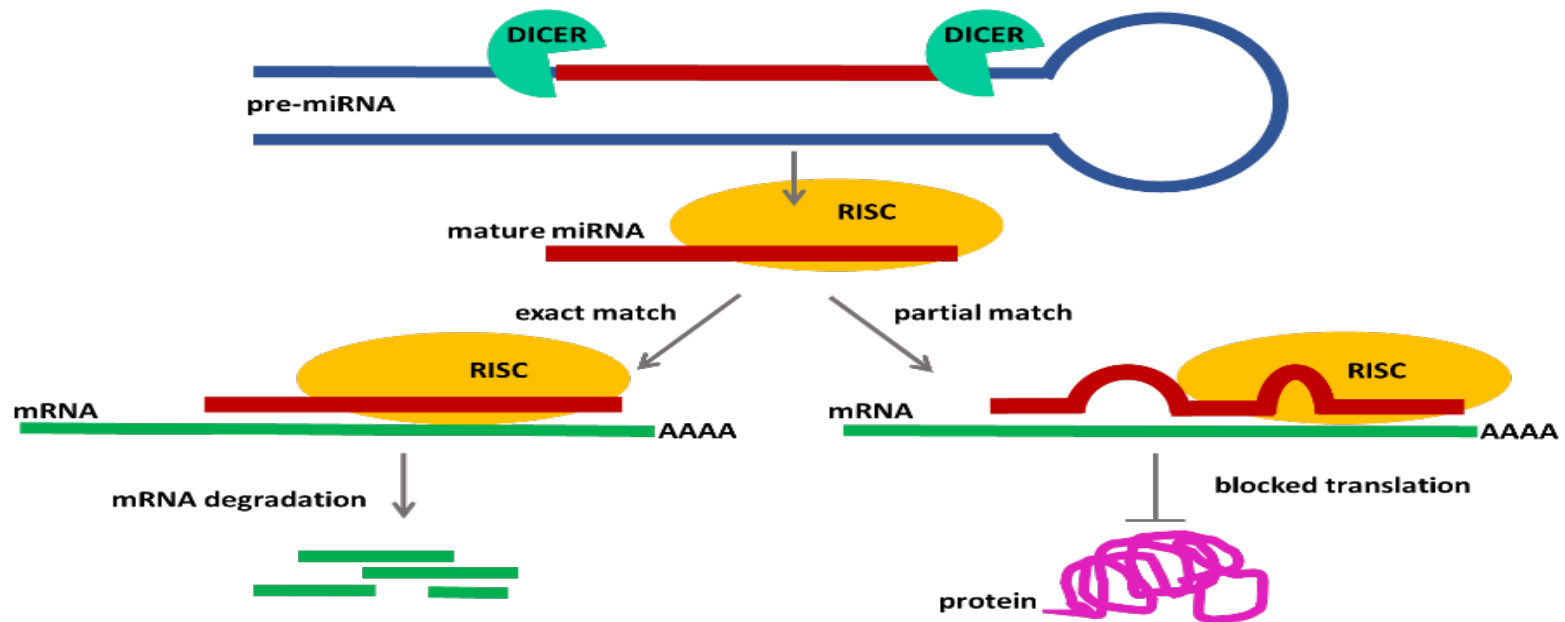
# What is controlling this differential expression?



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# miRNAs block translation of target mRNAs or mark target mRNAs for degradation

