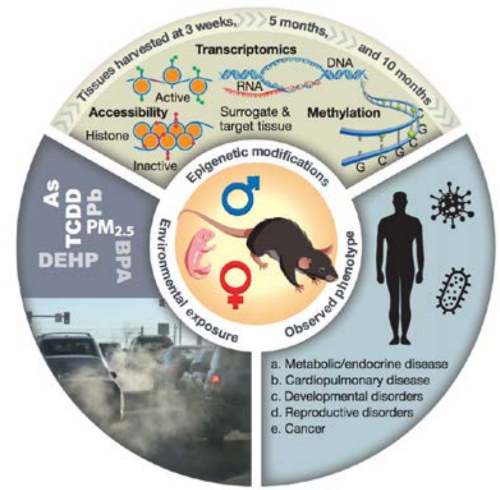
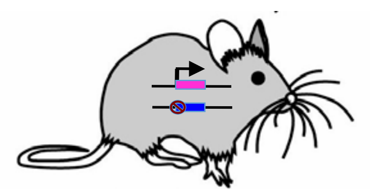
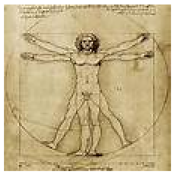
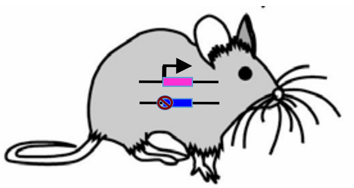
In Utero EDC Exposure May Reprogram the Adult Mouse Brain: A Role for Epigenetics

Marisa Bartolomei
University of Pennsylvania









Developmental Origins of Health and Disease

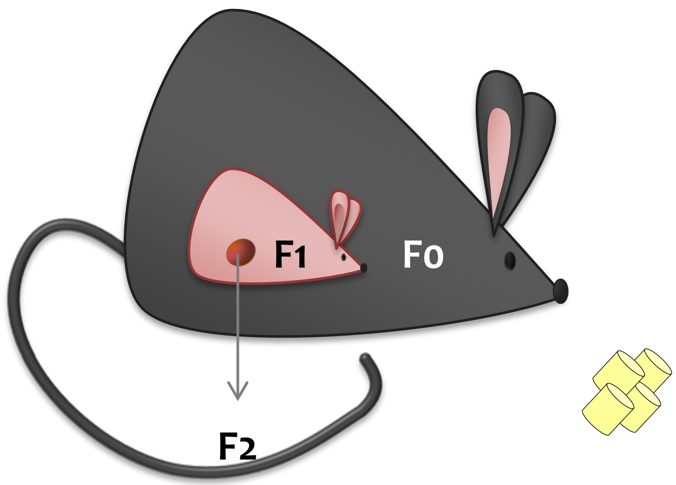
Adverse early life event

Epigenetics

Adult disease

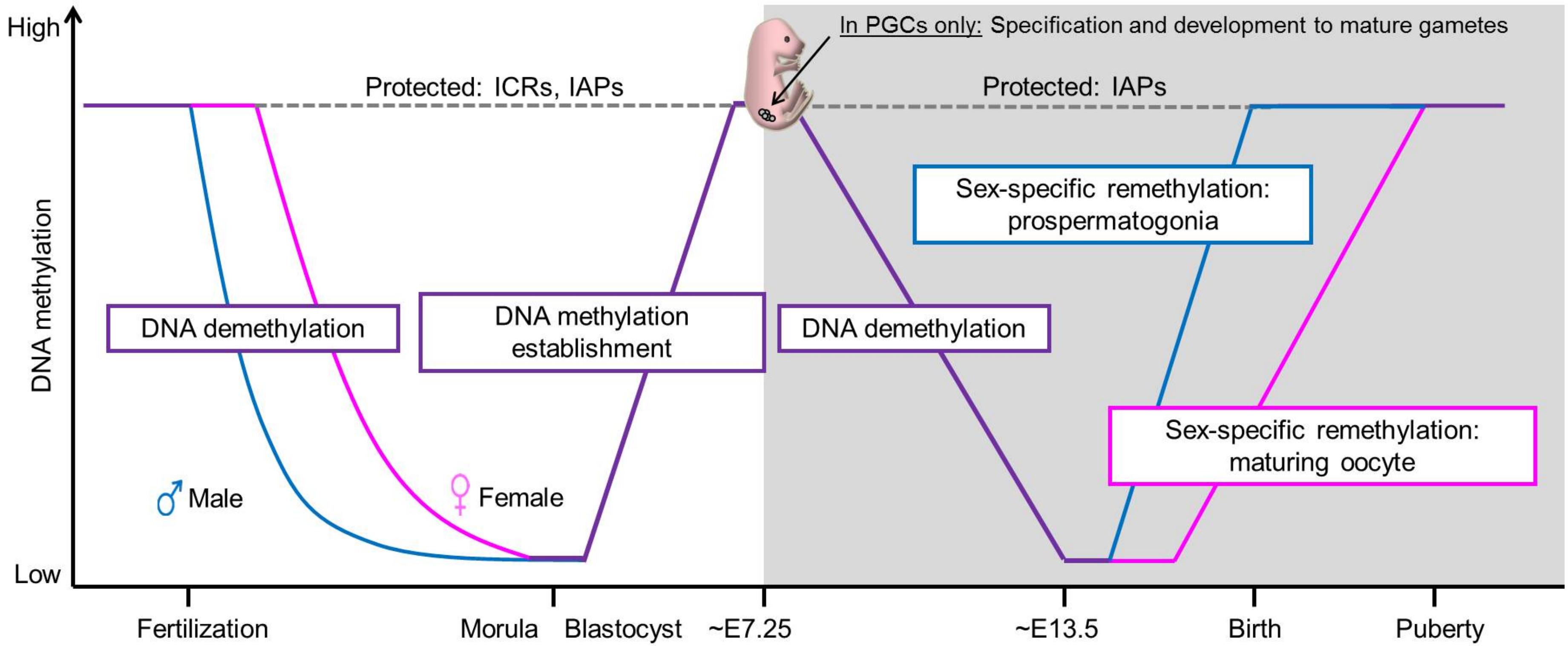
Metabolism

Brain Development & Behavior



Susiarjo *et al*. (2015, 2017) Endocrinology;
Xin *et al*. (2015) Semin in Cell and Dev Biol

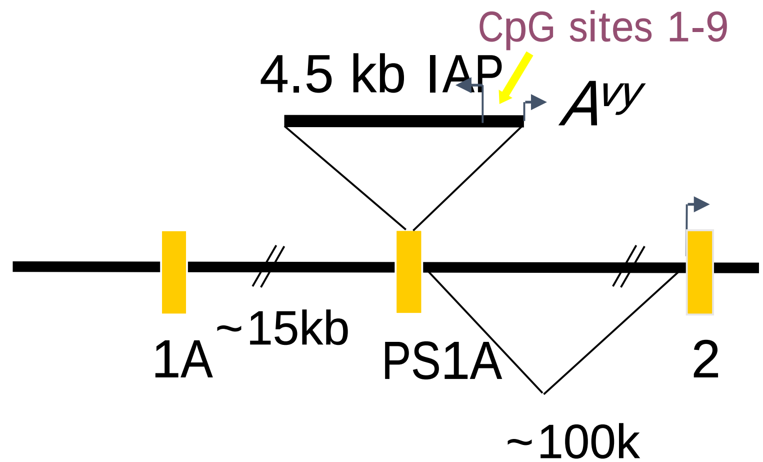
Early Development Represents a Vulnerable Window to Environmental Perturbations



