Thermal adaptation in chicks: epigenetic regulation of resilience and vulnerability

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Tatiana Kisliouk, Shelly Druyan, Amit Haron

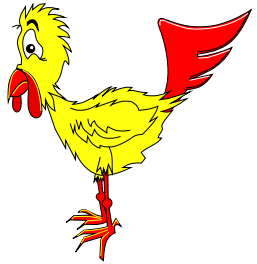
Stress-resilience or vulnerability

Determining whether a stressful event will lead to future stress-resilience or vulnerability depends on a delicate balance of a probably adjustable stress response set-point.

Heat resilience

OR

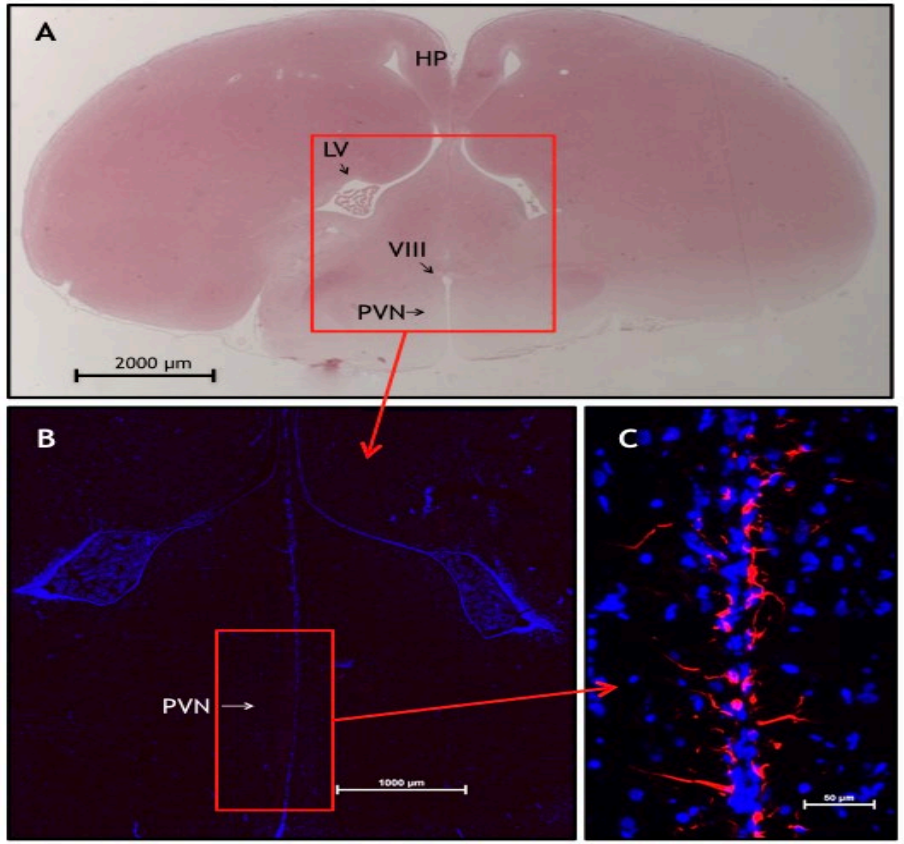
Heat vulnerability



Early life epigenetic regulation of CRH expression pattern in the PVN affects long-term stress response

The chick is a perfect model

- The ability to manipulate the embryo
- No dependency on maternal guidance.
- The ability to learn immediately after hatching.
- Their behavioral repertoire can be easily measured.
- The hypothalamus regulation is very similar to the mammalian

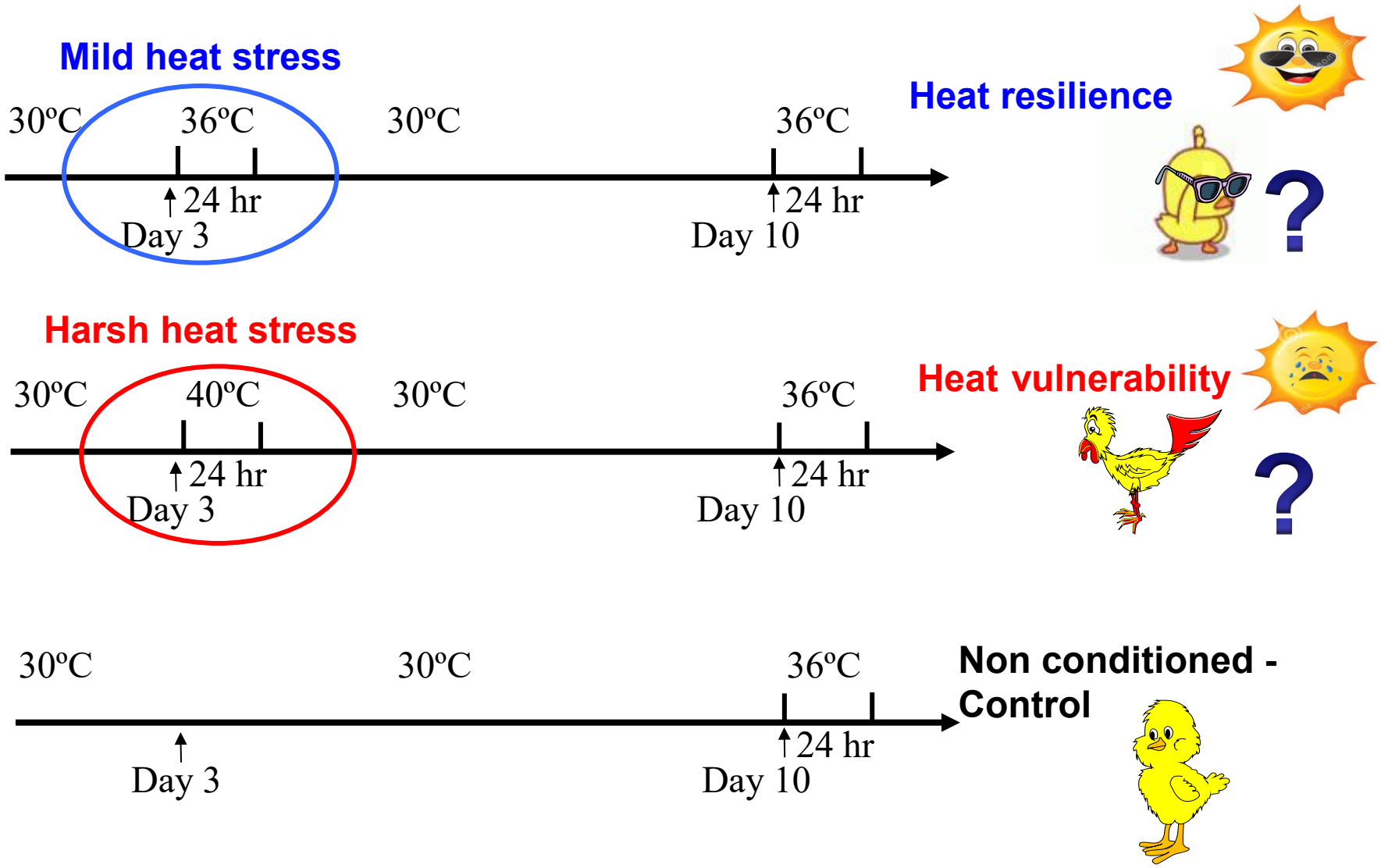


Cramer et al. Molecular Psychiatry 2019

The critical period for thermal control establishment in chicks

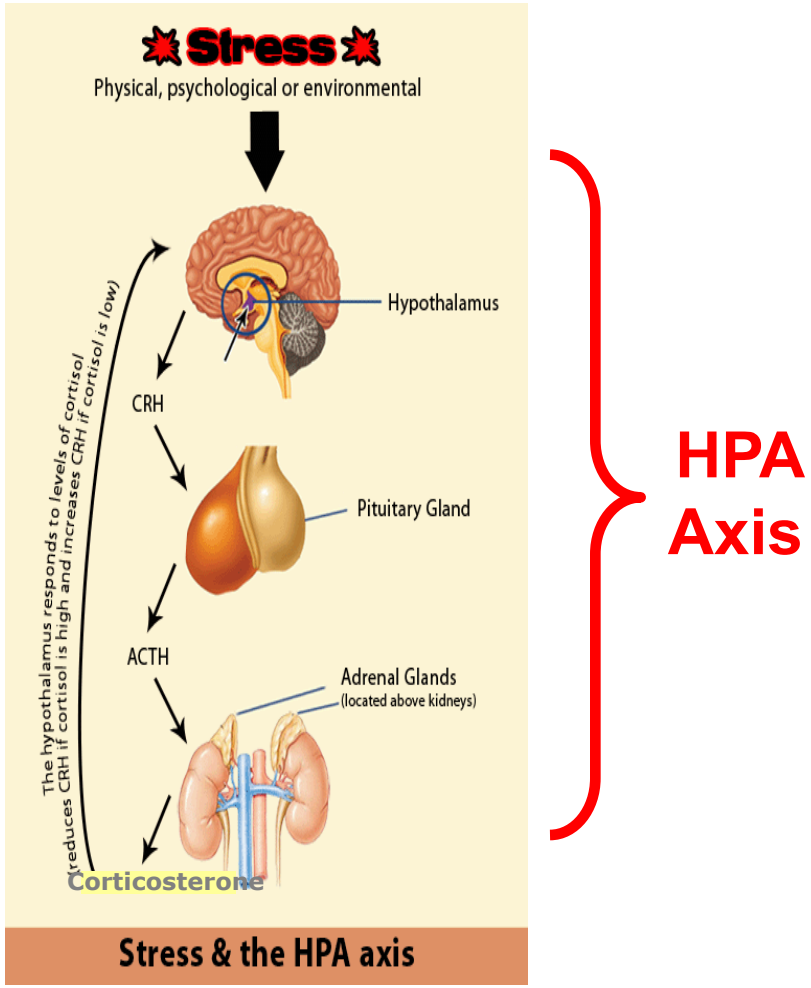
- Thermal control establishment completes developing during a post natal critical period.
- In chicks, the critical developmental period is between day 3-5 post hatching.