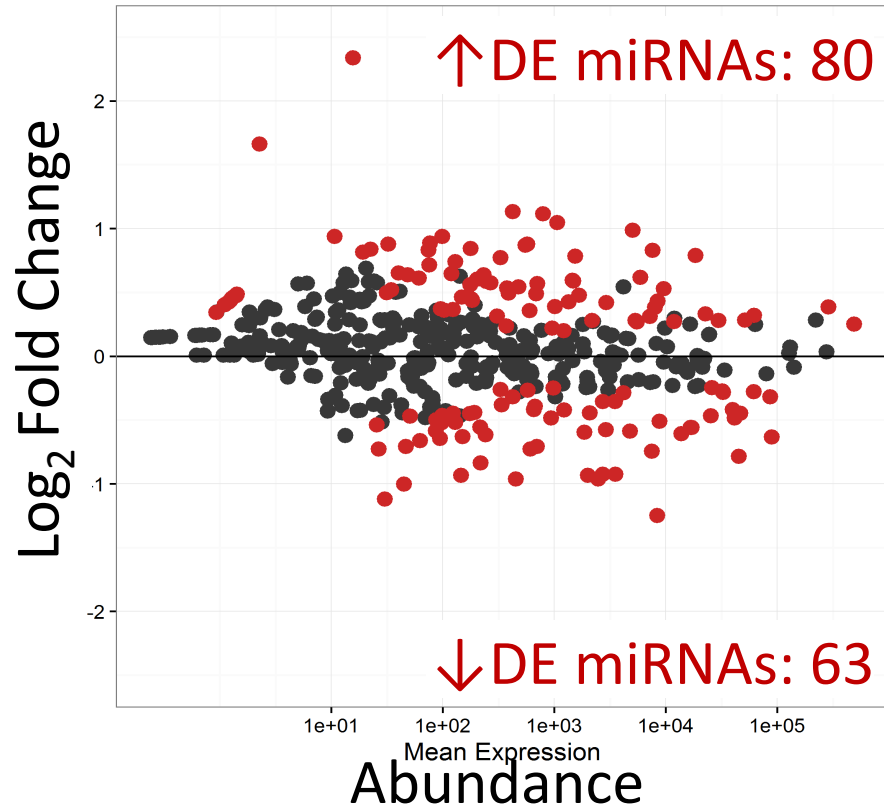


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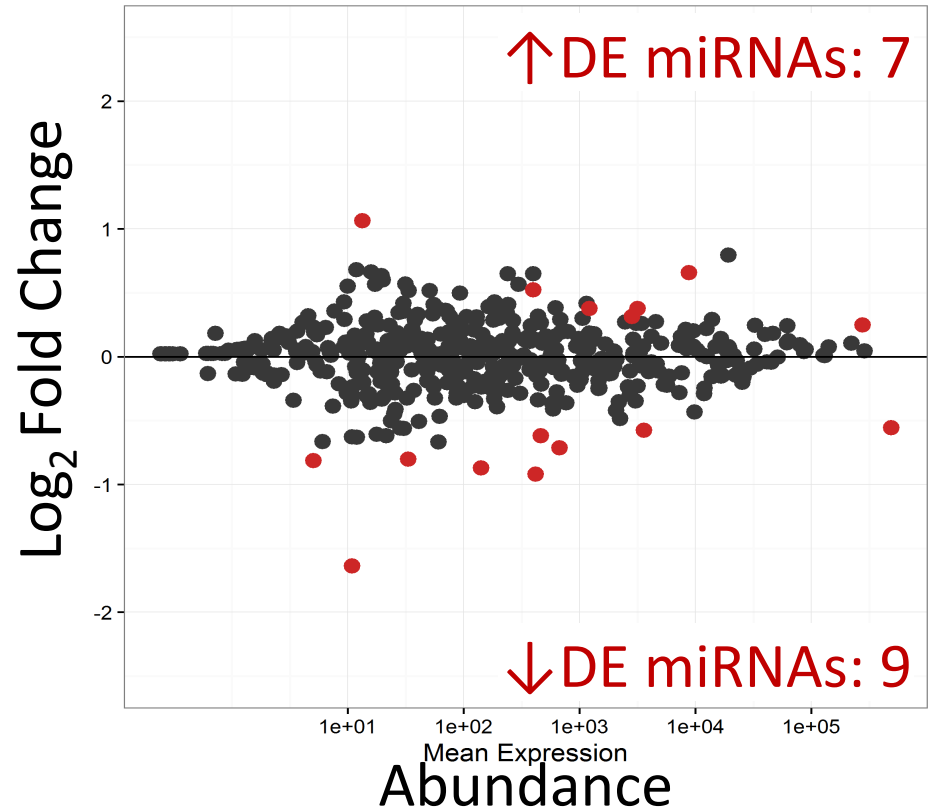


# BD exposure-induced changes in lung miRNAs

C57BL6



CAST



● Differentially expressed ( $q < 0.1$ )

● Not significant



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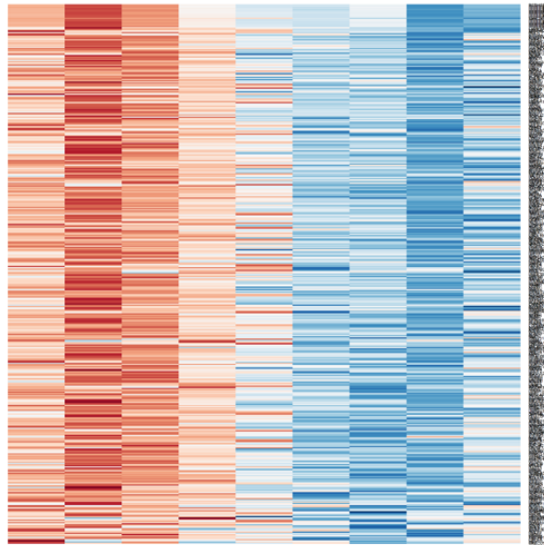




# Gene targets of miRNAs – C57BL6



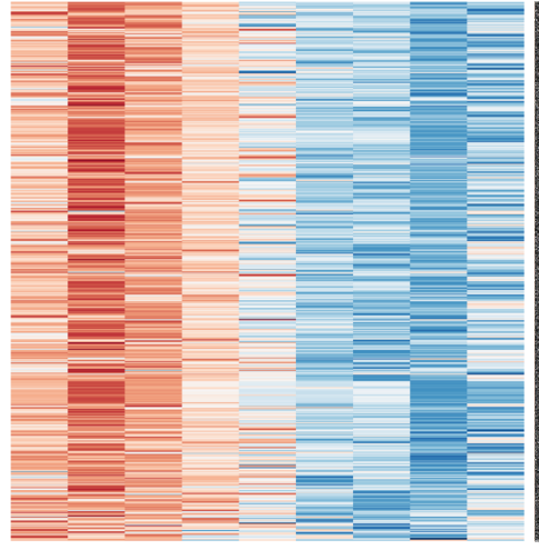
C57BL/6J Lung: mRNA targets of miR-34/449



Clean air-exposed BD-exposed



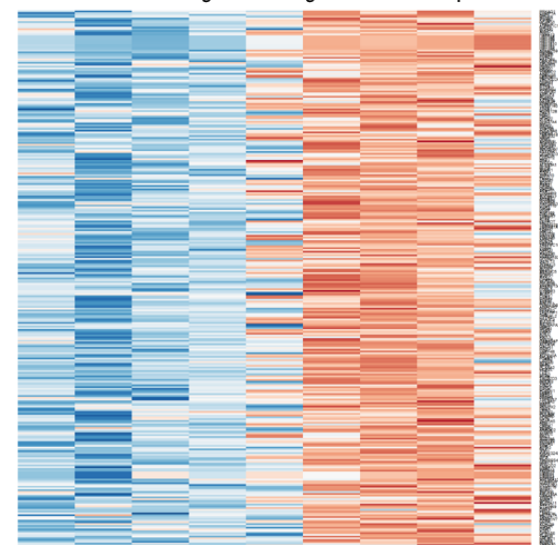
C57BL/6J Lung: mRNA targets of miR-326-3p



Clean air-exposed BD-exposed



C57BL/6J Lung: mRNA targets of miR-150-5p



Clean air-exposed BD-exposed



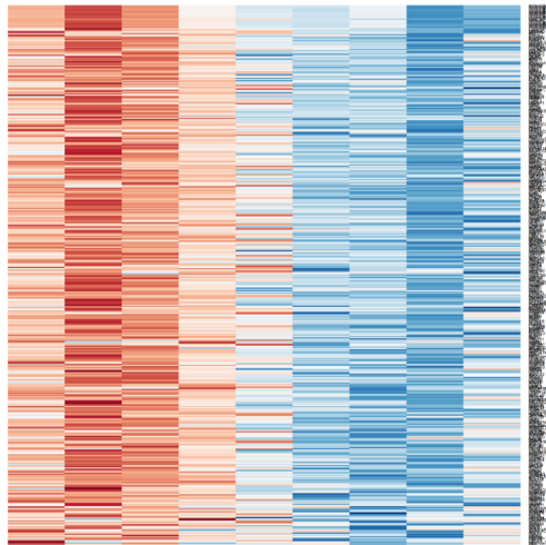
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# Gene targets of miRNAs – C57BL6