Bisphenol A Alters DNA Methylation

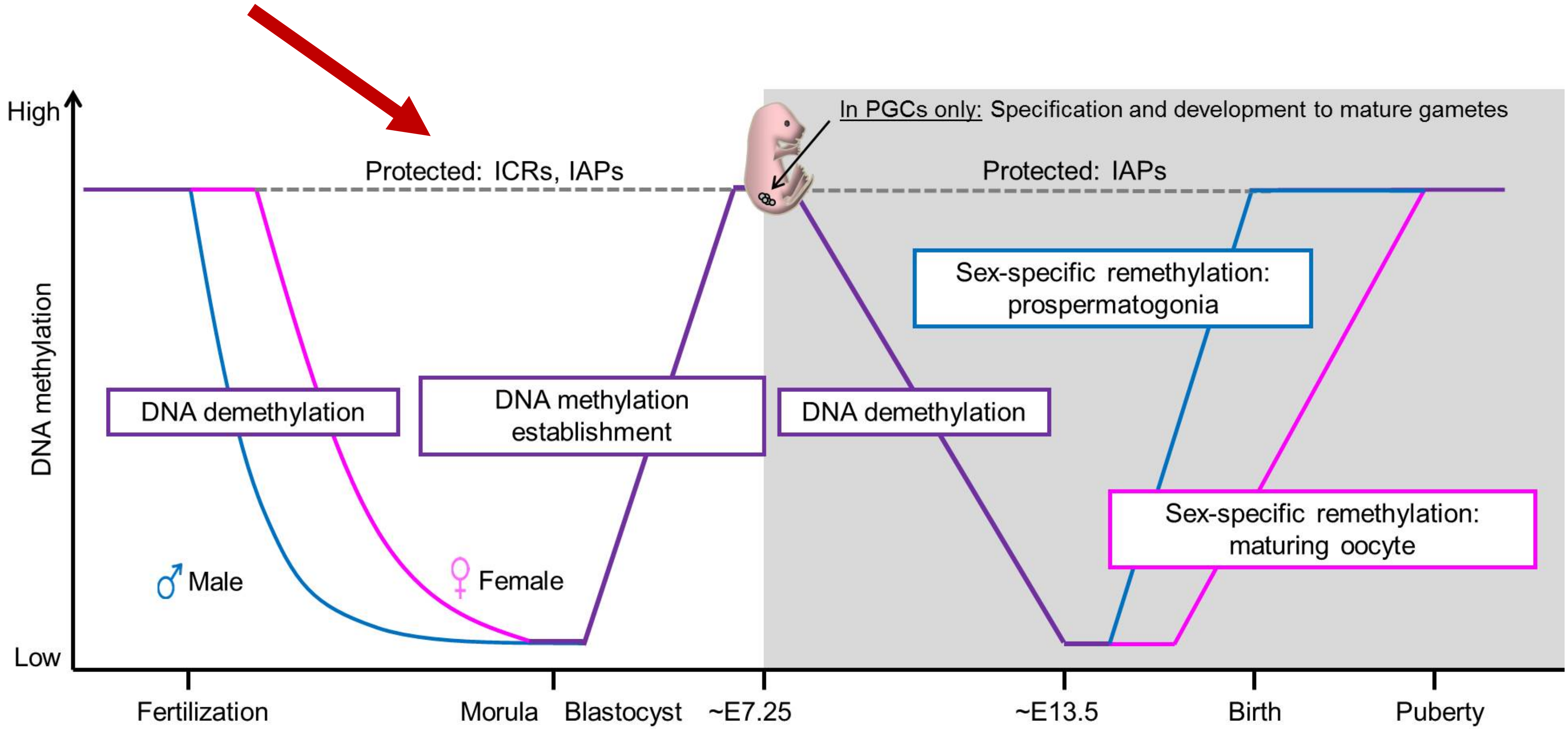
Agouti expression and coat color

hypomethylation

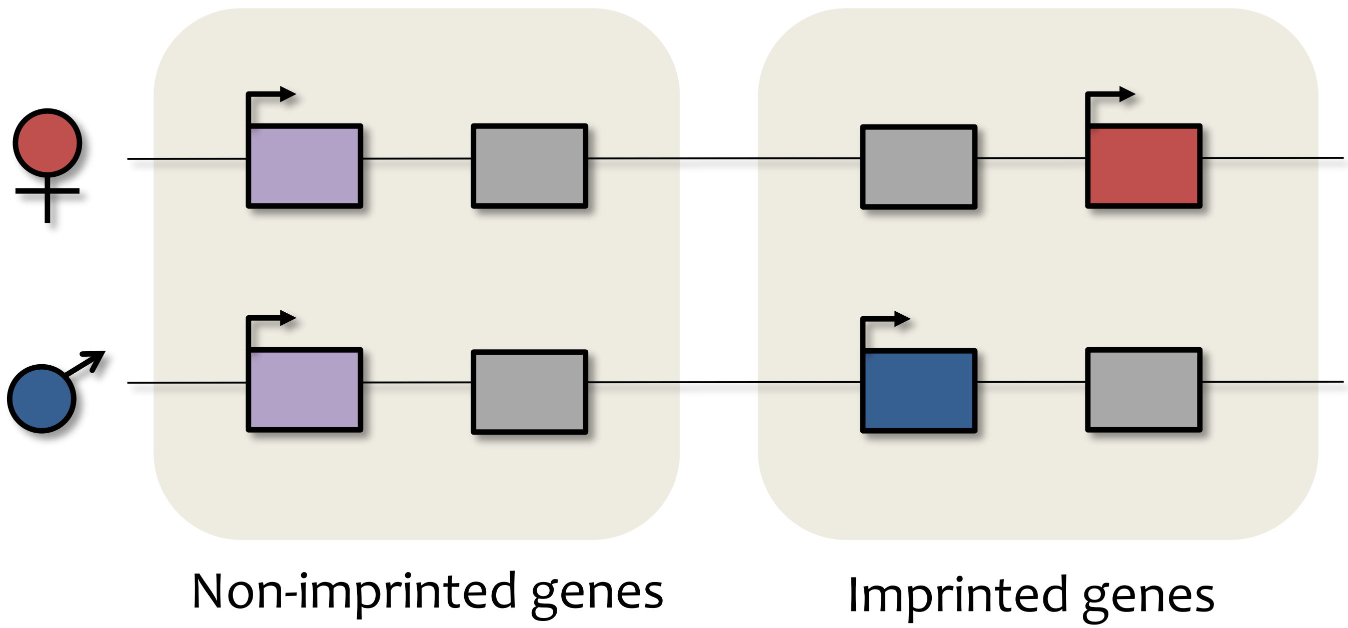


Dolinoy et.al., (2007)
PNAS 104:13056-13061

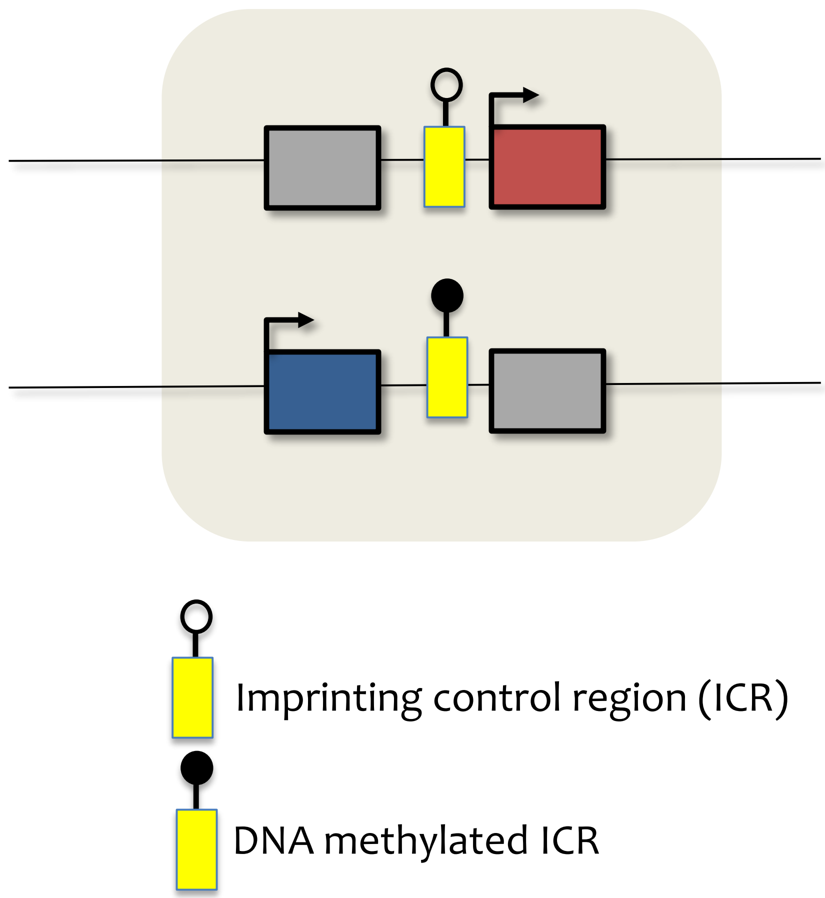
Early Development Represents a Vulnerable Window to Environmental Perturbations



Genomic Imprinting is Mammalian-Specific and Results in Monoallelic, Parent-of-Origin-Specific Expression

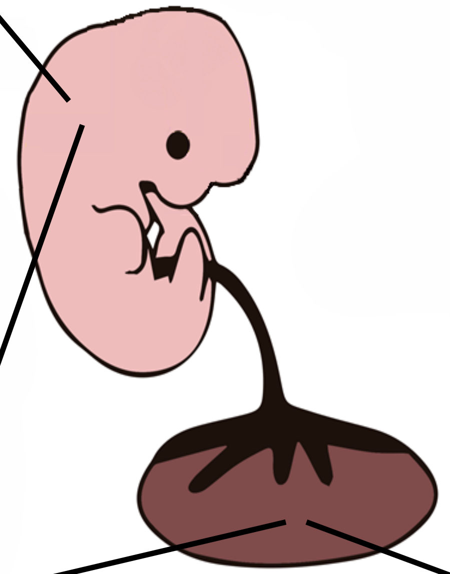


Imprinted Genes Reside in Clusters and are Regulated by ICRs that are DNA Methylated on a Single Parental Allele



Functions of Imprinted Genes

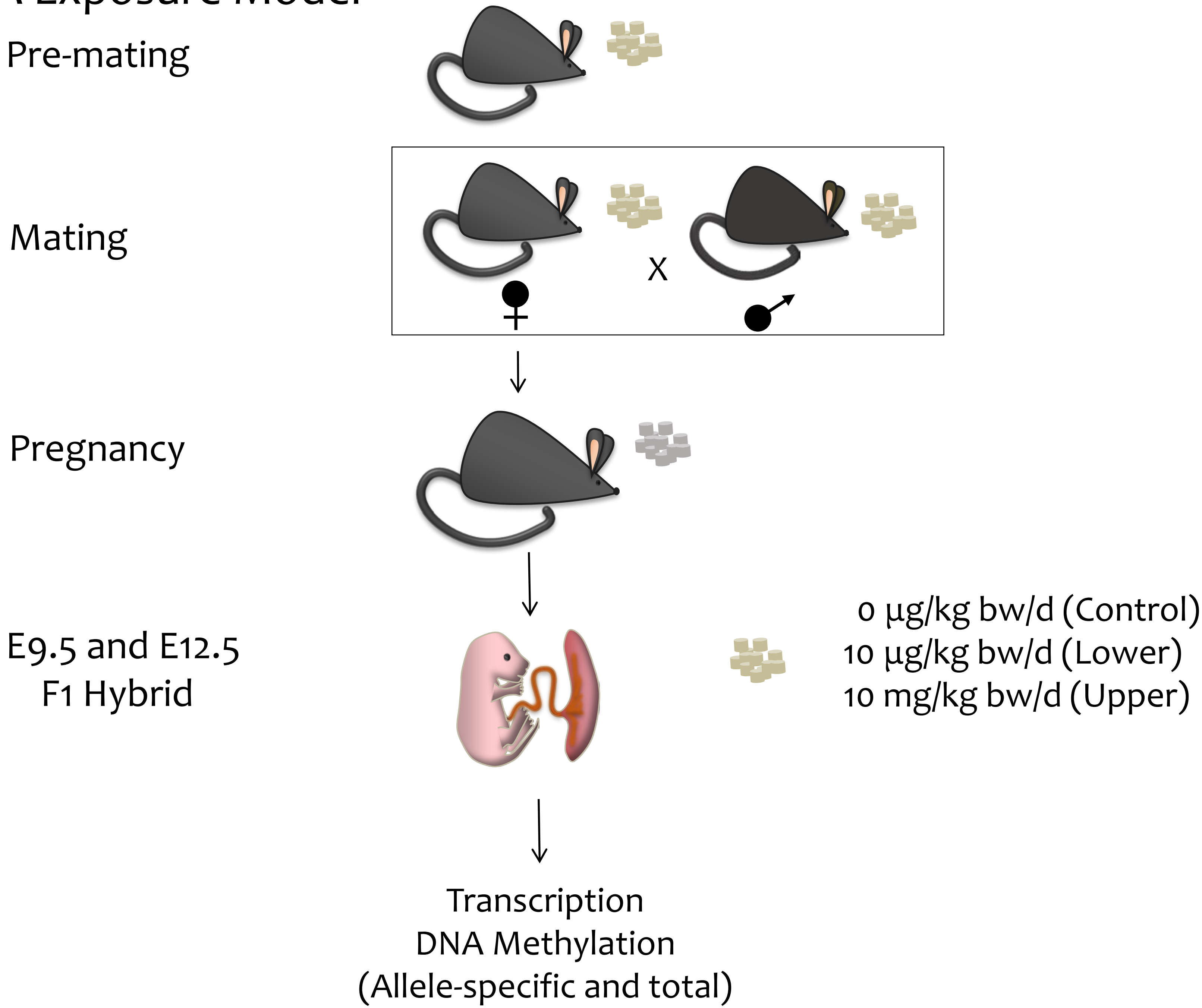
Gene	Direction of Imprint	Function of Gene Product	Role in Embryo Growth/Behavior
IGF2	Paternal	Positive regulator of growth	Growth
H19	Maternal	Negative regulator of growth	Suppression of growth
IGF2R	Maternal	Negative regulator of growth	Suppression of growth
GRB10	Maternal	Negative regulator of growth	Suppression of growth
GRB10	Paternal (neuron-specific)	Signal adaptor	Aggression
UBE3A	Maternal (neuron-specific)	Ubiquitin ligase & transcriptional co-activator	Memory, learning, motor function
PEG3	Paternal	Zinc finger protein; control of apoptosis	Sex-specific behavior
NDN	Paternal	Regulator of neuronal growth and differentiation	Spatial learning; socialization
NESP	Maternal	Secretory pathway	Exploratory behavior
GNAS	Maternal	Signal transduction	Cognition & sleep