Changing stress set point can lead to either stress resilience or to stress vulnerability



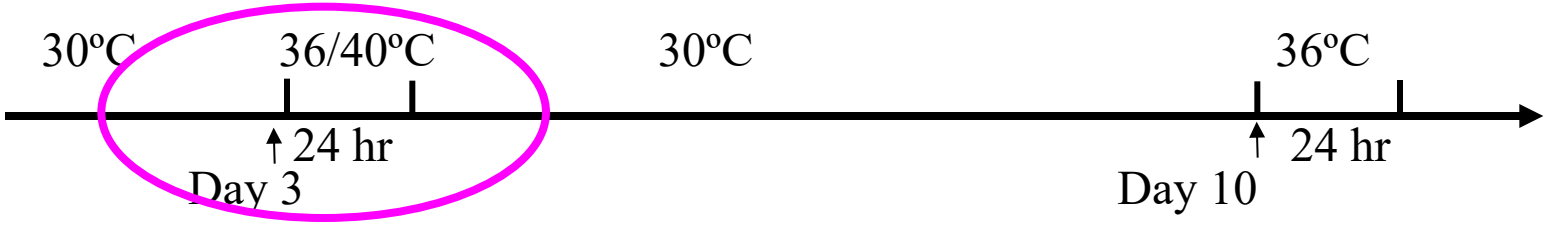
CRH- Corticotropin Releasing Hormone

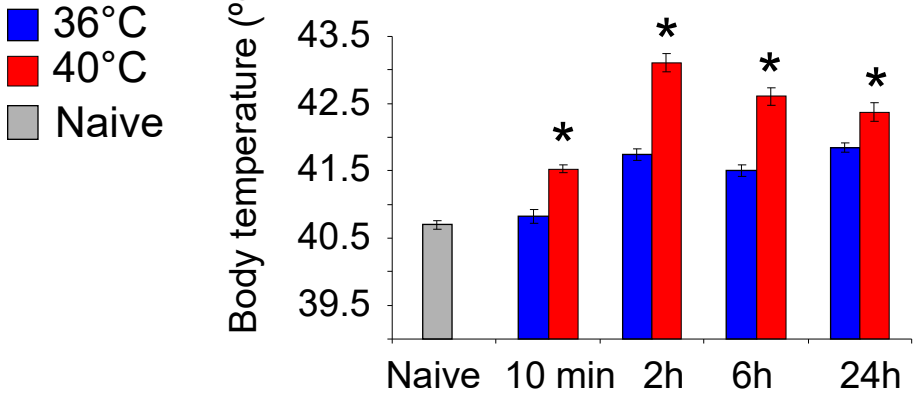
- Produced in the PVN
- Released to the Pituitary gland
- Pituitary gland secretes ACTH
- ACTH increases production and release of Corticosteroids such as Corticosterone from the adrenal glands

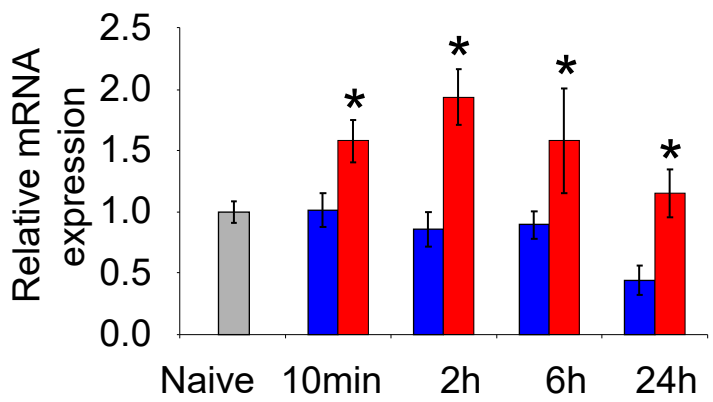


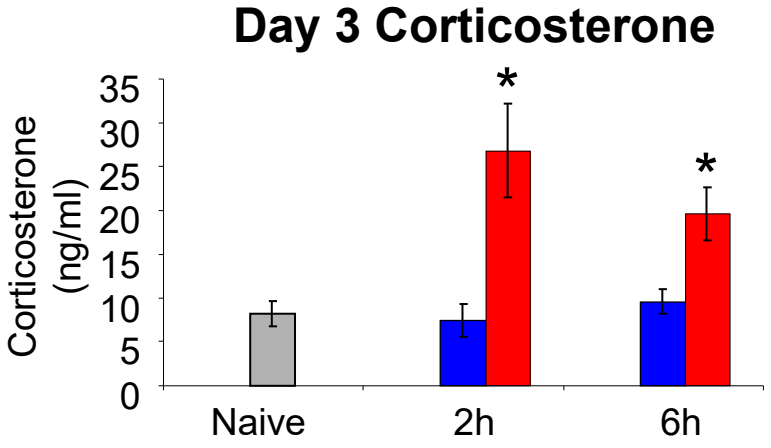
HPA Axis

Changing stress set point can lead to either stress resilience or stress vulnerability