C57BL/6J Lung: mRNA targets of miR-34/449



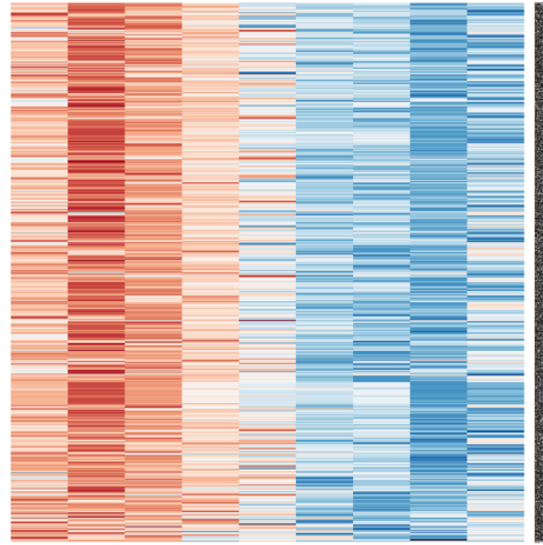
Clean air-exposed BD-exposed

Hemostasis

Notch-HLH Transcription



C57BL/6J Lung: mRNA targets of miR-326-3p



Clean air-exposed BD-exposed

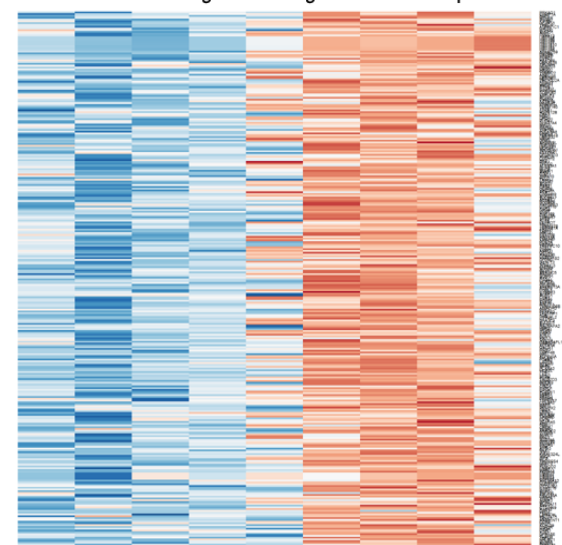
Hemostasis

Immune system

Regulation of Signaling



C57BL/6J Lung: mRNA targets of miR-150-5p



Clean air-exposed BD-exposed

Glucuronidation

(Phase II Metabolism)

Membrane Transport



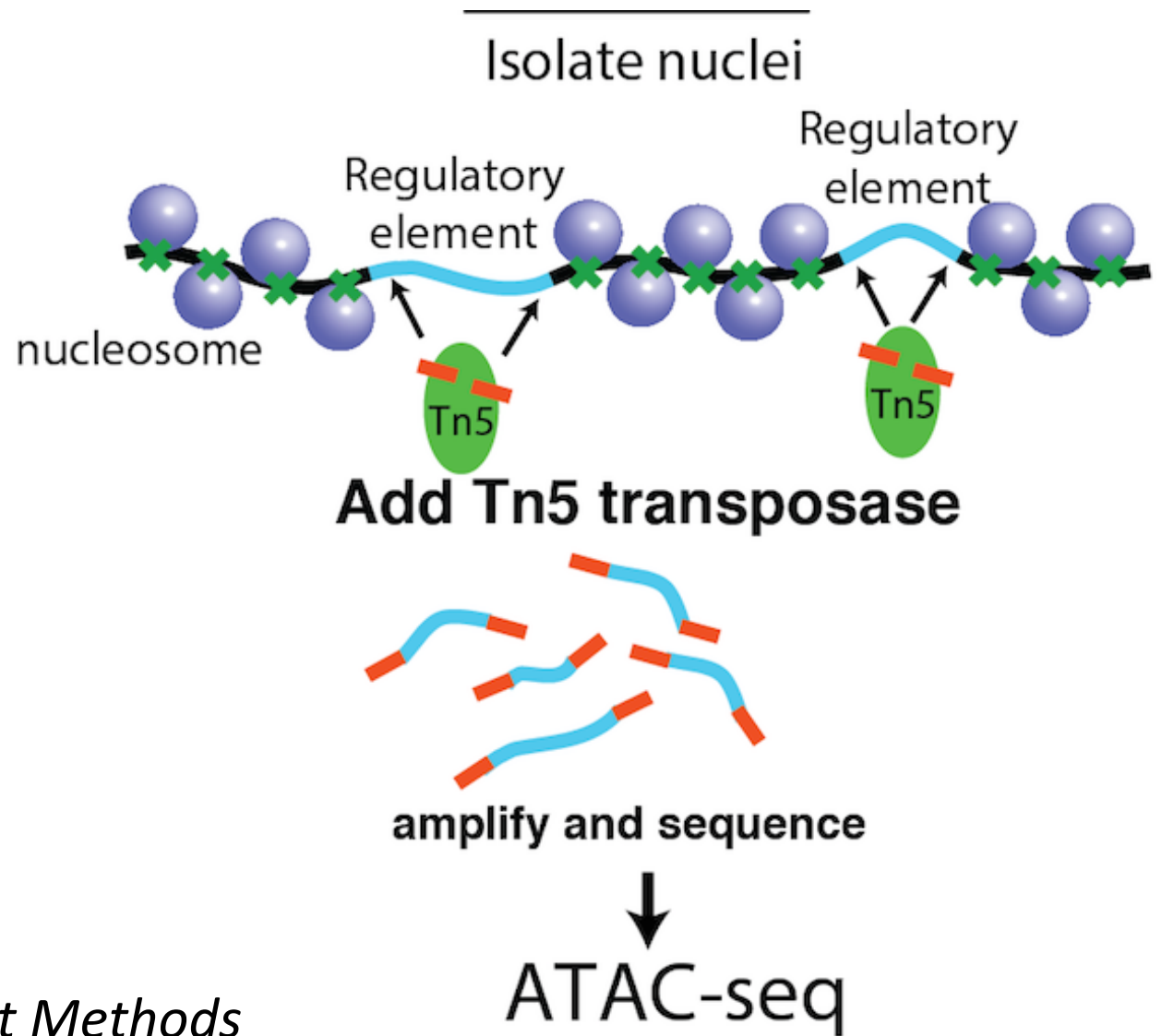
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# Assay for Transposase Accessible Chromatin