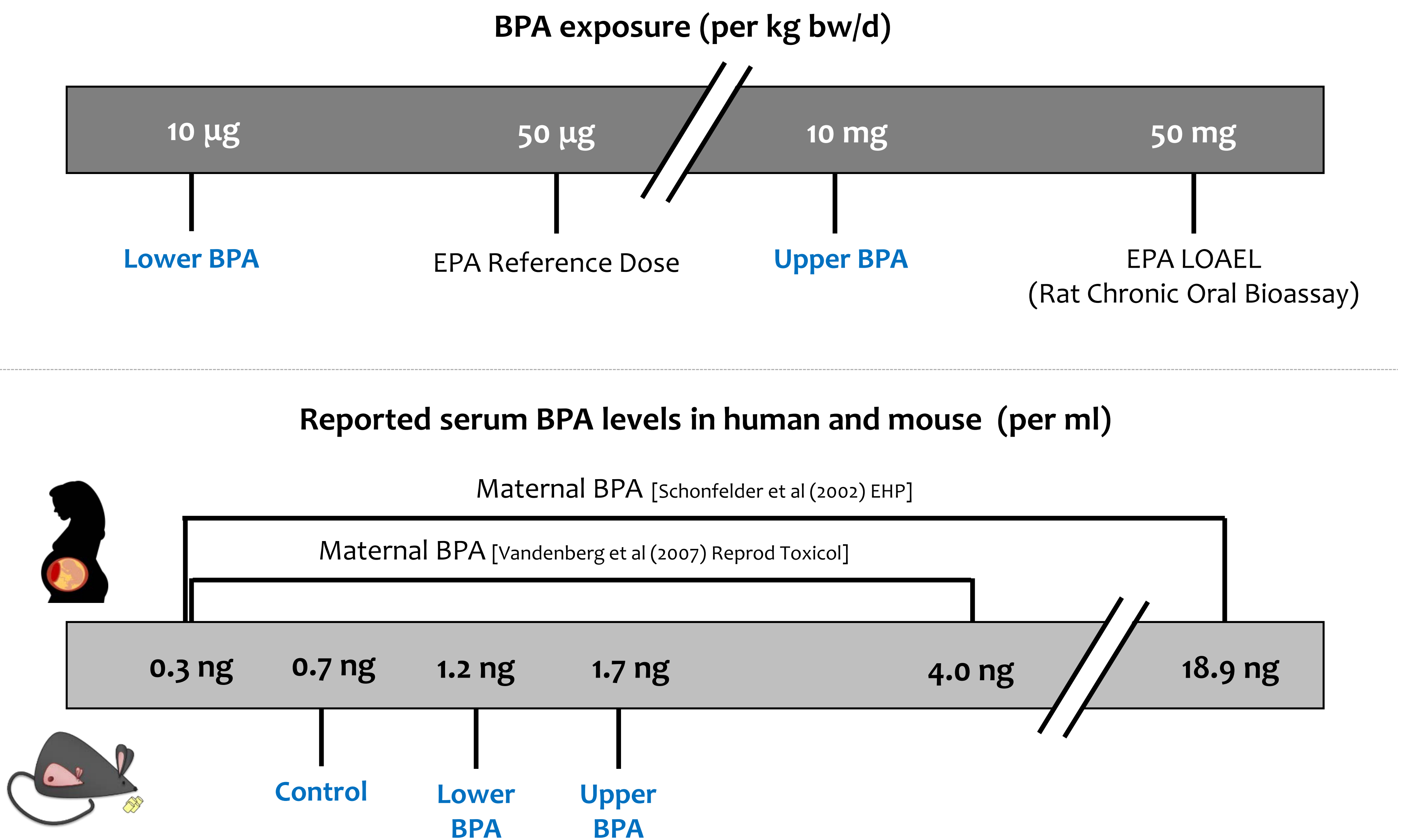
Gene	Direction of Imprint	Function of Gene Product	Role in Placenta
PEG3	Paternal	Zinc finger protein; control of apoptosis	Growth
PEG1	Paternal	Hydrolase	Growth
MASH2	Maternal	Helix-loop-helix transcription factor	Spongiotrophoblast development
PHLDA2	Maternal	Pleckstrin homology domain protein	Spongiotrophoblast restriction
CDKN1C	Maternal	Cell cycle regulator	Spongiotrophoblast restriction
SLC22A3	Maternal	Cation transporter	Nutrient transfer

From Kalish et al, 2014

BPA Exposure Model

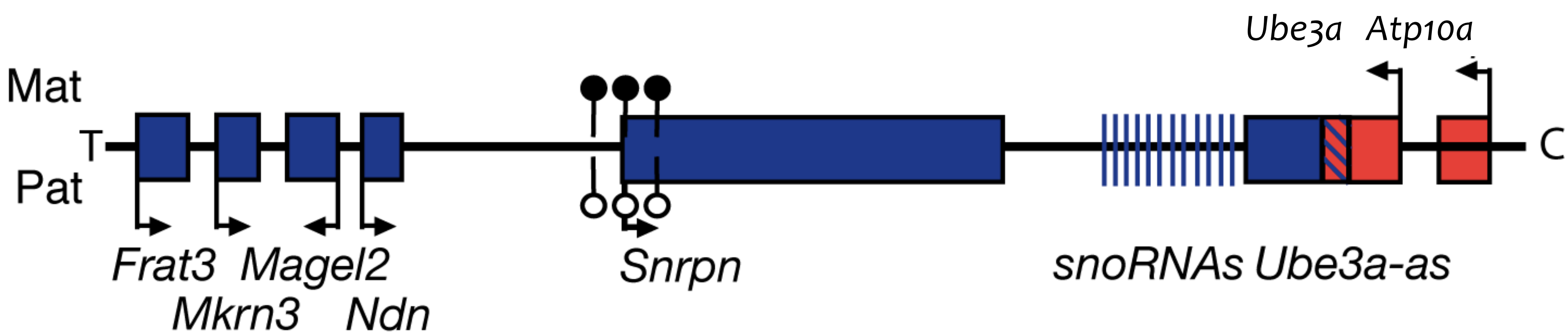


Early Life BPA Exposure is Representative of Human Exposure Levels

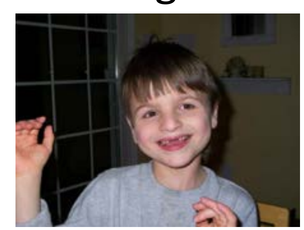


Susiarjo et al (2013) PLoS Genetics; Susiarjo et al (2015) Endocrinology

The *Snrpn* Domain**



**Prader Willi and Angelman Syndromes Critical Regions



Expressed maternal allele

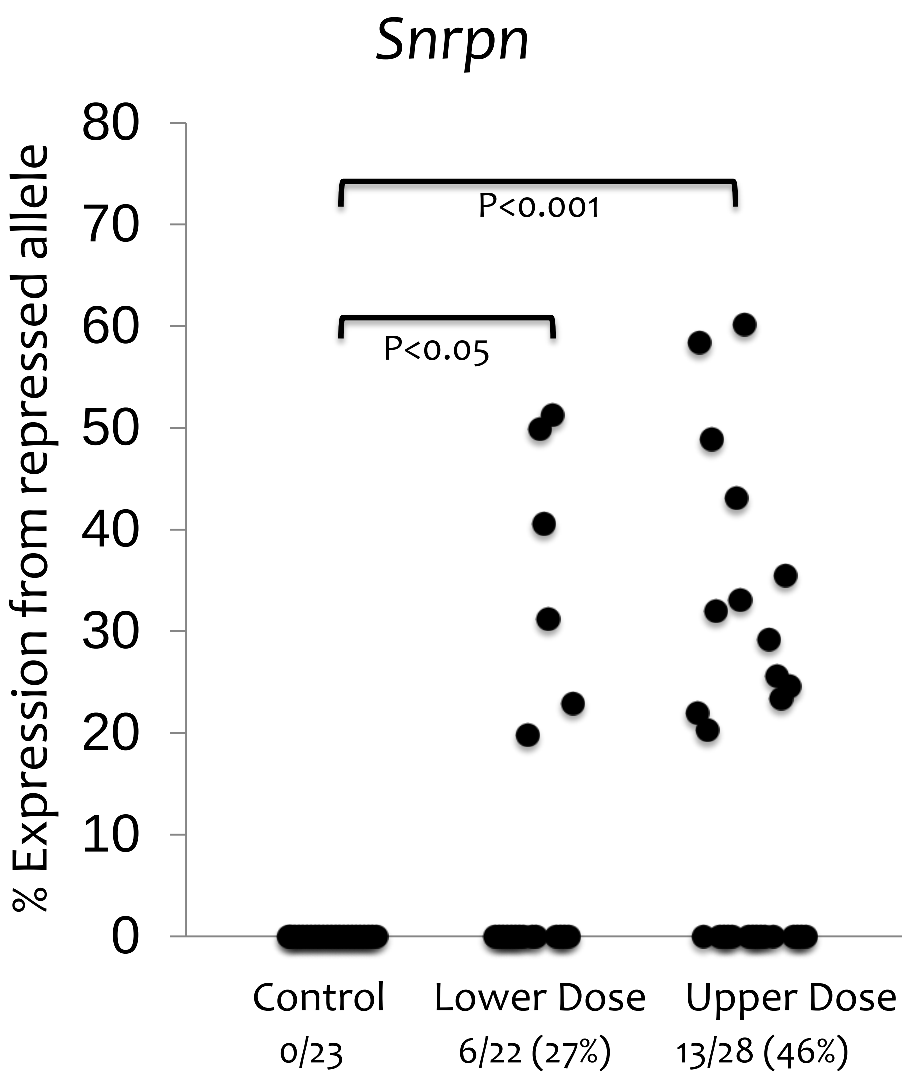
Expressed paternal allele

Unmethylated DMR

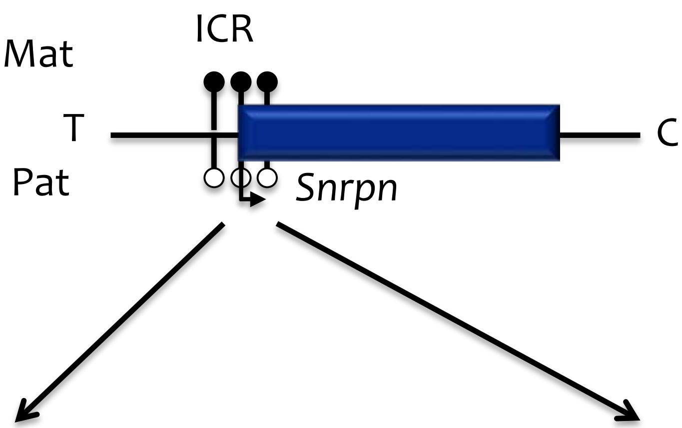
Methylated DMR

Susiarjo et al (2013) PLoS Genetics

Increased Proportion of E9.5 Placentae with Biallelic Expression of *Snrpn* in BPA-Exposed Mice