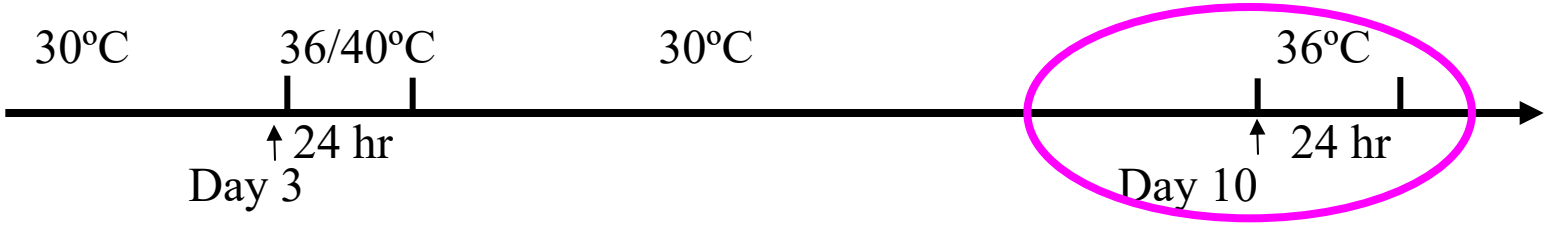


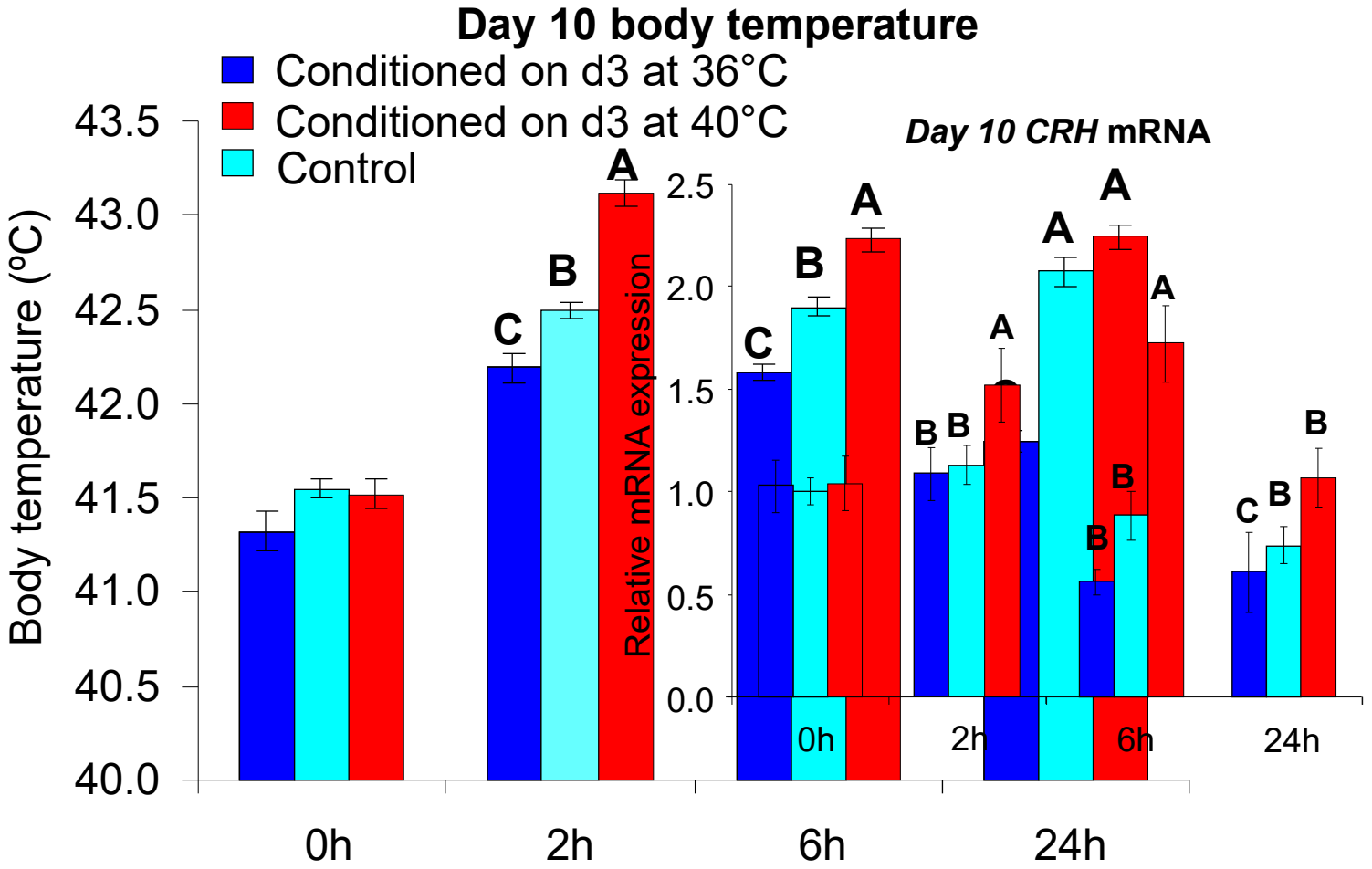


Cramer et al., 2015

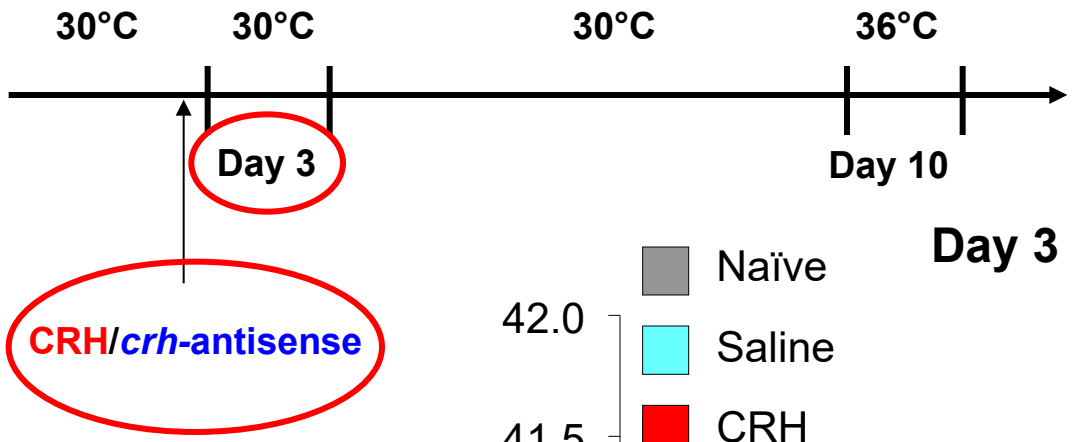
N= 32-35 Per column

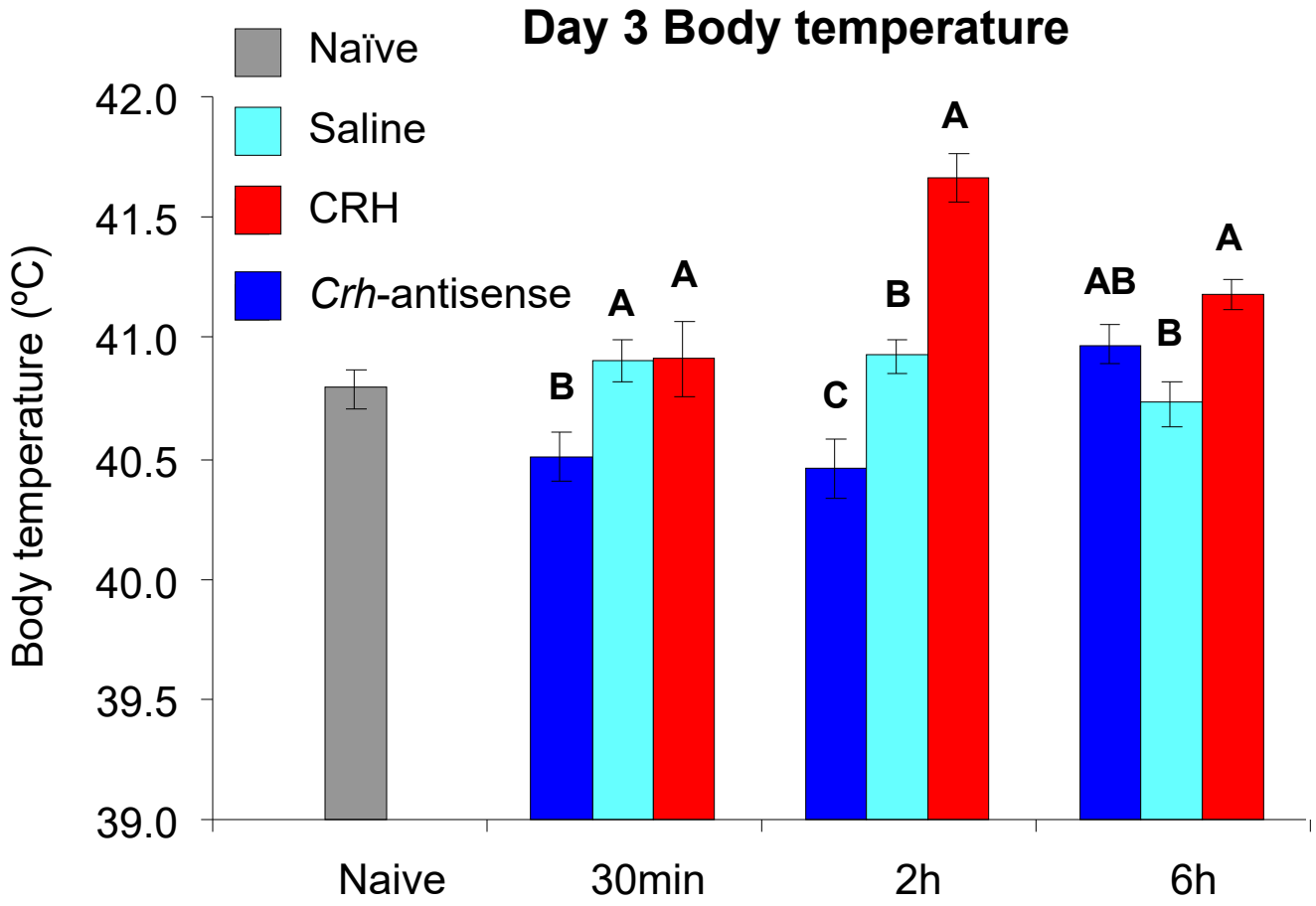
Exposure to different intensities of heat-stress on day 3 resulted in either a heat-resilient or vulnerable response 1 week later





Intracranial injection of CRH or *crh*-antisense led to opposite effects on the body temperature