Crawford Lab

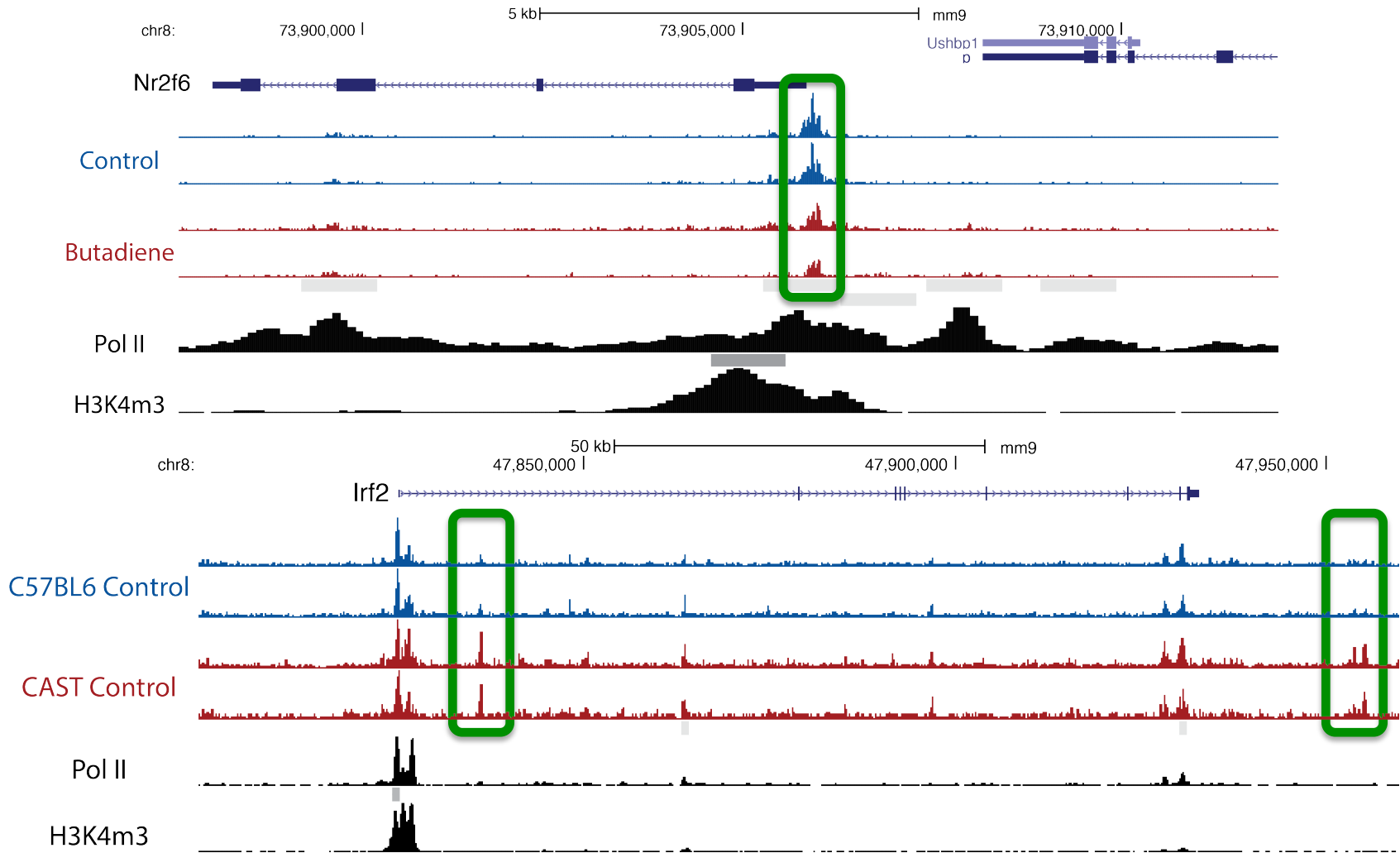
Buenrostro, et al. *Nat Methods*



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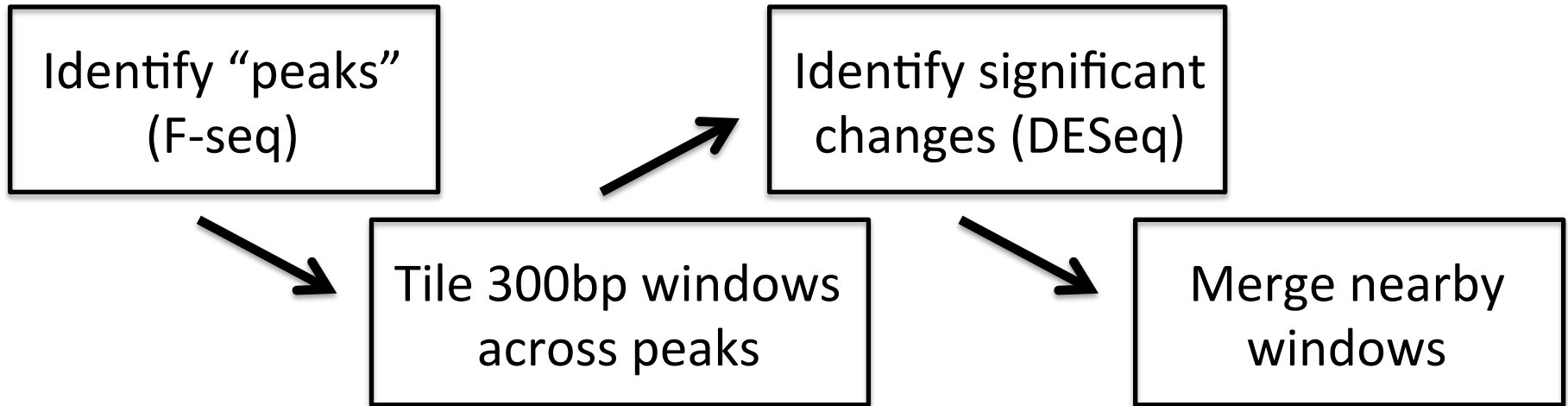
# Treatment and strain-specific chromatin