BPA Exposure Reduced Methylation of 16 CpGs in the Maternal ICR in Placentas



AATCTGTGTGATGCTTGCAATCACTTGGGAGCAATT
TTTTAAAAAAATTAAATGTATTTAGTAATAGGCAATT
ATATCCATTATTCCAGACTGACAGTGATTTTTTTTTA
AATACA**CG**CTCAAATTTC**CG**CAGTAGGAATGCTCAA
GCATTCCTTTTGGTAGCTGCCTTTTGGCAGGACATT
C**CG**GTCAGAGGGACAGAGACCCCTGCATTG**CG**GC
AAAAATGTG**CG**CATGTGCAGCCATTGCCTGGGA**CG**
CATG**CG**TAGGGAGC**CGCGCG**ACAAACCTGAGCCA
TTG**CG**GCAAGACTAG**CG**CAGAGAGGAGAGGGAGC
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TGCAA**CG**CAATGGAG**CG**AGGAAGGTCAGCTGGGC
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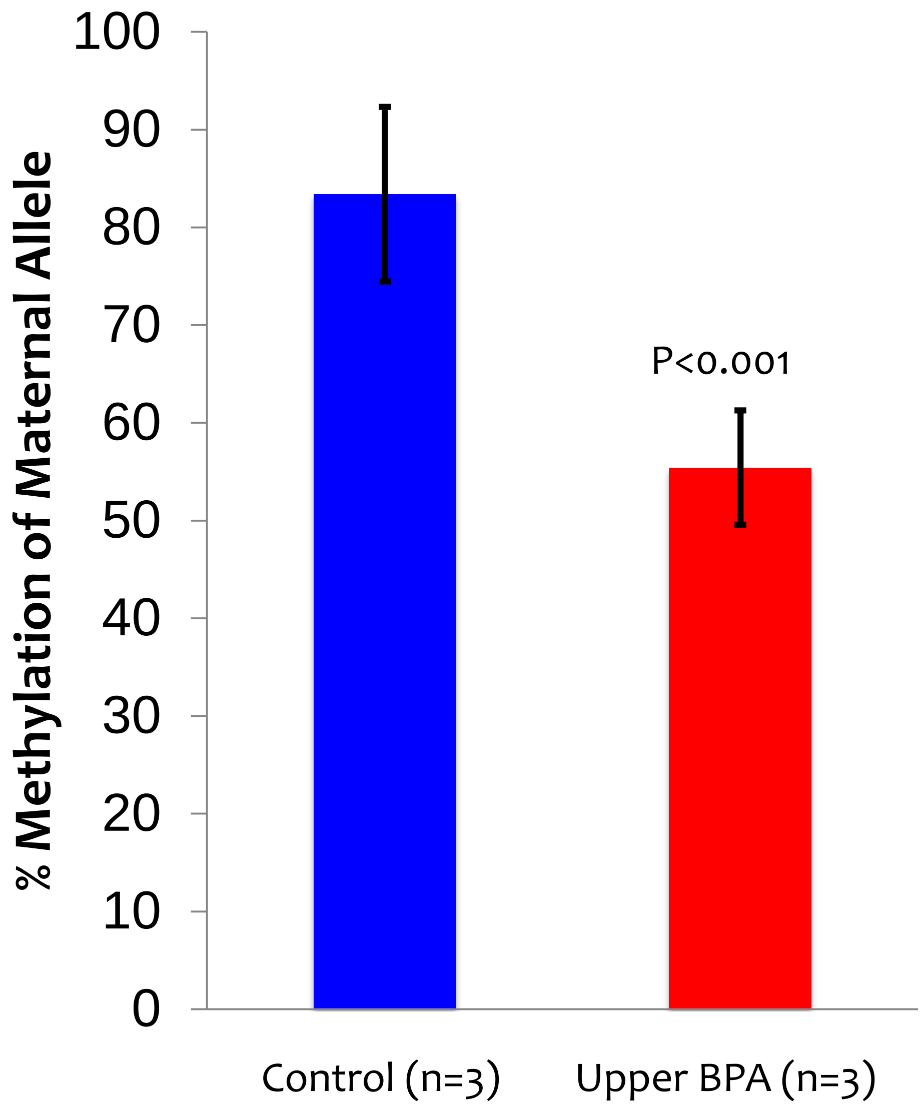
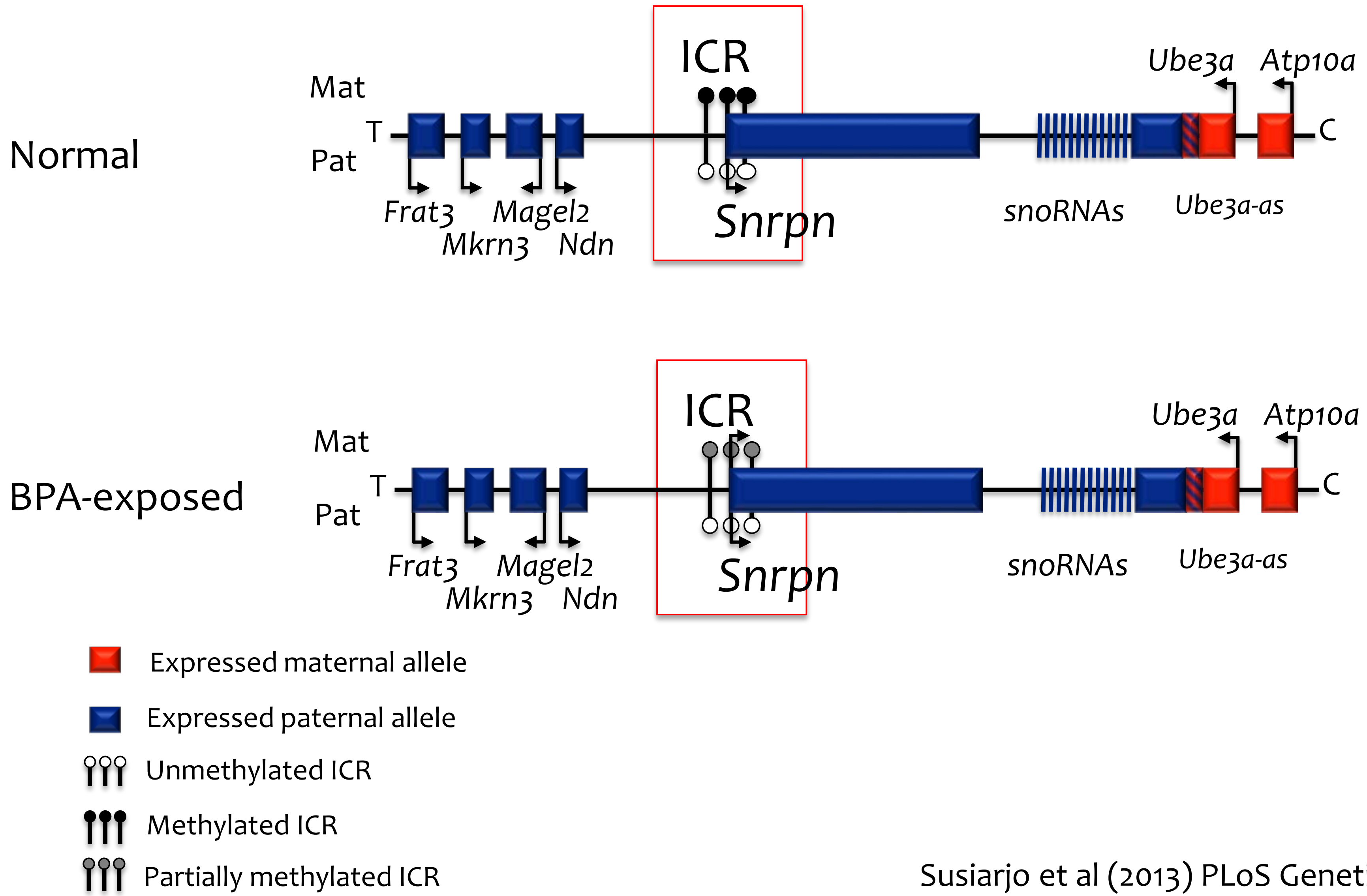


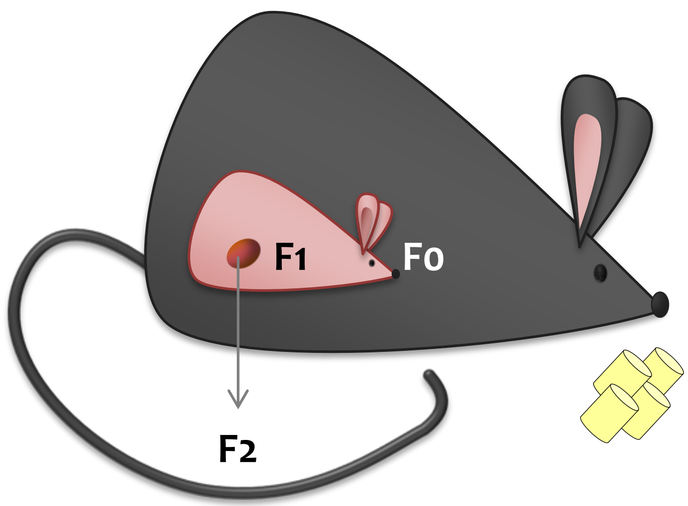
The Imprinted *Snrpn* Locus Experiences Loss of ICR Methylation and Biallelic Expression in BPA-Exposed Offspring (placentas and **embryonic brain**)