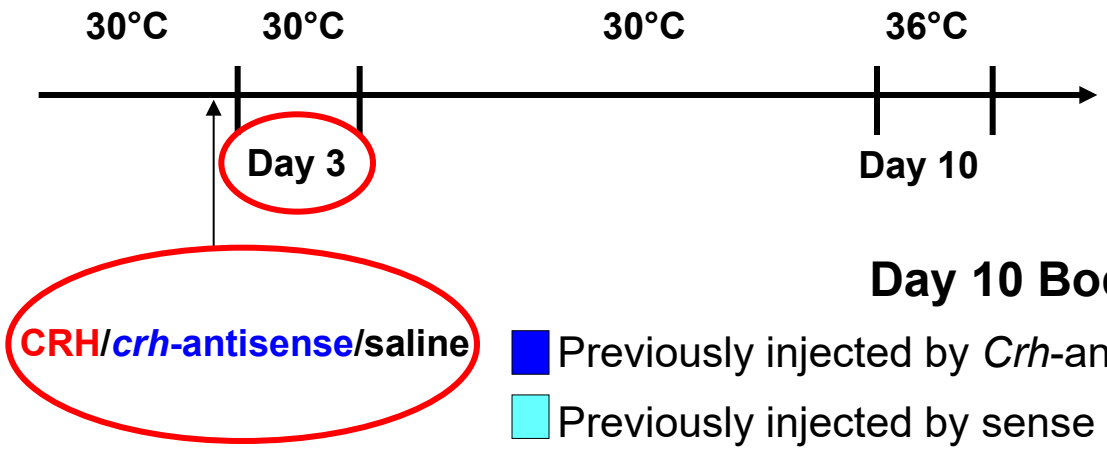


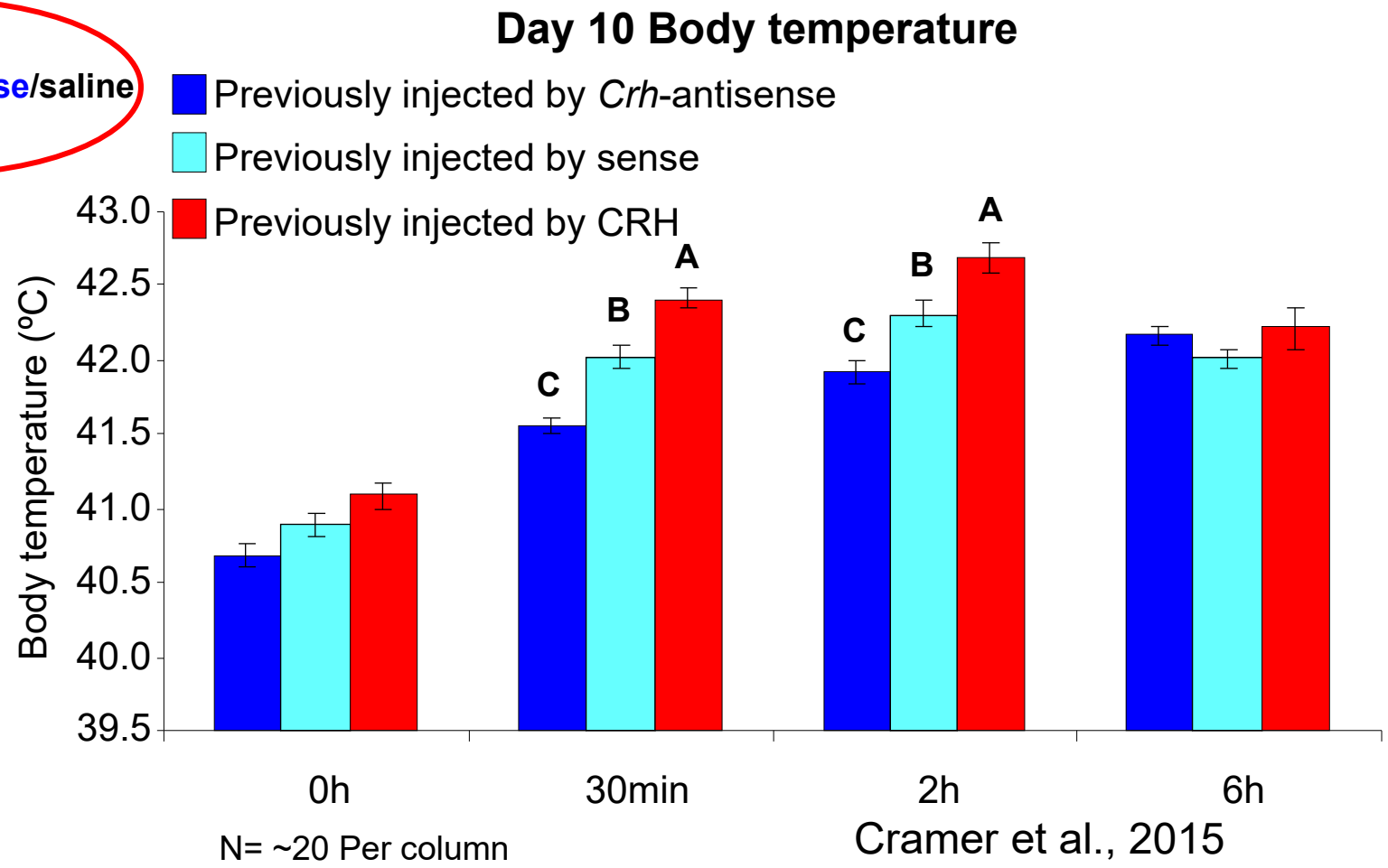


N= ~20 Per column

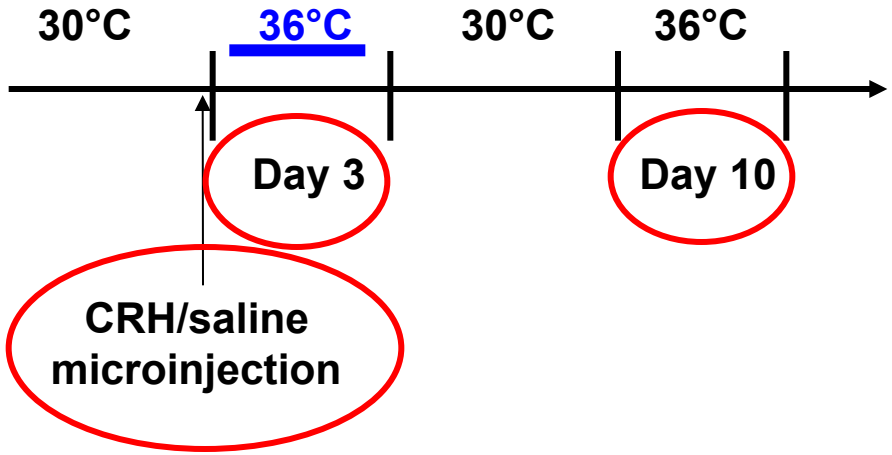
Cramer et al., 2015

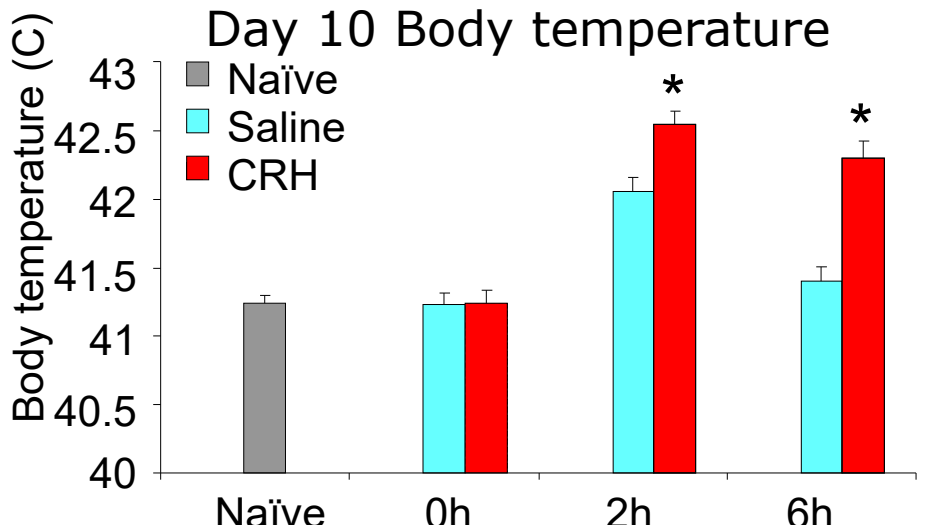
Intracranial injection of CRH or *crh* antisense produces a long-term opposite effect on body temperature response

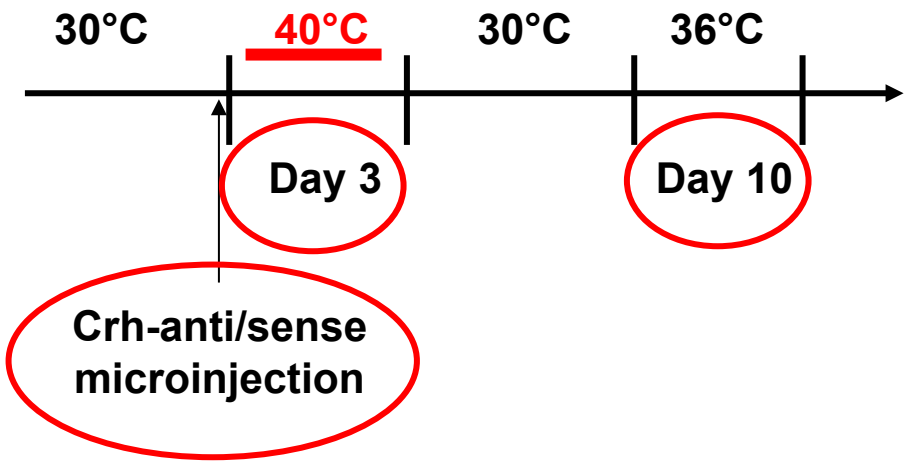


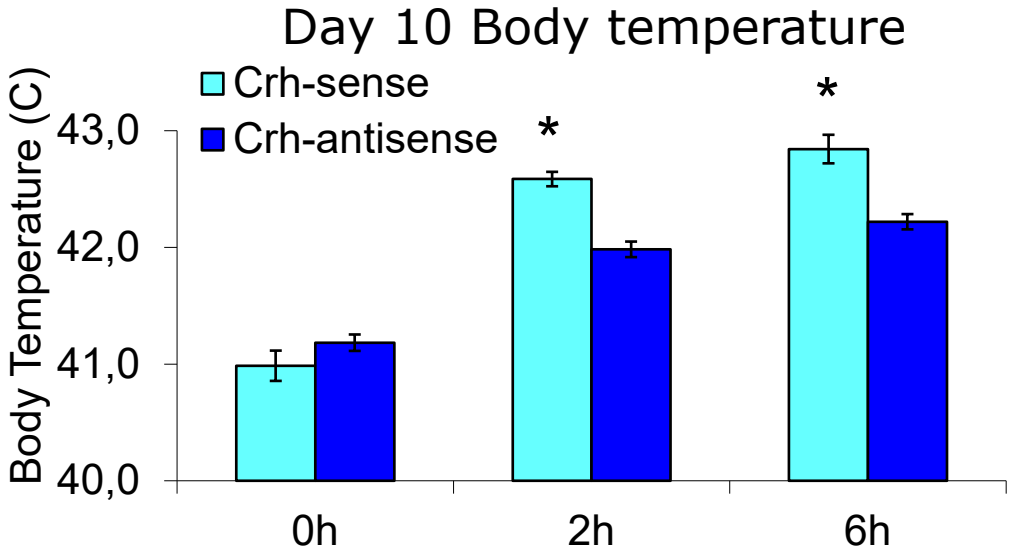


Vulnerability and resilience can be reversed by inhibiting or activating CRH









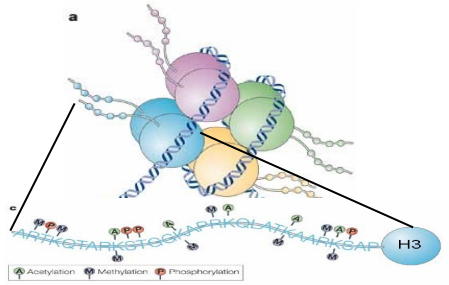
What is epigenetics?

Epigenetics refers to how behavior and environment can cause changes that affect the way genes work. Unlike genetic changes (mutations), epigenetic changes are reversible and do not change the sequence of DNA bases, but they can change how a DNA sequence is read.

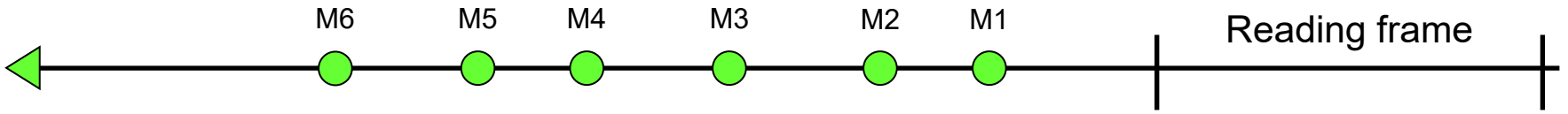
- Epigenetic changes are common during development
- But they occur throughout life
- They can be heritable

"The epigenetic code" – Five regulatory steps

1. The condensation of the packing of the chromatin determines which genes will be transcribed.



2. In the promoter and intron areas of the different genes there is a code which is based on the methylation of CpG dinucleotides that determine the expression level of the gene.