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# Chromatin Alterations on Exposure to BD

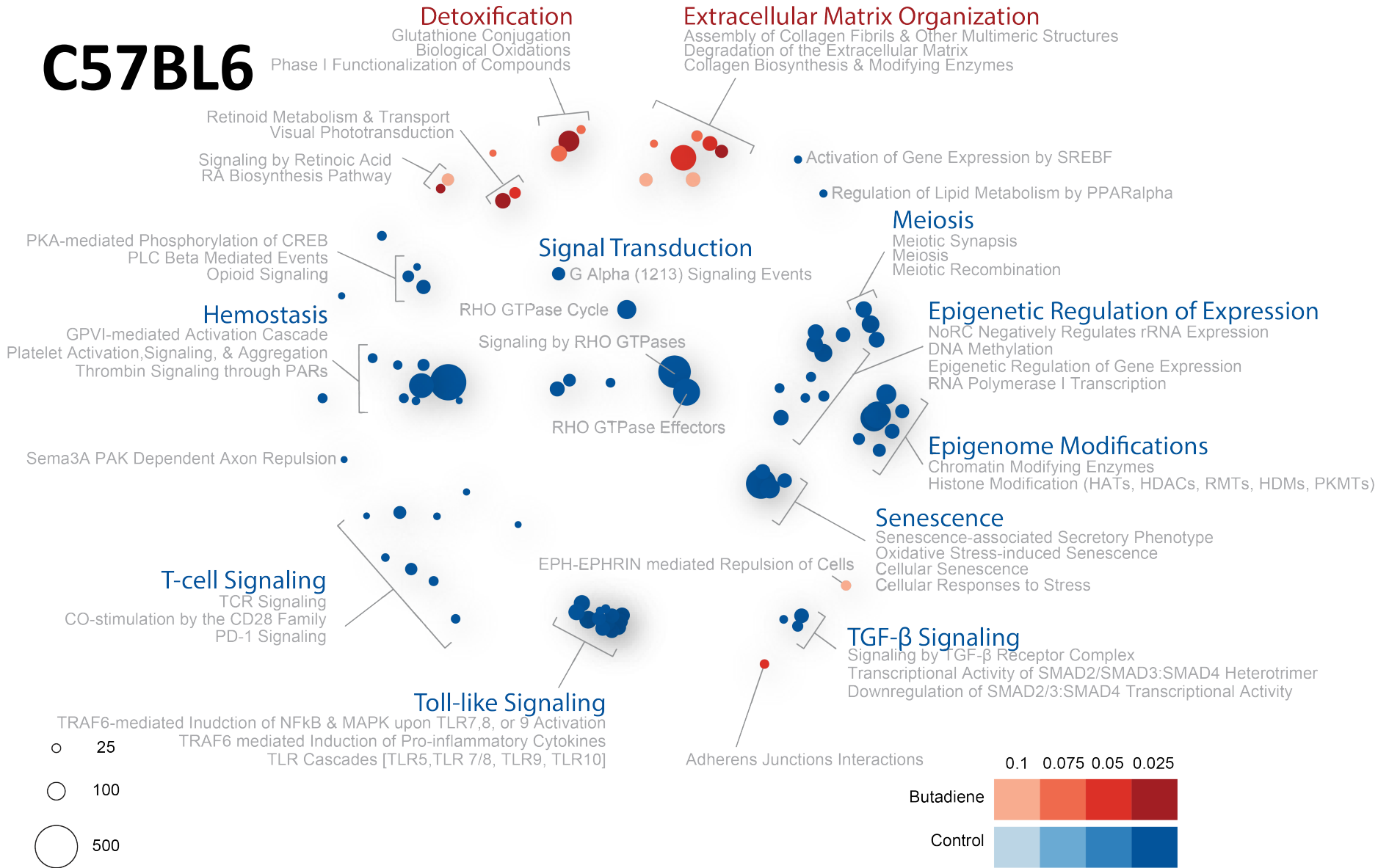


	<u>Windows Tested</u>	<u>Significant Windows</u>	<u>Mapped Genes</u>
<u>C57BL6</u>	325,103	2,610	3,274
<u>CAST</u>	326,645	0	0





# C57BL6



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# C57BL/6

**Detoxification**  
 Glutathione Conjugation  
 Biological Oxidations  
 Phase I Functionalization of Compounds

**Extracellular Matrix Organization**  
 Assembly of Collagen Fibrils & Other Multimeric Structures  
 Degradation of the Extracellular Matrix  
 Collagen Biosynthesis & Modifying Enzymes

Retinoid Metabolism & Transport  
 Visual Phototransduction  
 Signaling by Retinoic Acid  
 RA Biosynthesis Pathway

● Activation of Gene Expression by SREBF  
 ● Regulation of Lipid Metabolism by PPARalpha

**Signal Transduction**  
 ● G Alpha (1213) Signaling Events

**Meiosis**  
 Meiotic Synapsis  
 Meiosis  
 Meiotic Recombination

**Hemostasis**  
 GPVI-mediated Activation Cascade  
 Platelet Activation, Signaling, & Aggregation  
 Thrombin Signaling through PARs

RHO GTPase Cycle  
 Signaling by RHO GTPases  
 RHO GTPase Effectors

**Epigenetic Regulation of Expression**  
 NoRC Negatively Regulates rRNA Expression  
 DNA Methylation  
 Epigenetic Regulation of Gene Expression  
 RNA Polymerase I Transcription