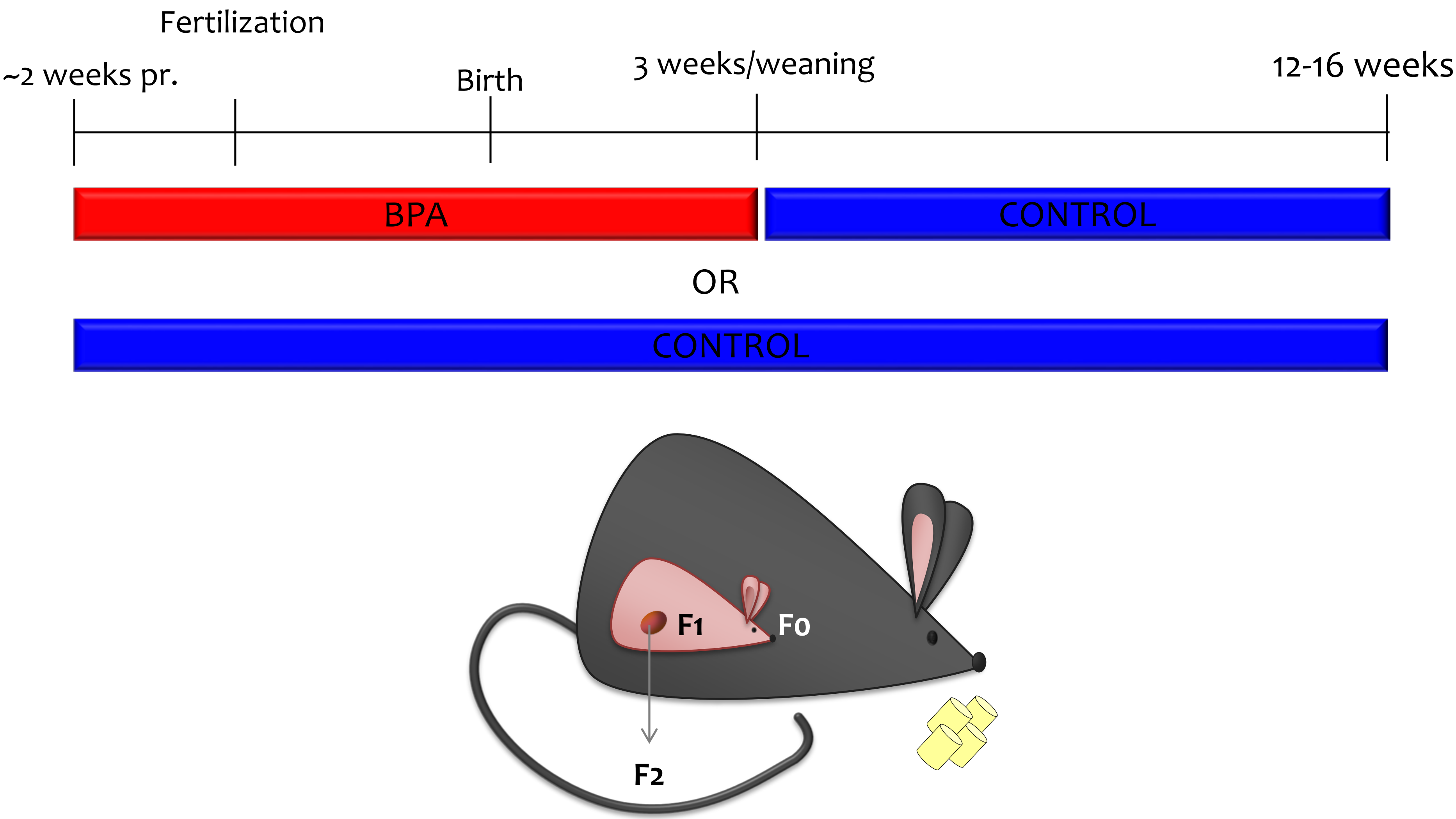
Susiarjo et al (2013) PLoS Genetics

Is BPA Exposure Linked to Long-term Phenotypic Abnormalities?

F1 and F2 offspring



Timeline of BPA Exposure to Generate F1 Offspring



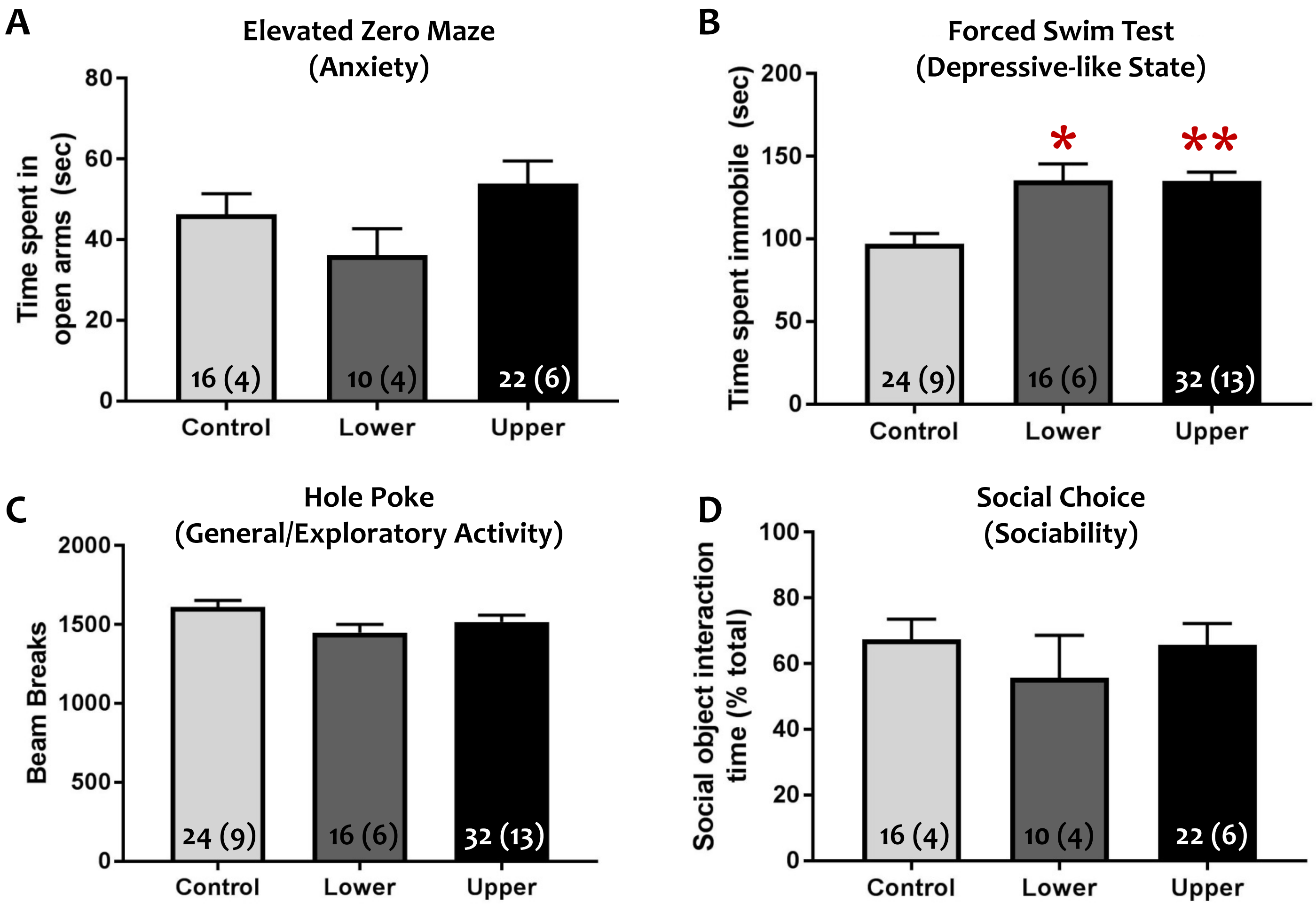
Developmental Origins of Health and Disease

Adverse early life event → Adult disease

Metabolism

Brain Development
Behavior

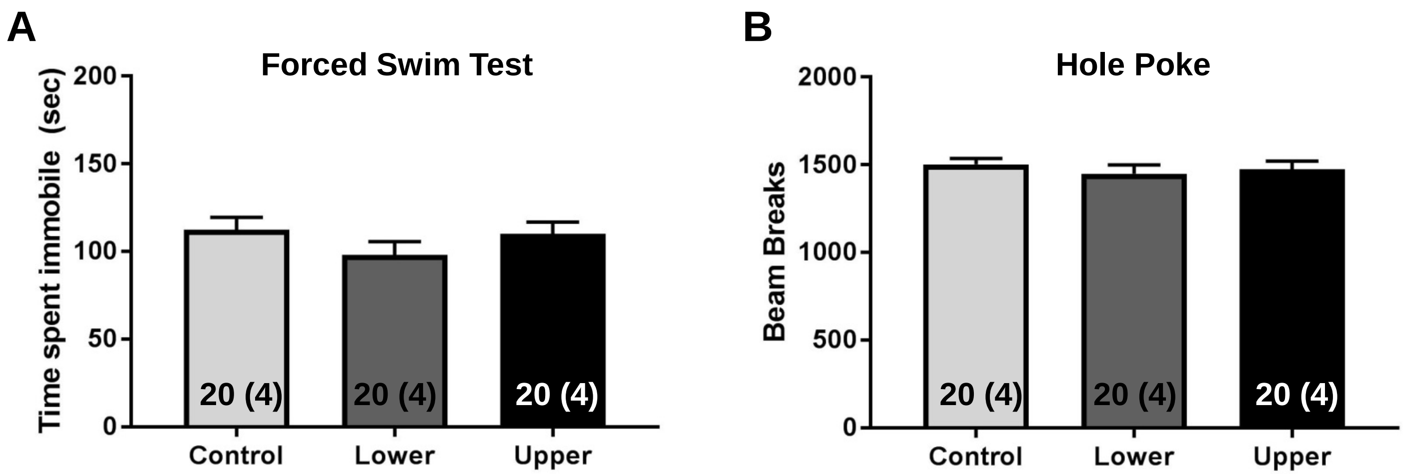
Early Life Exposure to BPA Increases Behavioral Despair in Adulthood



Stats: Linear mixed effects with Kenward Rogers correction; * $P < 0.05$, ** $P < 0.01$