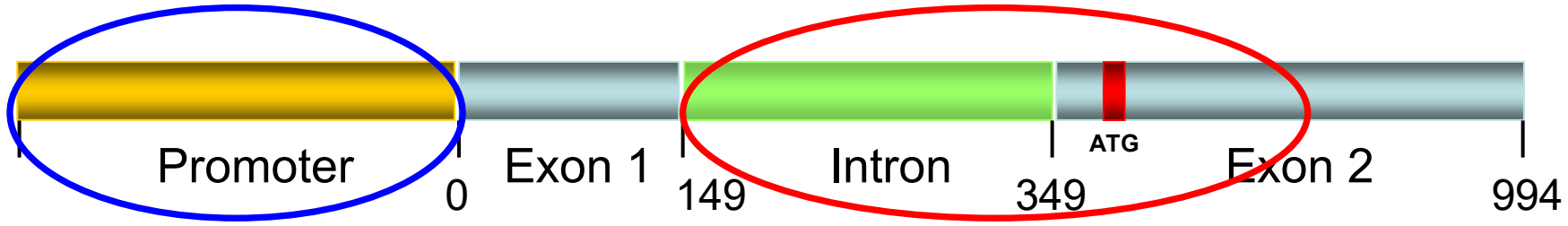


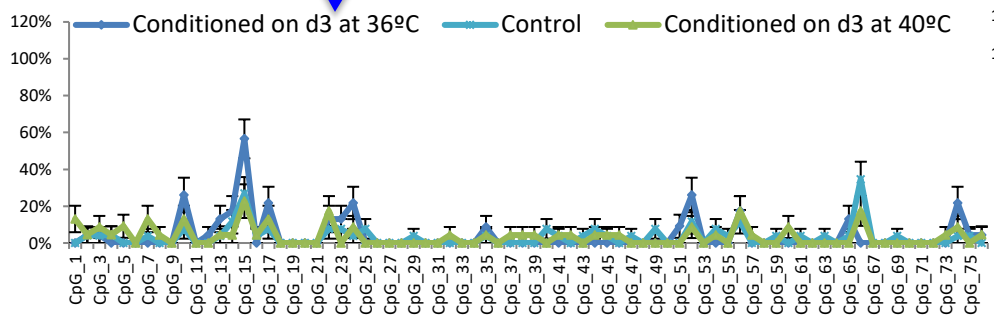
3. Noncoding RNA such as MicroRNA affect translation

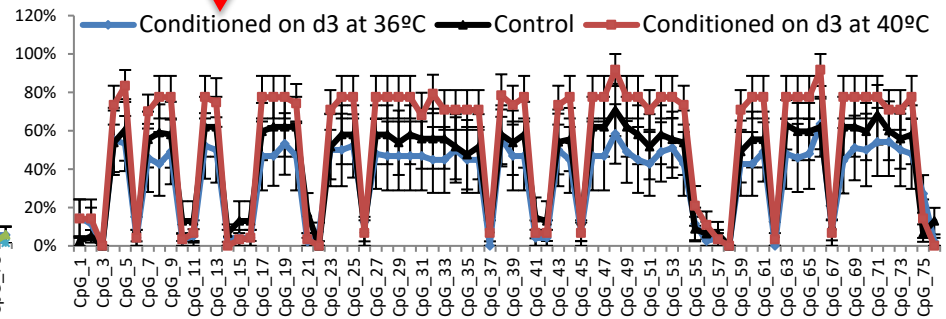
4. Epitranscriptomics

5. 3D chromatin organization

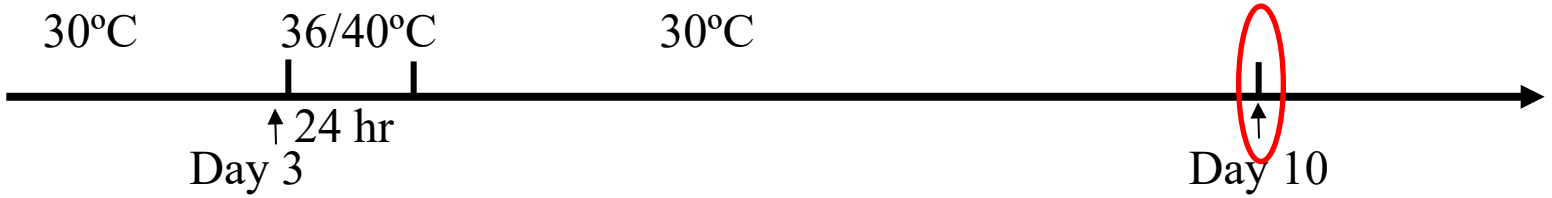
There are large differences in the DNA (CpG) methylation pattern at different regions of the CRH gene



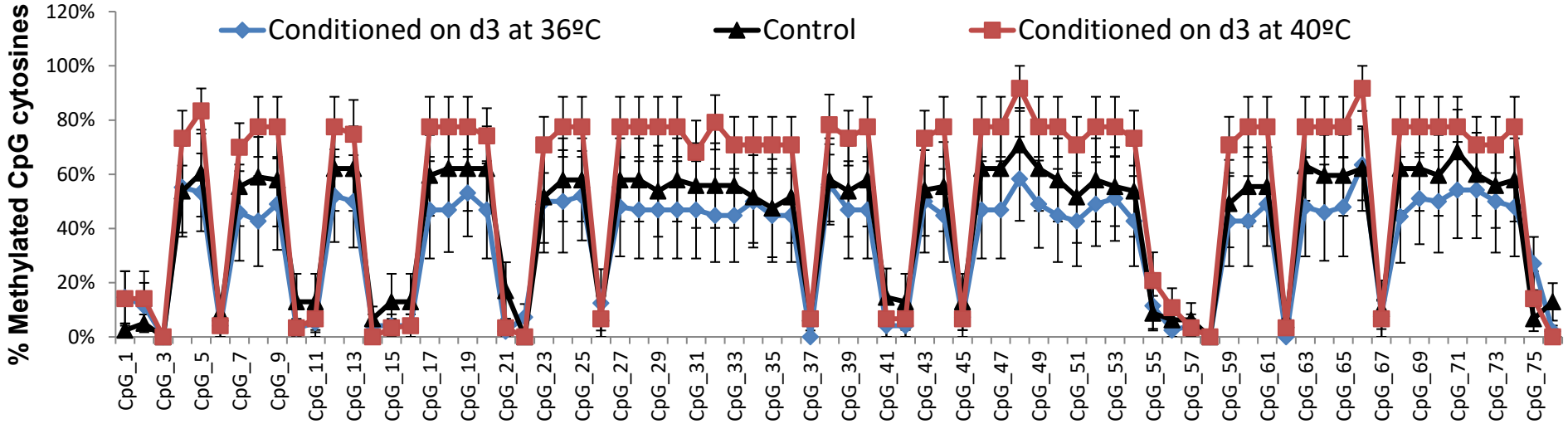




There are large differences in the CpG methylation pattern of the CRH intron between resilient and vulnerable chicks

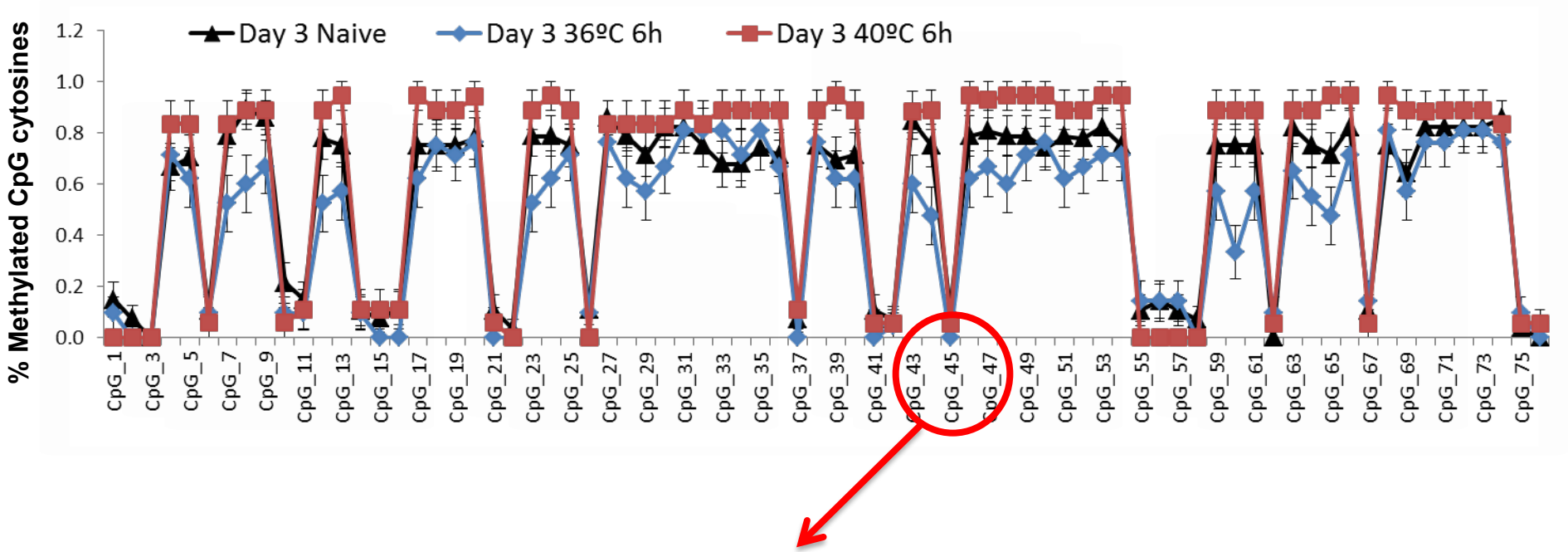


5-methylcytosine (5mC) pattern of the *CRH* intron a week after heat conditioning