Sema3A PAK Dependent Axon Repulsion ●

**Epigenome Modifications**  
 Chromatin Modifying Enzymes  
 Histone Modification (HATs, HDACs, RMTs, HDMs, PKMTs)

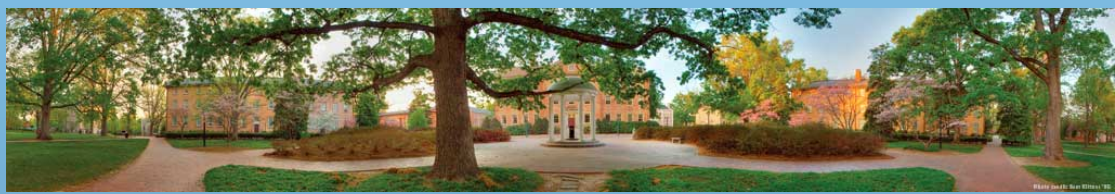
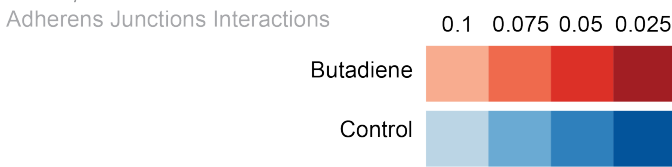
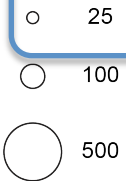
**T-cell Signaling**  
 TCR Signaling  
 CO-stimulation by the CD28 Family  
 PD-1 Signaling

EPH-EPHRIN mediated Repulsion of Cells

**Senescence**  
 Senescence-associated Secretory Phenotype  
 Oxidative Stress-induced Senescence  
 Cellular Senescence  
 Cellular Responses to Stress

**Toll-like Signaling**  
 TRAF6-mediated Inudction of NFkB & MAPK upon TLR7,8, or 9 Activation  
 TRAF6 mediated Induction of Pro-inflammatory Cytokines  
 TLR Cascades [TLR5, TLR 7/8, TLR9, TLR10]

**TGF-β Signaling**  
 Signaling by TGF-β Receptor Complex  
 Transcriptional Activity of SMAD2/SMAD3:SMAD4 Heterotrimer  
 Downregulation of SMAD2/3:SMAD4 Transcriptional Activity



# What drives differences between C57BL6 and CAST in response to butadiene exposure?



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# Gene expression and chromatin landscape differ at basal state

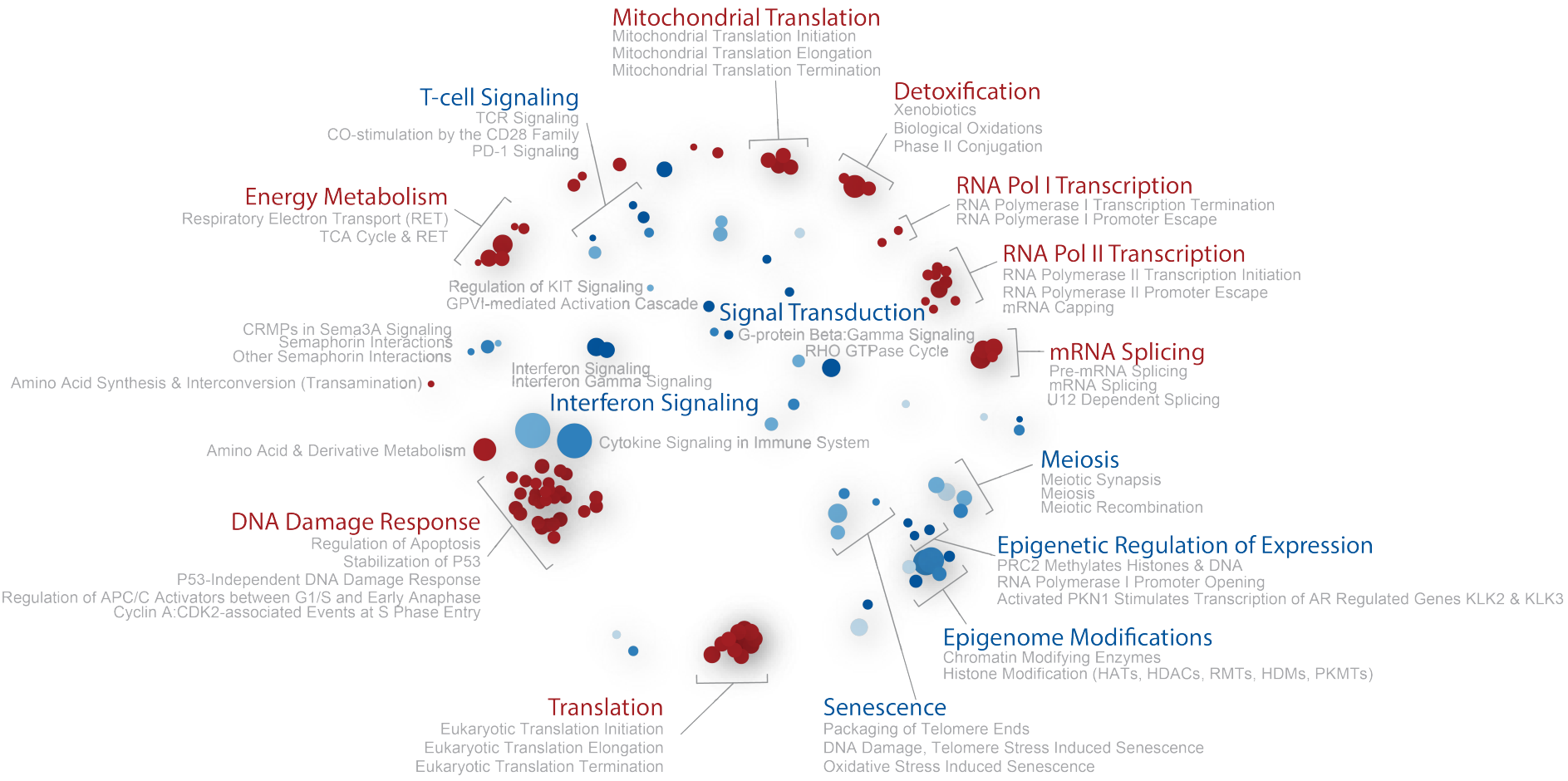
- mRNA expression:

	<u>Total Genes</u>	<u>Differential</u>
<u>C57BL6 vs CAST</u>	20868	10250
<u>C57BL6 exposure</u>	20868	4116

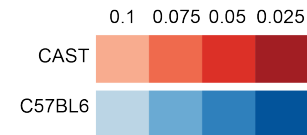
- Chromatin Accessibility:

	<u>Windows Tested</u>	<u>Significant Windows</u>	<u>Mapped Genes</u>
<u>C57BL6 vs CAST</u>	304,385	19,220	11,184
<u>C57BL6 exposure</u>	325,103	2,610	3,274





# C57BL6 vs. CAST

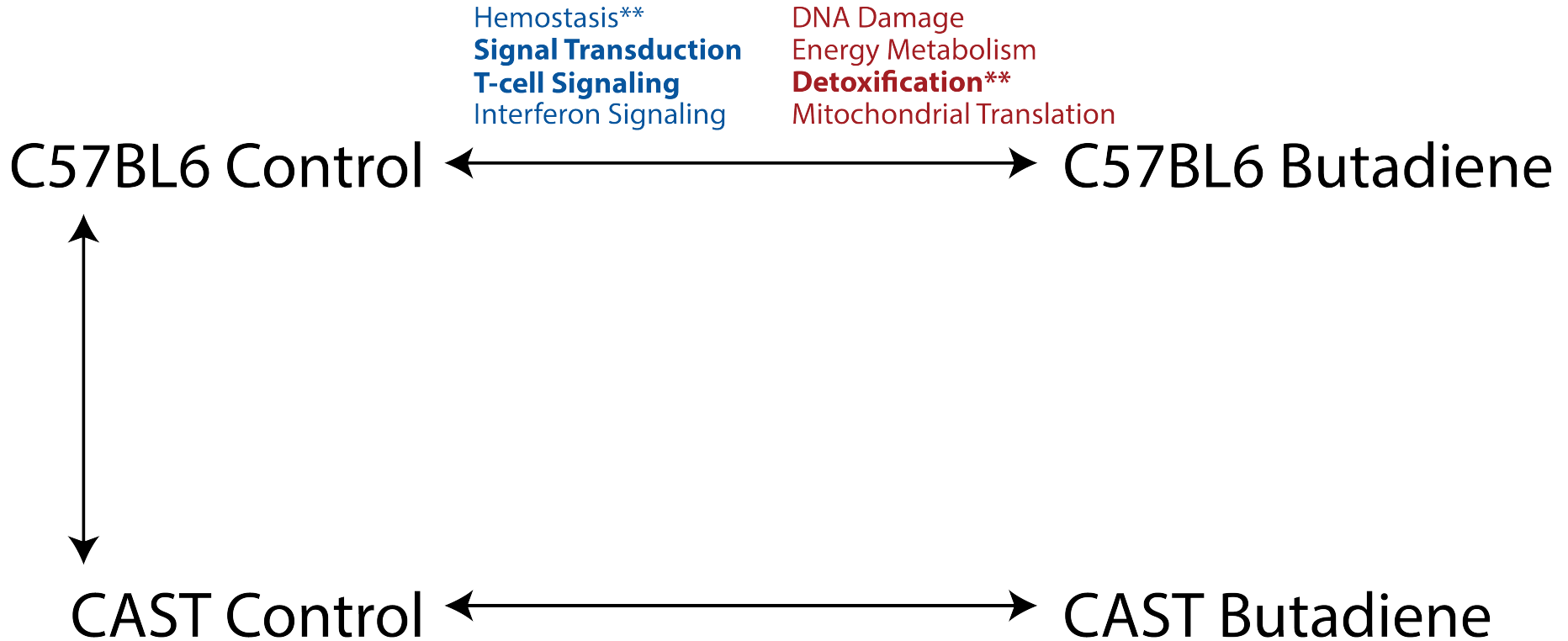


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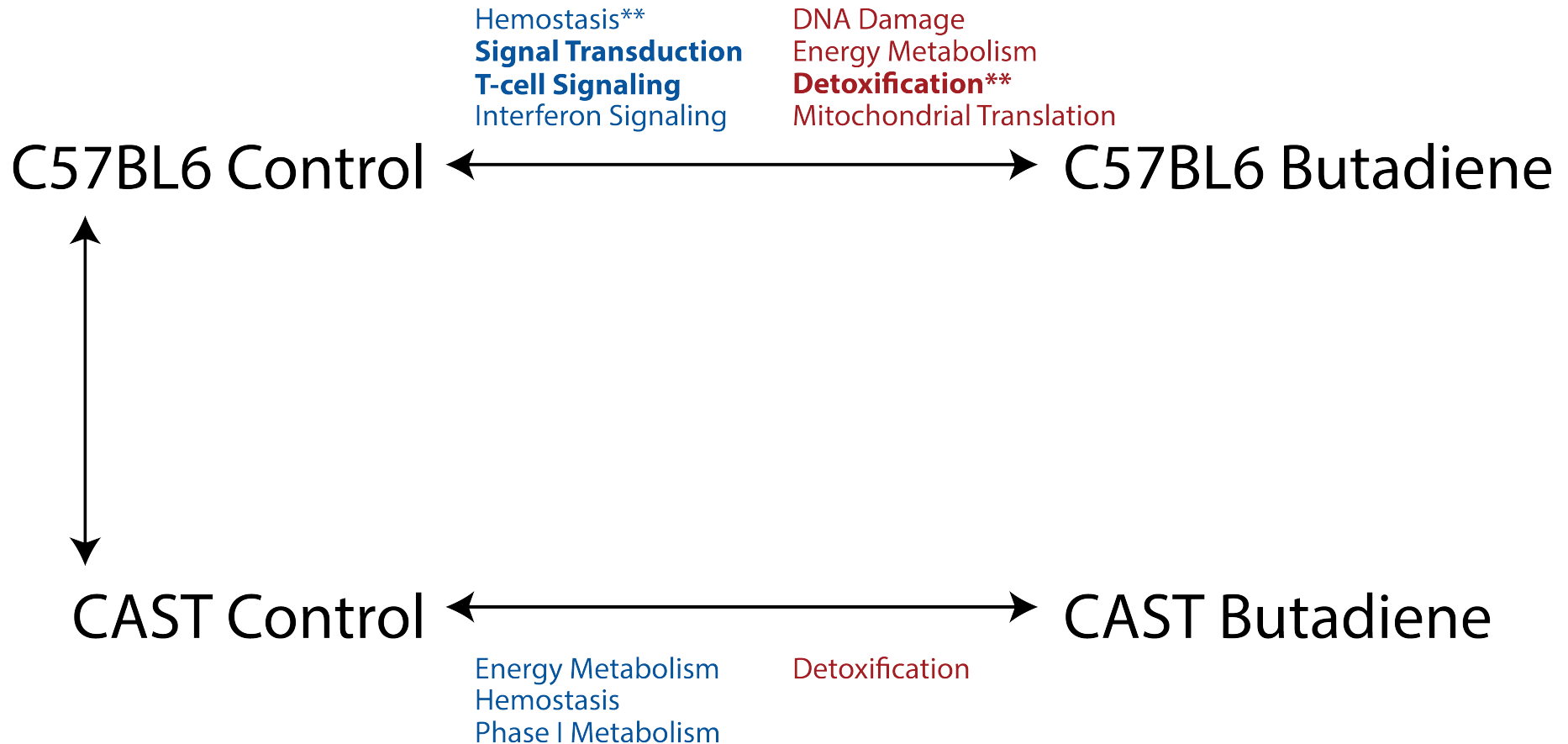