Xin et al (2018) Hormones and Behavior

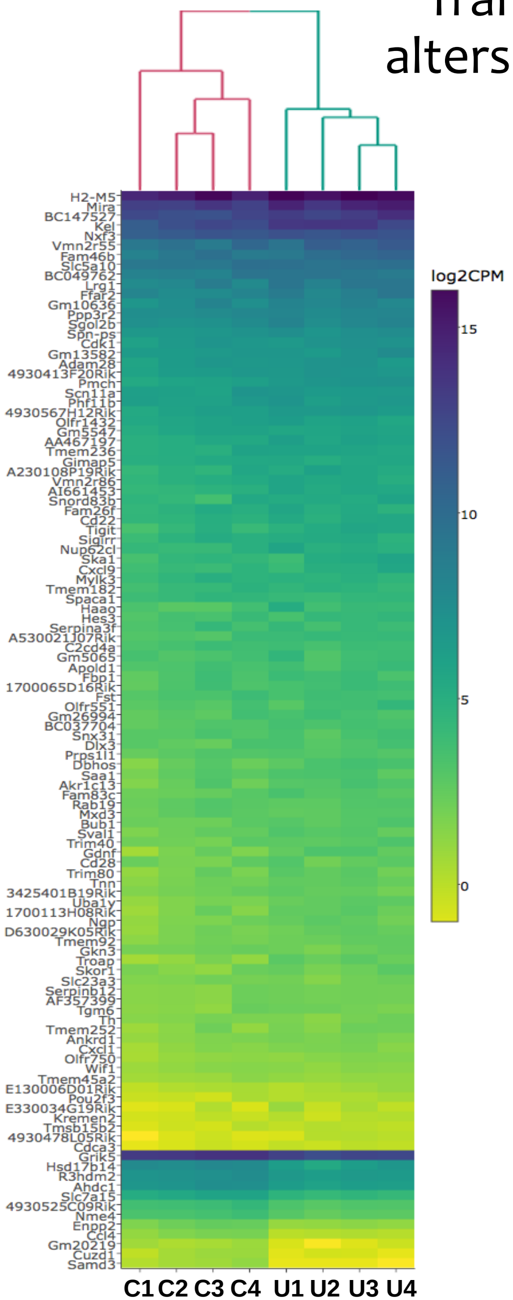
Affective Behavioral Changes are Limited to the F1 Generation



Stats: Linear mixed effects with Kenward Rogers correction; * P < 0.05

Xin et al (2018) Hormones and Behavior

Transcriptome Analysis Suggests Early Life Exposure to BPA alters Neurotransmitter Systems in Adult F1 Male Hippocampus



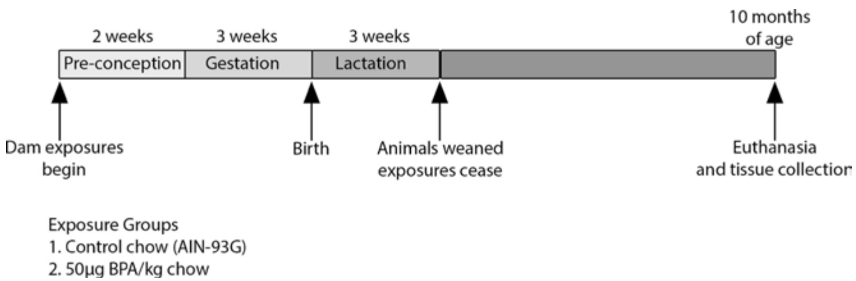
Name (IPA, Upregulated Genes)	P-value
Catecholamine Biosynthesis	9.10E-03
DNA damage-induced 14-3-3 Signaling	2.41E-02
GADD45 Signaling	3.89E-02
Mitotic Roles of Polo-Like Kinase	4.18E-02
Role of CHK Proteins in Cell Cycle Checkpoint Control	4.18E-02

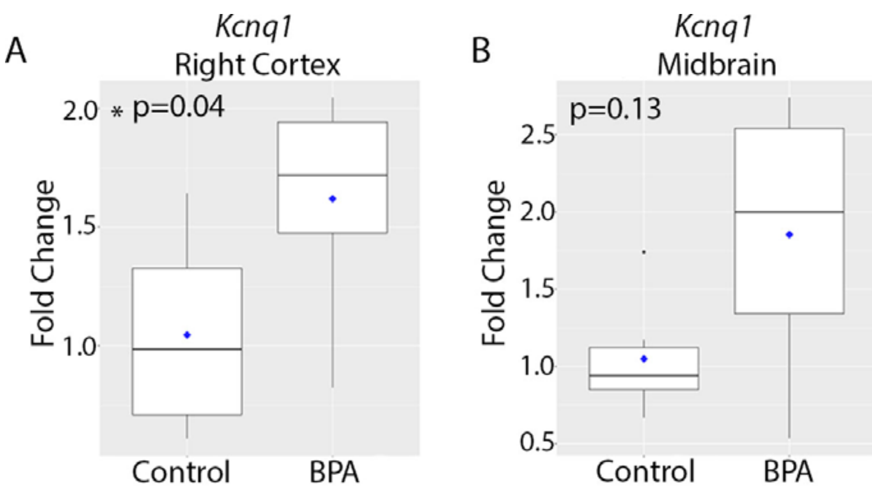
Name (IPA, Downregulated Genes)	P-value
Pyrimidine Deoxyribonucleotides De Novo Biosynthesis I	1.22E-03
Pyrimidine Ribonucleortides De Novo Biosynthesis	4.86E-03
Pyrimidine Ribonucleotides Interconversion	4.86E-03
Salvage Pathways of Pyrimidine Ribonucleotides	2.18E-02
Glutamate Receptor Signaling	2.83E-02

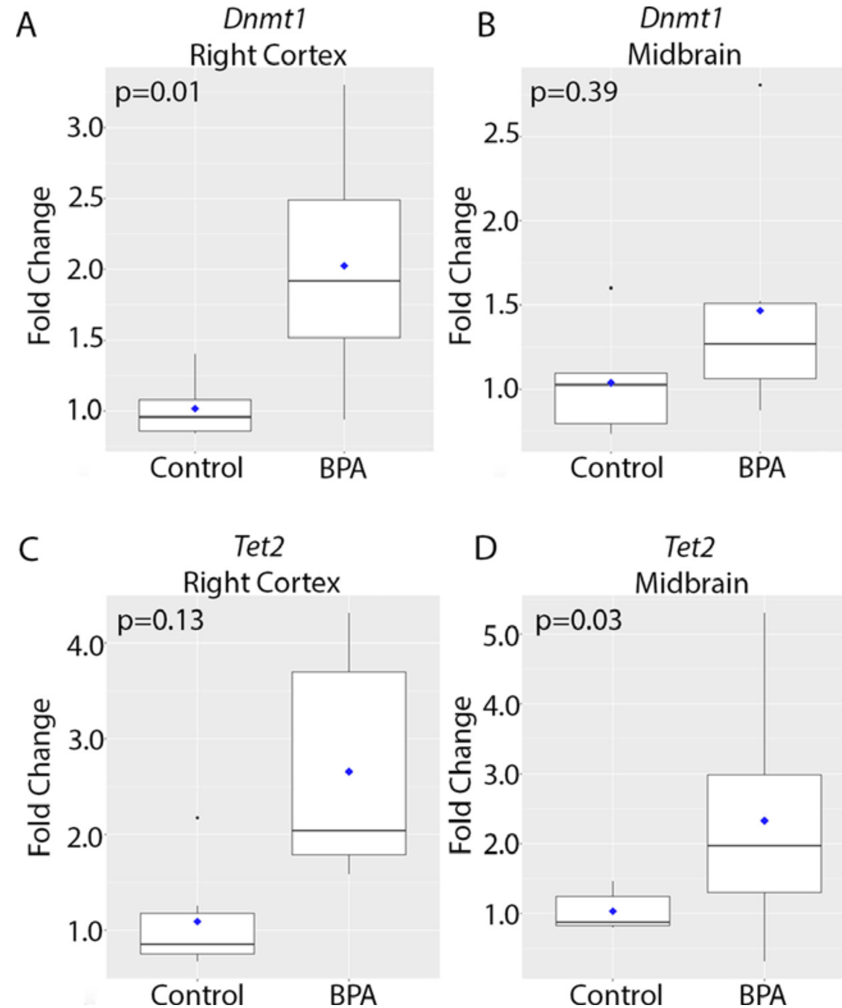
Xin et al (2018) Hormones and Behavior

Yemin Lan

Perinatal BPA Exposure Leads to Region-Specific Changes in Expression of the Imprinted Gene *Kcnq1* and Epigenetic Modifiers in the Brains of Adult Offspring

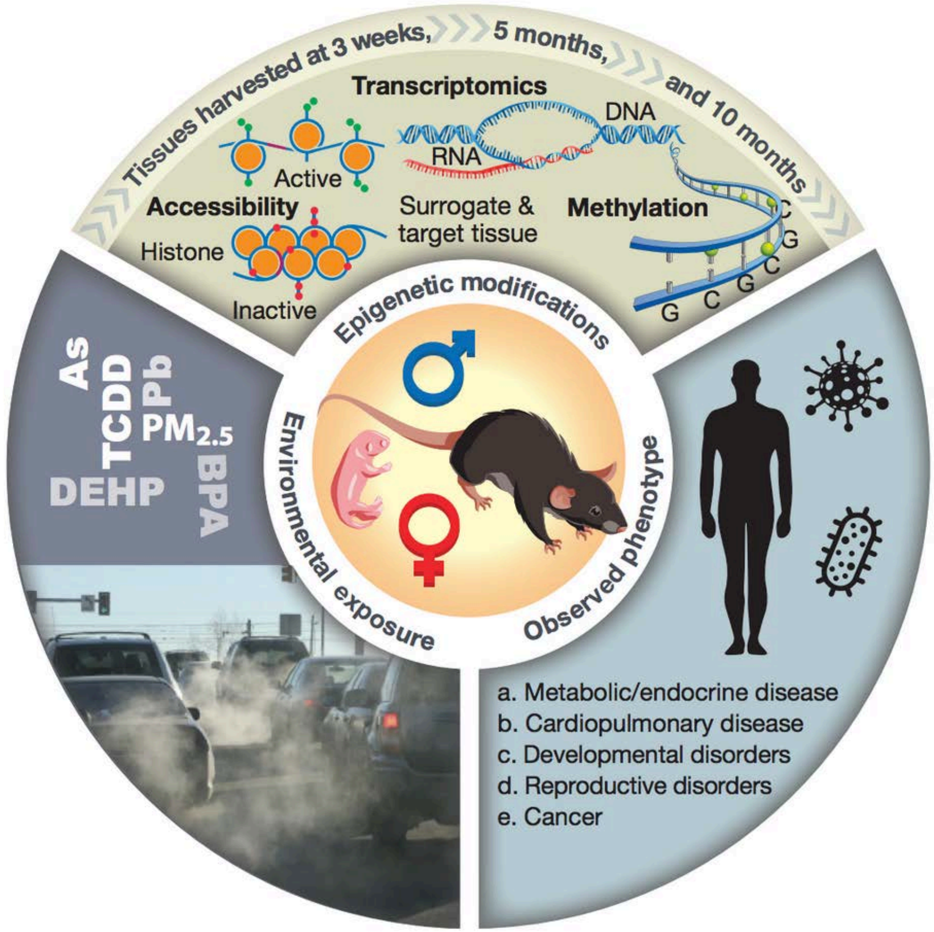






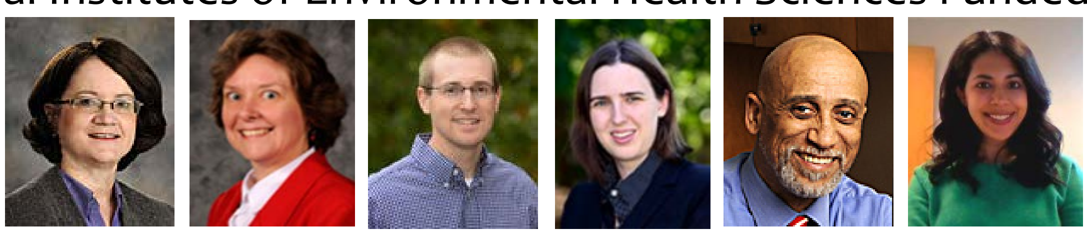
Malloy, Svoboda et al. (2019) Frontiers in Genetics