N= 7-10 Per treatment

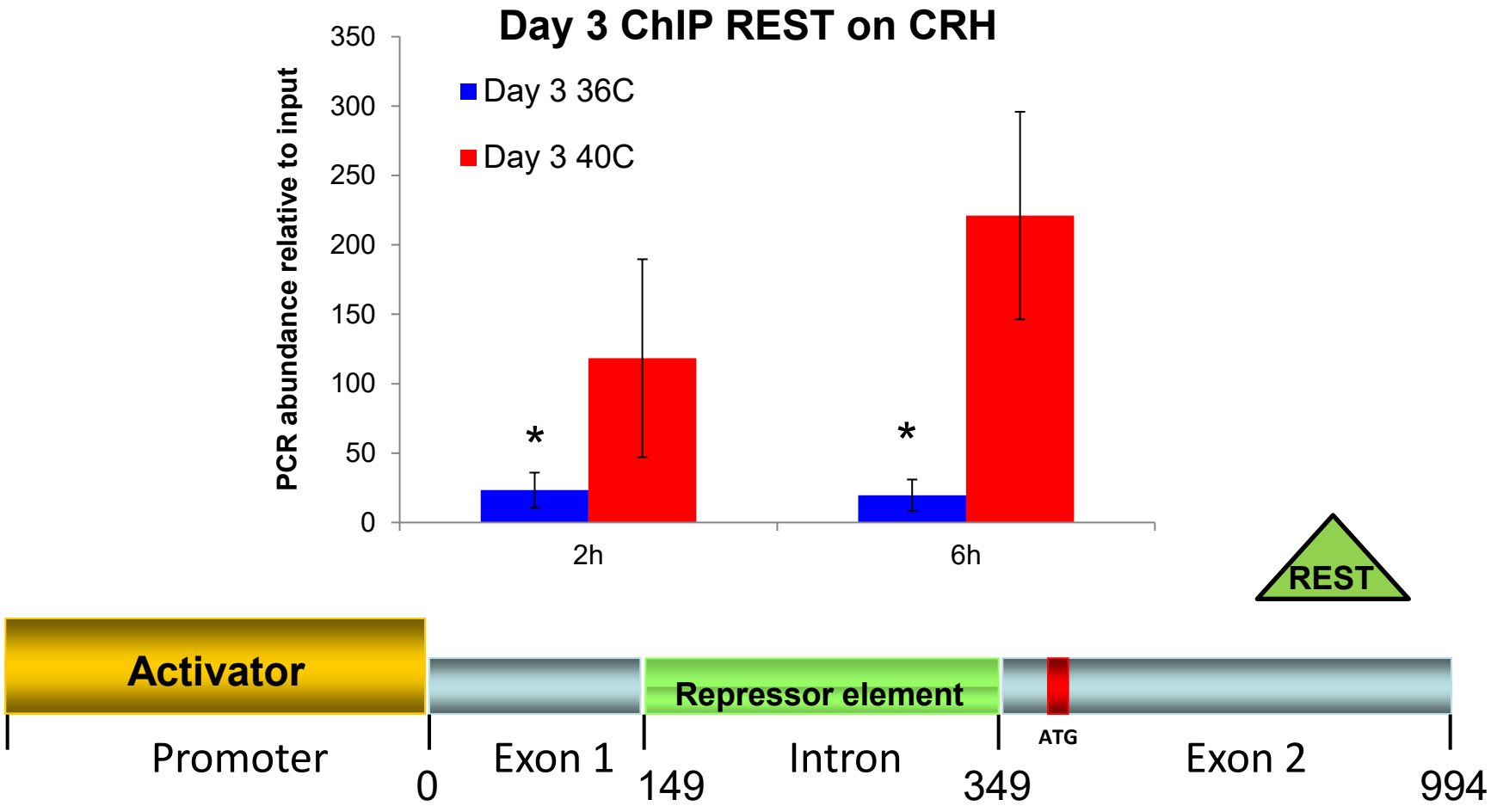
Significant association between DNA methylation and the binding of REST transcription factor



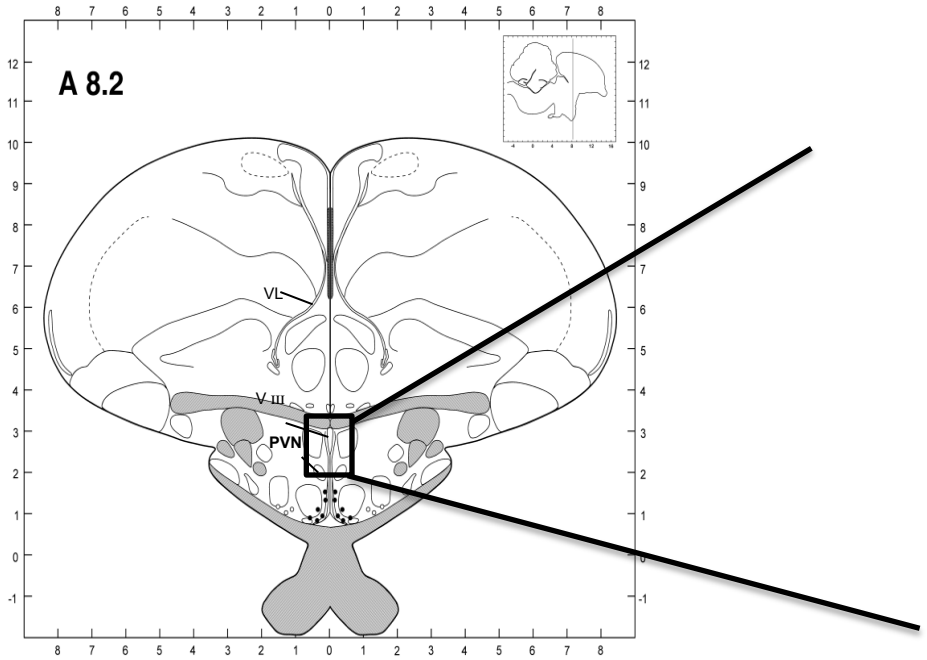
REST transcription factor binding site

REST - RE1-Silencing Transcription factor (REST),
also known as Neuron-Restrictive Silencer Factor (NRSF)

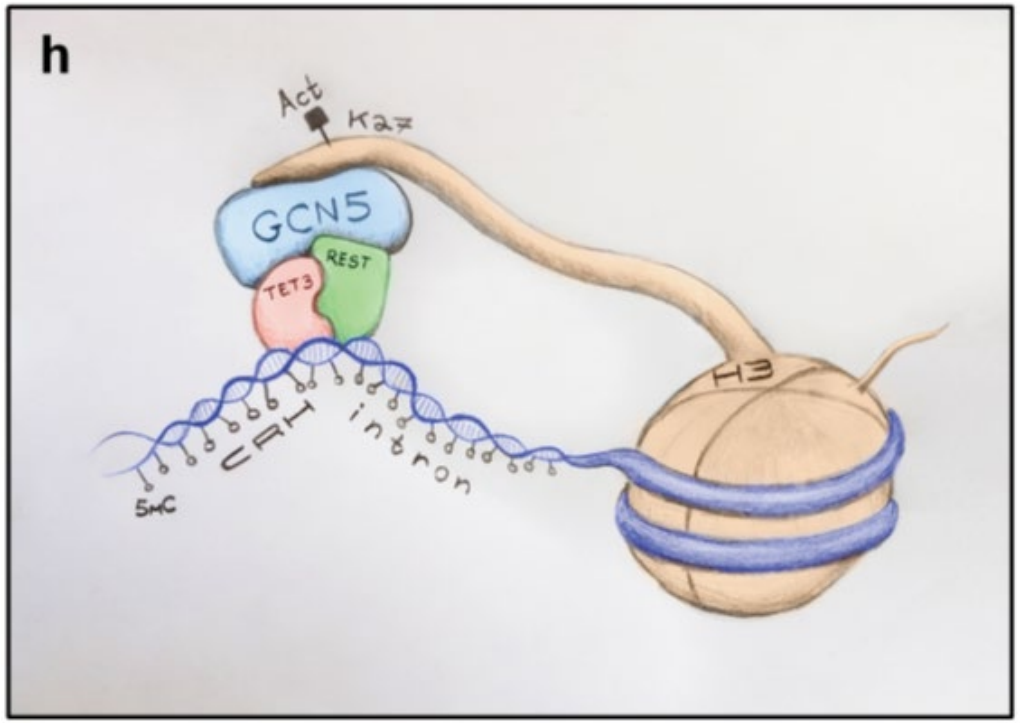
REST acts as a repressor on the intron thus activating the CRH gene expression



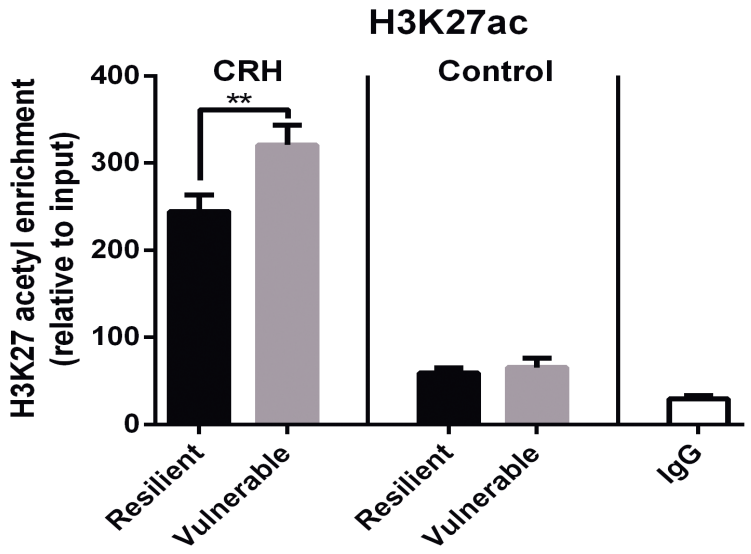
Cross talk between DNA methylation/hydroxymethylation and histone post translation modification