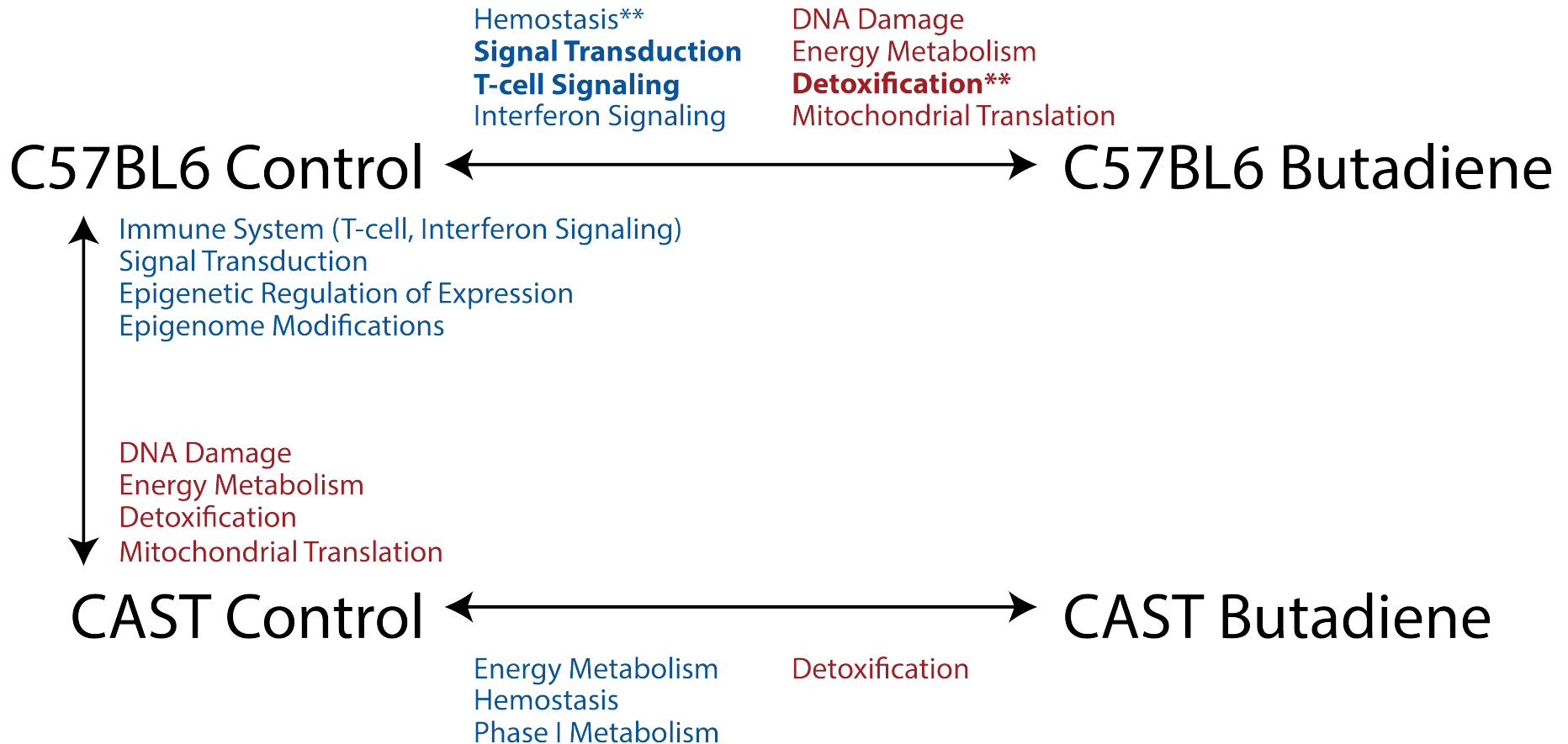
# Summary



# Summary



# Summary



# Epigenetic data in toxicology studies enables

- Understanding better how expression phenotypes arise in cells and tissues
- Understanding how basal epigenetic state contributes to altered susceptibility
- Investigations into how genetic variation contributes to phenotype through effects on epigenome



# Acknowledgements

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Jeremy Wang

**Matt Weiser**

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Grace Chappell

Greg Crawford (Duke)

Alexias Salas



University Cancer  
Research Fund



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