TaRGET II Consortium



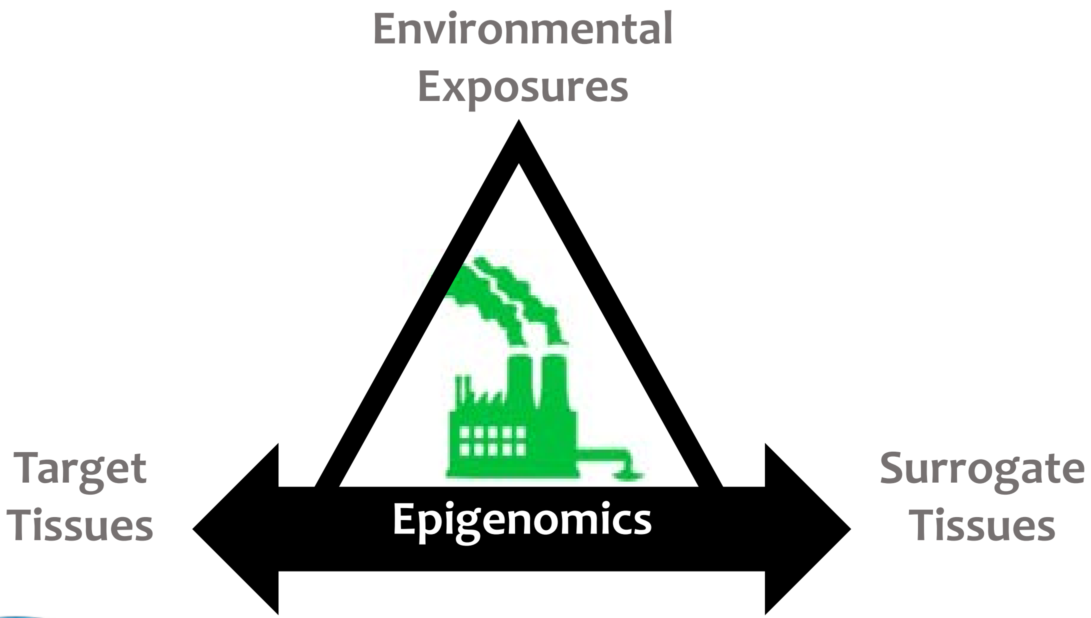
Wang et al, 2018, Nature Biotechnology

National Institutes of Environmental Health Sciences Funded Program



C Lawler, K McAllister, C Duncan, A Garton, F Tyson**, A. Ramsaran

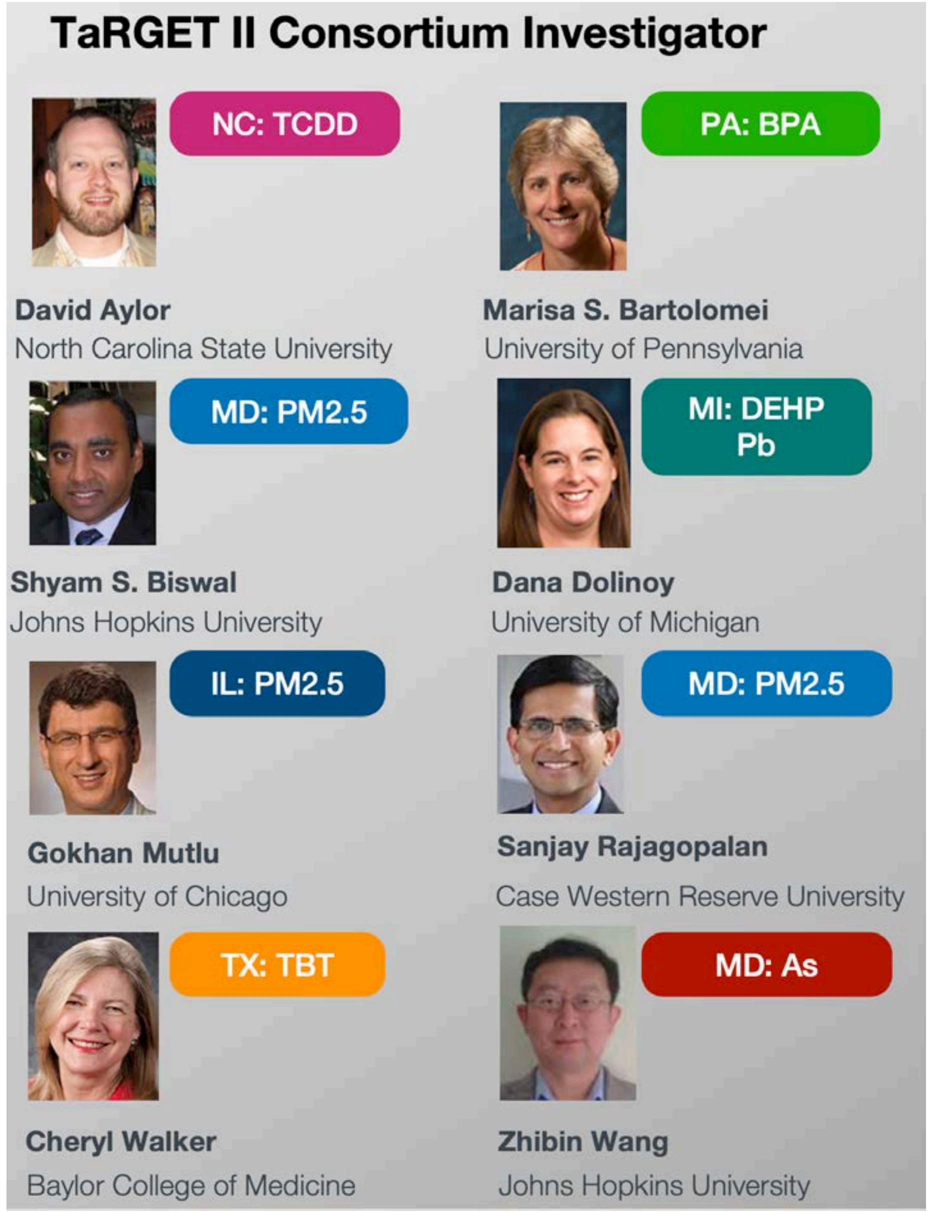
Goals of the TaRGET II Consortium



Goals
- Explore exposure-induced epigenomic signatures
- Perform surrogate analysis
- Provide a data resource for the research community

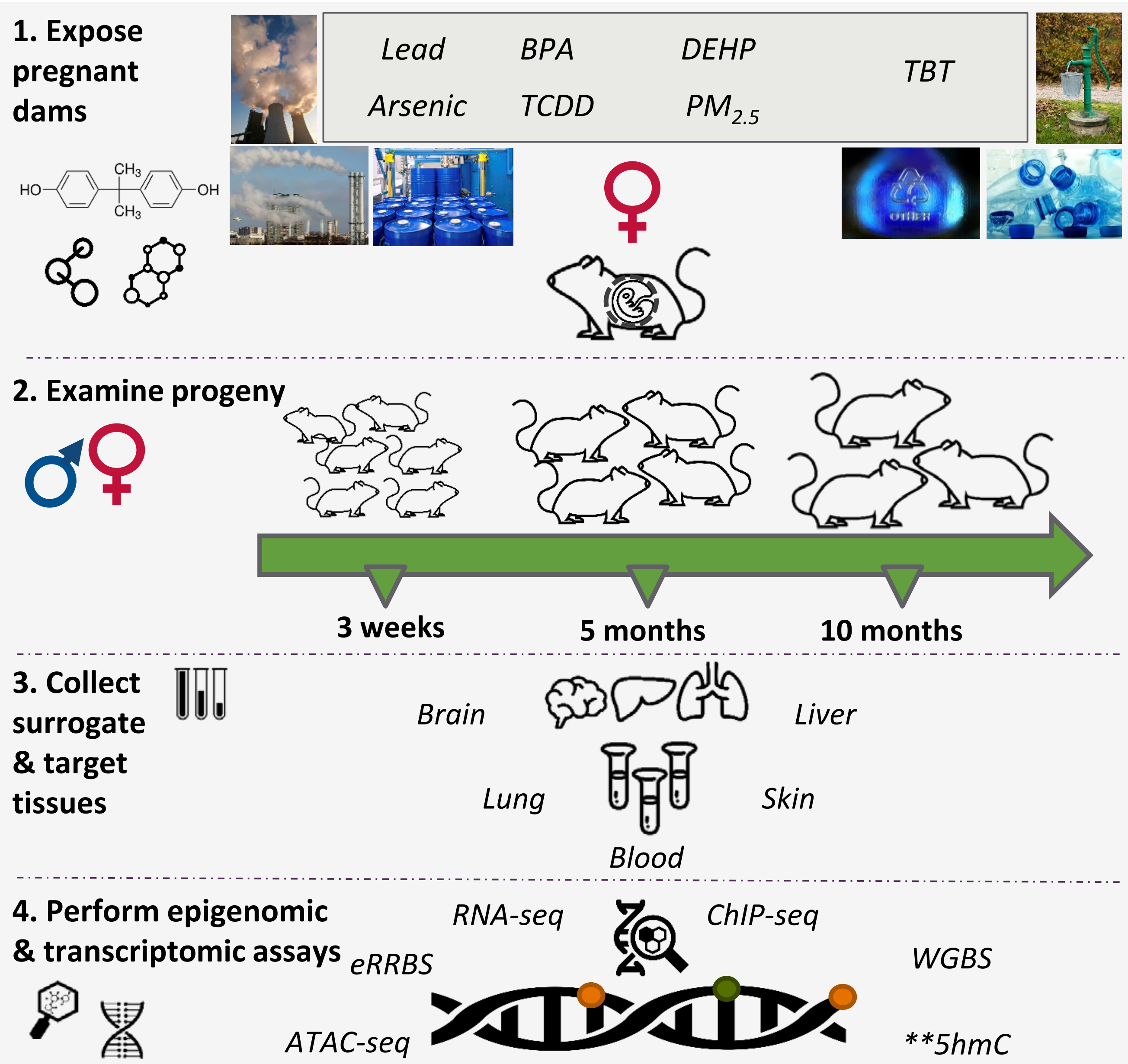
Adapted from F. Tyson, NIEHS

TaRGET II Consortium



Modified from B. Zhang

TaRGET II Approach for Identifying Epigenomic Signatures



Observed Phenotypes

- Metabolic/endocrine disease
- Cardiopulmonary disease
- Developmental disorders
- Reproductive disorders
- Cancer
- Cognitive/behavioral phenotypes