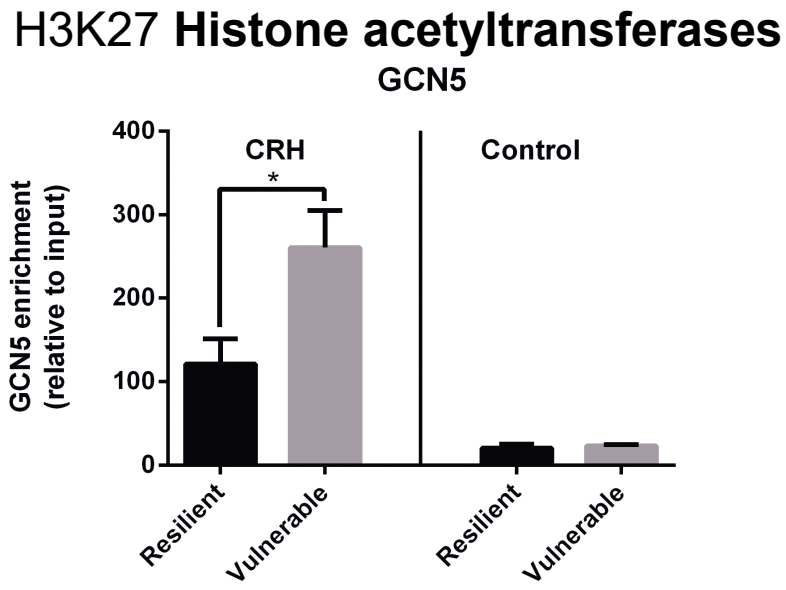


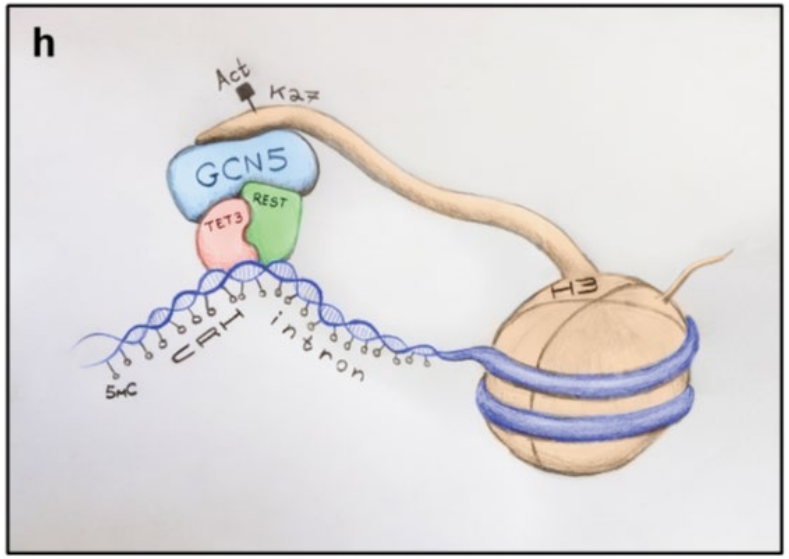
CRH intron



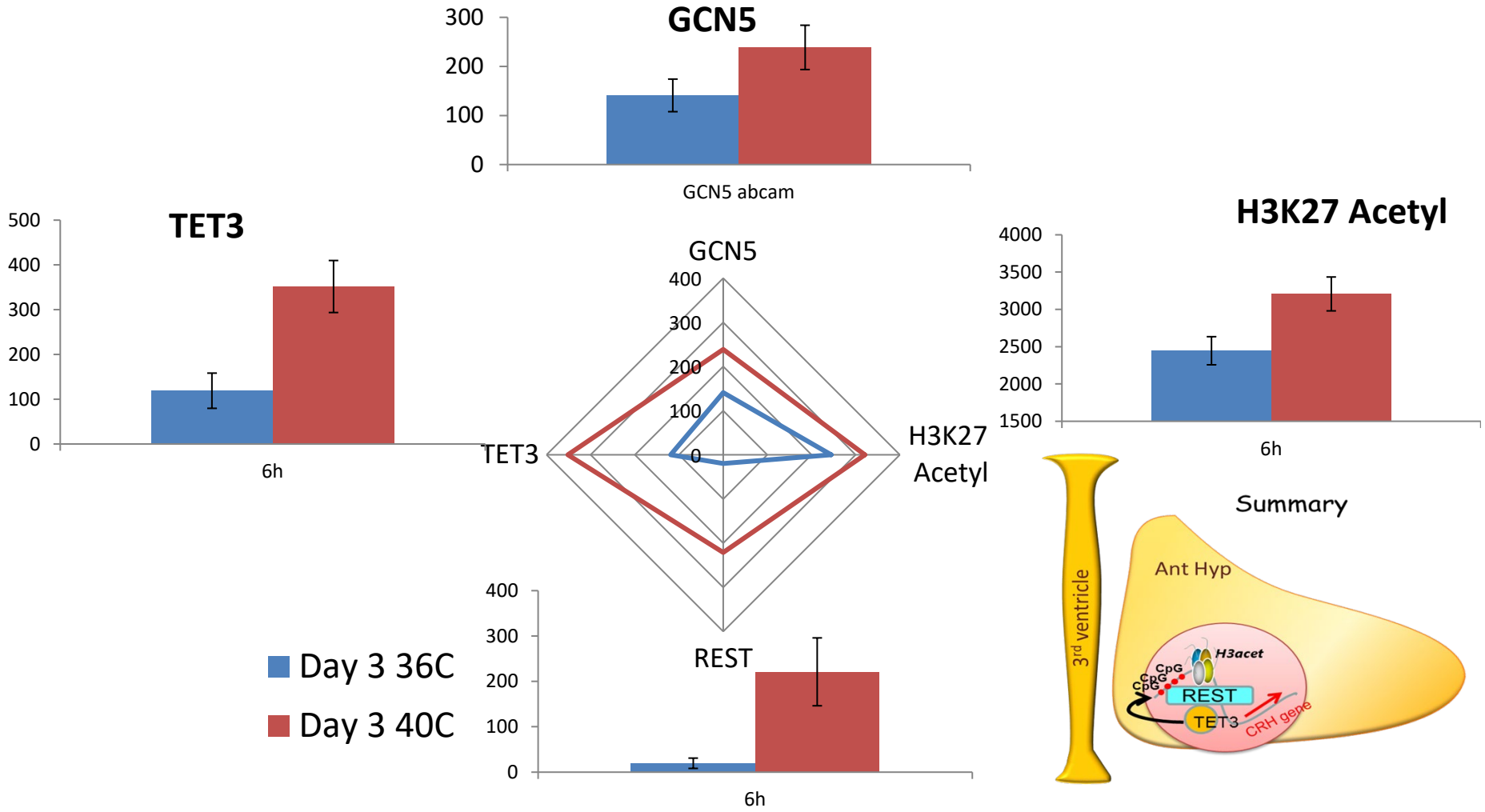
Histone 3 Lys 27 acetylation is induced in vulnerable chicks at the first intron of CRH