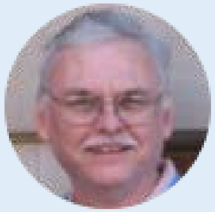
Modified from F. Tyson

TaRGET II Consortium

Data Coordination Center @ Washington University in St. Louis

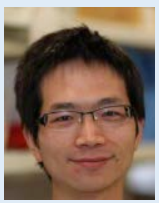
Department of Genetics

PI: Ting Wang



Co-PI: Mike Province

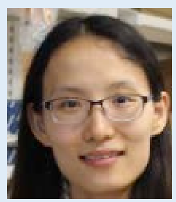
Co-I: Heather Lawson



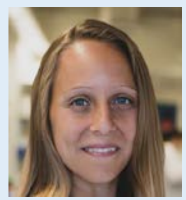
Daofeng Li

Deepak Puruchotham

Xiaoyu Zhuo

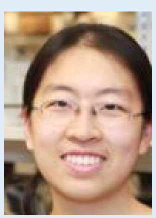


Wanqing Shao

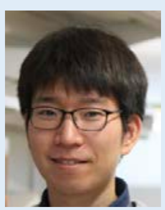


Erica Pehrsson

Xiaoyun Xing



Yiran Ho



Ju Heon Maeng

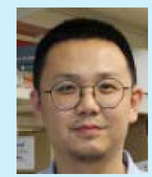
Alan Du

Yujie Chen

Department of Developmental Biology

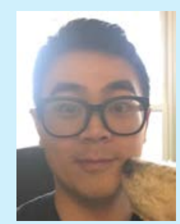
Co-I: Bo Zhang

Paul Gontarz



Benpeng Miao

Shuhua Fu

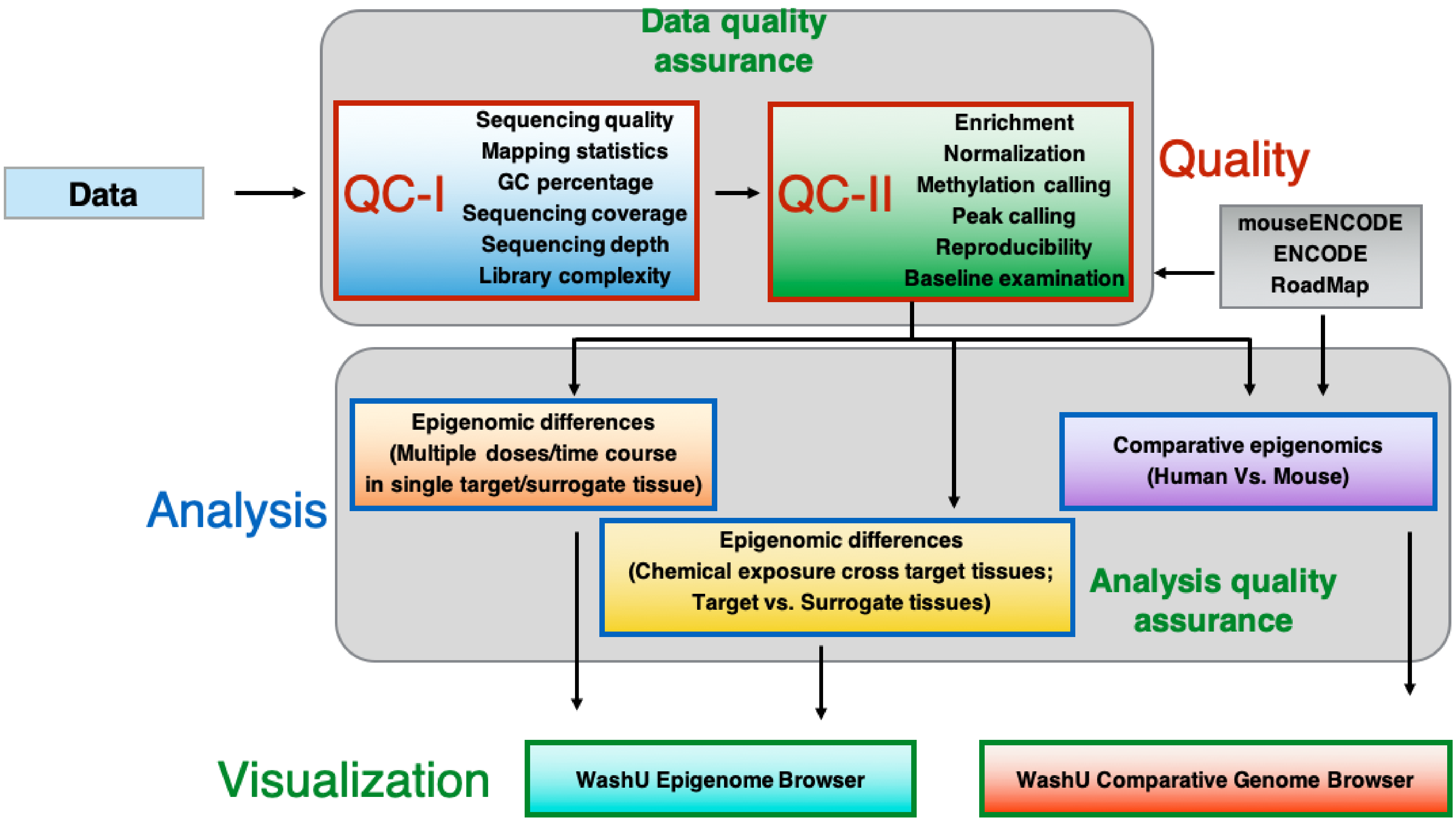


Shaopeng Liu

https://data.targetepigenomics.org

Modified from B. Zhang

The DCC Focuses on Genomics data, Including Data Quality Control, Data Integrative Analysis and Data Sharing and Visualization



https://data.targetepigenomics.org

Modified from B. Zhang

TaRGET II Consortium Data (Phase I) was Released 5/20/2020

Series GSE146508

Title	Transcriptome and open chromatin analysis of 5-month mouse liver and blood by the TaRGET II consortium
Organism	Mus musculus
Experiment type	Expression profiling by high throughput sequencing Genome binding/occupancy profiling by high throughput sequencing
Summary	The TaRGET (Toxicant Exposures and Responses by Genomic and Epigenomic Regulators of Transcription) program is a research consortium funded by the National Institute of Environmental Health Sciences (NIEHS). The goal of the collaboration is to address the role of environmental exposures in disease pathogenesis as a function of epigenome perturbation, including understanding the environmental control of epigenetic mechanisms and assessing the utility of surrogate tissue analysis in mouse models of disease-relevant environmental exposures (https://targetepigenomics.org).
Overall design	RNA-seq and ATAC-seq of liver and blood samples from 5-month mice that were subjected to disease-relevant environmental exposures. The TaRGET II consortium (https://targetepigenomics.org/about/)
Samples (769) More...	GSM4387308 Aylor_ATAC_Blood_Ctrl_5month_Female_biorep1 GSM4387309 Aylor_ATAC_Blood_Ctrl_5month_Female_biorep2 GSM4387310 Aylor_ATAC_Blood_Ctrl_5month_Female_biorep3

GEO accession: GSE146508

BioProject: PRJNA610793

Released data: 769

Modified from B. Zhang

Moving Forward--Neurotoxicology Resources

BPA, DEHP, Pb: Cortex RNA-seq, WGBS in progress

TCDD: Cortex, hippocampus, hypothalamus RNA-seq in progress

PM2.5: Cortex and hypothalamus RNA-seq and WGBS in progress

> Toxicol Sci. 2020 May 27;kfaa069. doi: 10.1093/toxsci/kfaa069. Online ahead of print.

Single Cell Analysis of the Gene Expression Effects of Developmental Lead (Pb) Exposure on the Mouse Hippocampus

Kelly M Bakulski [1], John F Dou [1], Robert C Thompson [2], Christopher Lee [1], Lauren Y Middleton [1], Bambarendage P U Perera [1], Sean P Ferris [2], Tamara R Jones [1], Kari Neier [1], Xiang Zhou [1], Maureen A Sartor [1 2], Saher S Hammoud [2], Dana C Dolinoy [1], Justin A Colacino [1]

https://data.targetepigenomics.org

Future Questions/Ideas

How appropriate is mouse as a model for human developmental disorders associated with exposures?

How important are epigenetic perturbations in abnormal phenotypes and in predicting outcomes? Which epigenetic marks?
Chromatin structure, DNA methylation

Can we use surrogate tissues to predict phenotypes of target tissues?
early exposures could affect all tissues but only manifest an abnormal phenotype in a subset

Is there an exposure signature? General or specific to a given agent?

We have only addressed single exposures in our studies. What about more complex exposures?

Christopher Krapp**
Duy Nguyen**
Yemin Lan**
Joanne Thorvaldsen
Lisa Vrooman
Aimee Juan
Suhee Chang
Blake Caldwell
Laren Riesche
Nicole Matos-Robles
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Eric Rhon-Calderon
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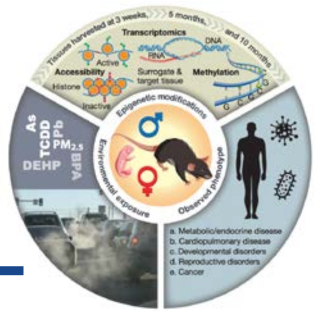
Frances Xin (Spark Therapeutics)
Martha Stefaniak

EPIGENETICS INSTITUTE Penn

Perelman School of Medicine UNIVERSITY of PENNSYLVANIA | CEET



NIEHS