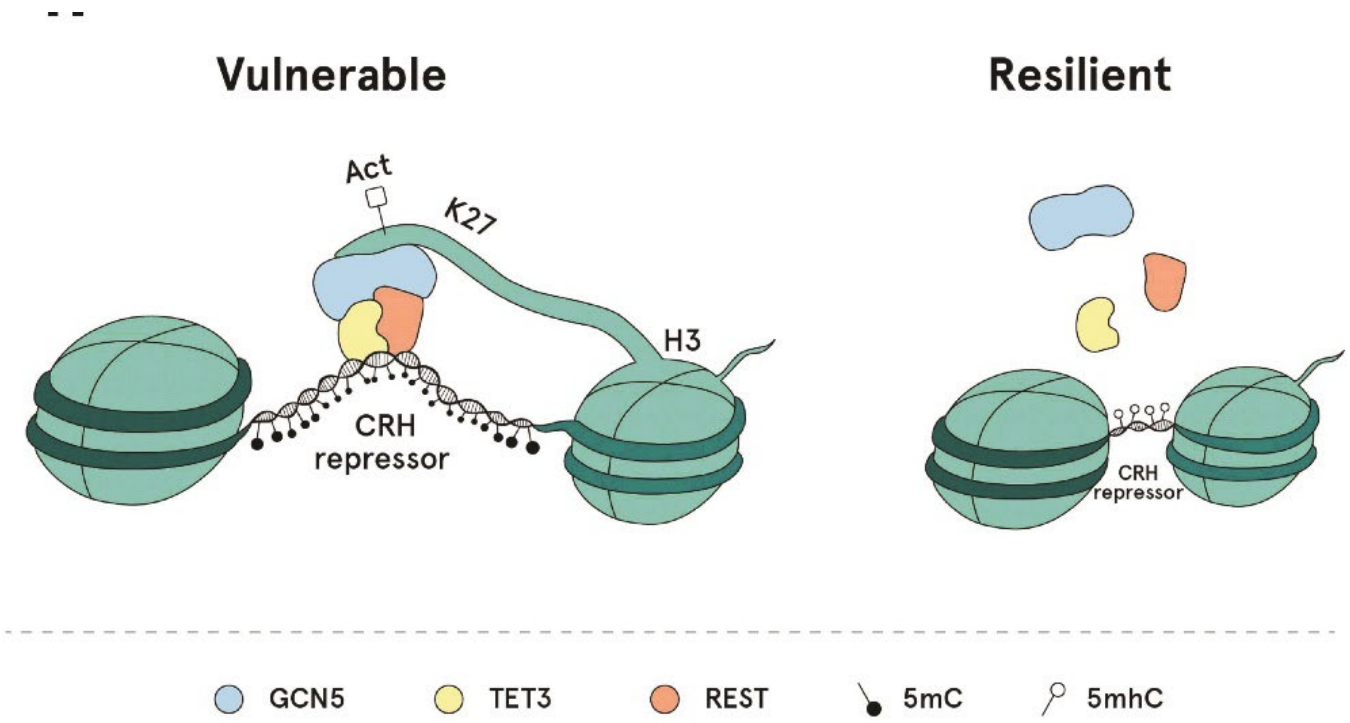




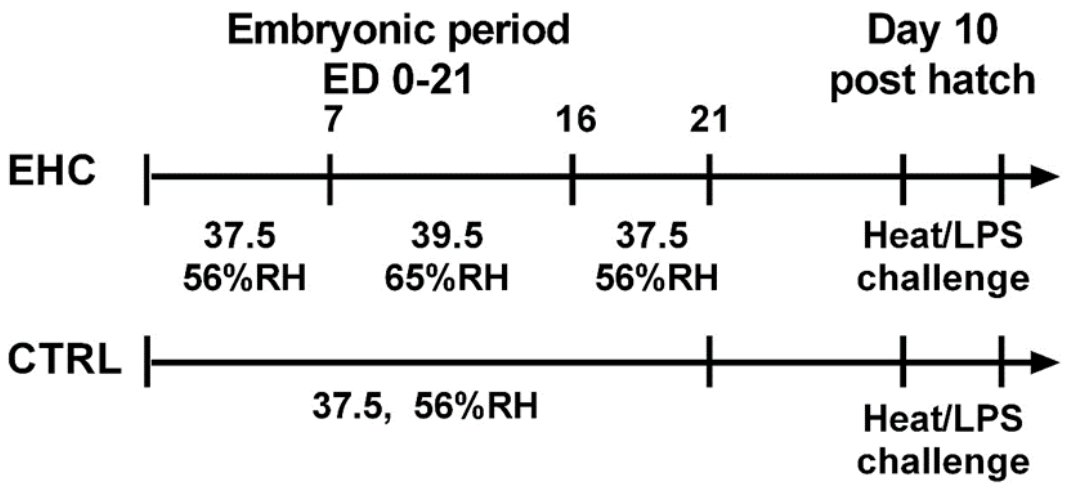
RE-1 silencing transcription factor (REST) acts as a repressor on the repressing element of the CRH gene, thus activating the CRH gene of animals trained to become vulnerable to heat stress. REST recruits TET3 to form a protein complex which binds together to the repressor, thereafter GCN5, histone acetylase enzyme, is elevated causing an increase in H3K27 acetylation.



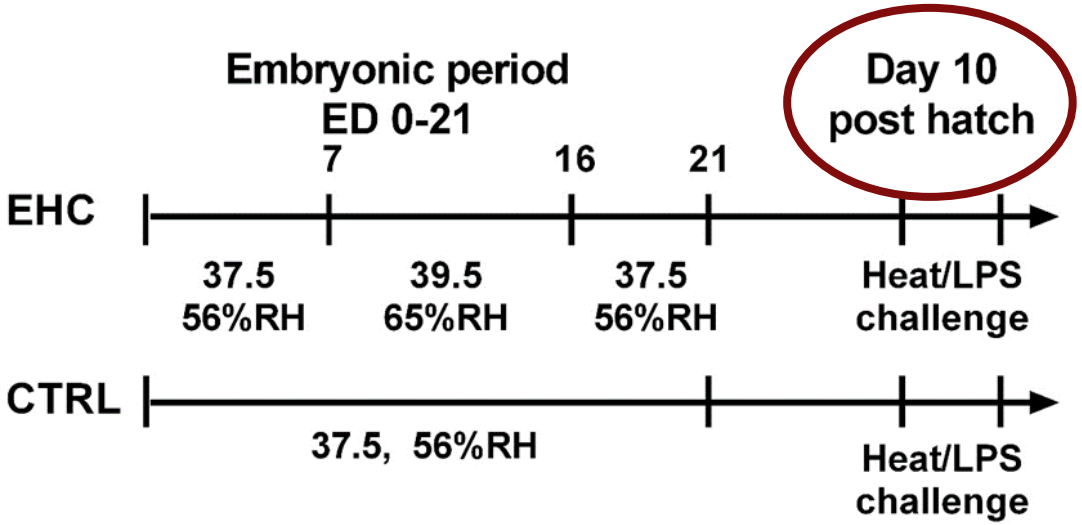
We conclude that heat stress encounter during early development triggers multi level-epigenetic mechanism involving both post-translational histone modification and DNA methylation in a regulatory segment of CRH determines a resilient or vulnerable response to stress later in life.

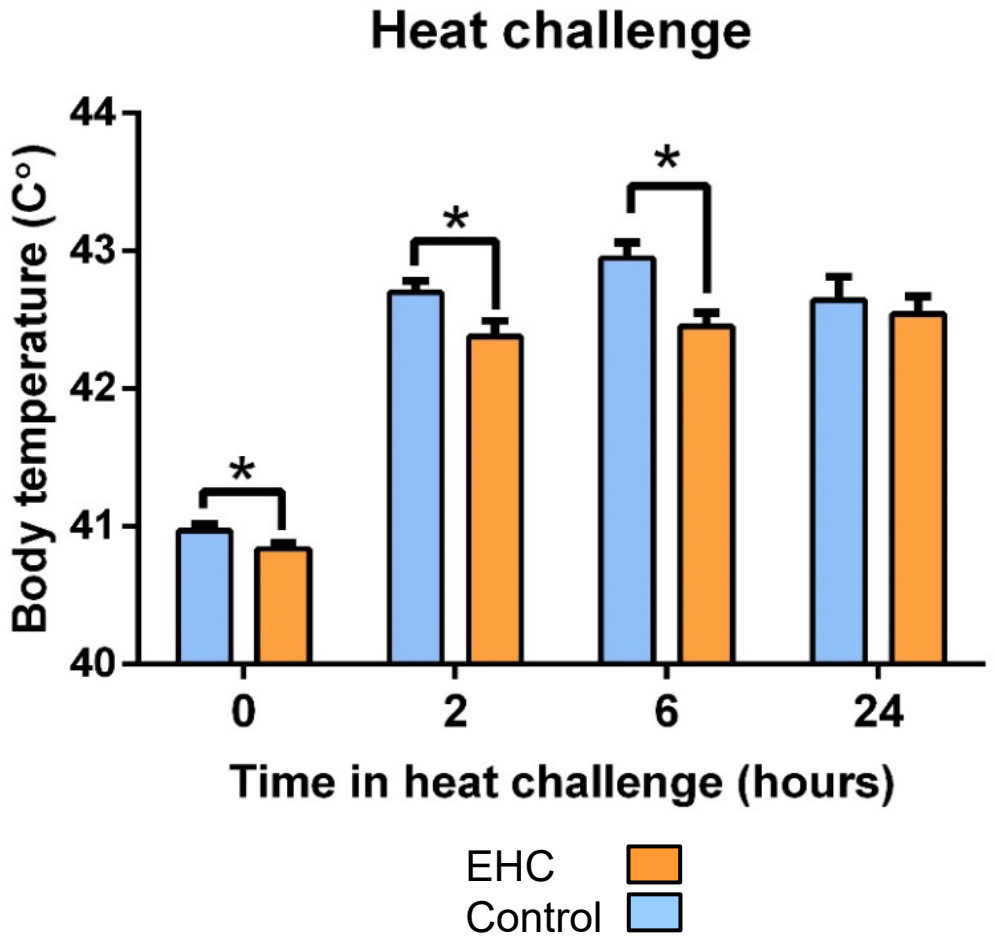


It is technically difficult to condition a 3 day old chicks in a commercial farming
As a solution Shlomo Yahav suggested conditioning the embryo in the egg



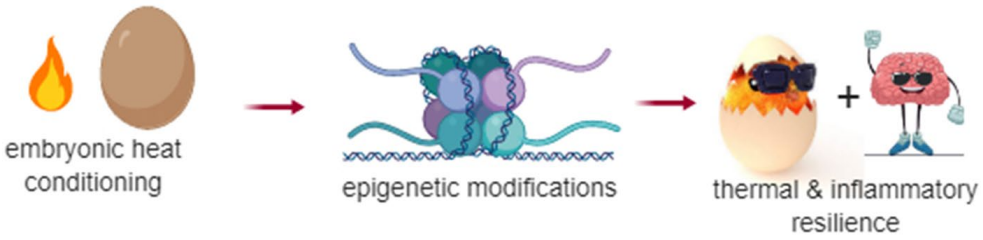
Chicks that were embryonically heat conditioned are resilient to heat stress throughout life.



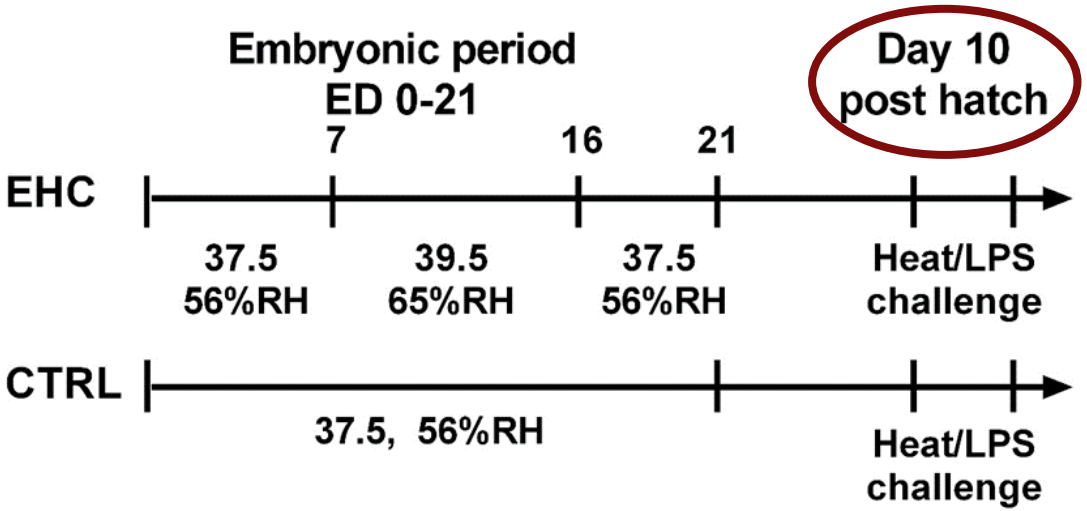


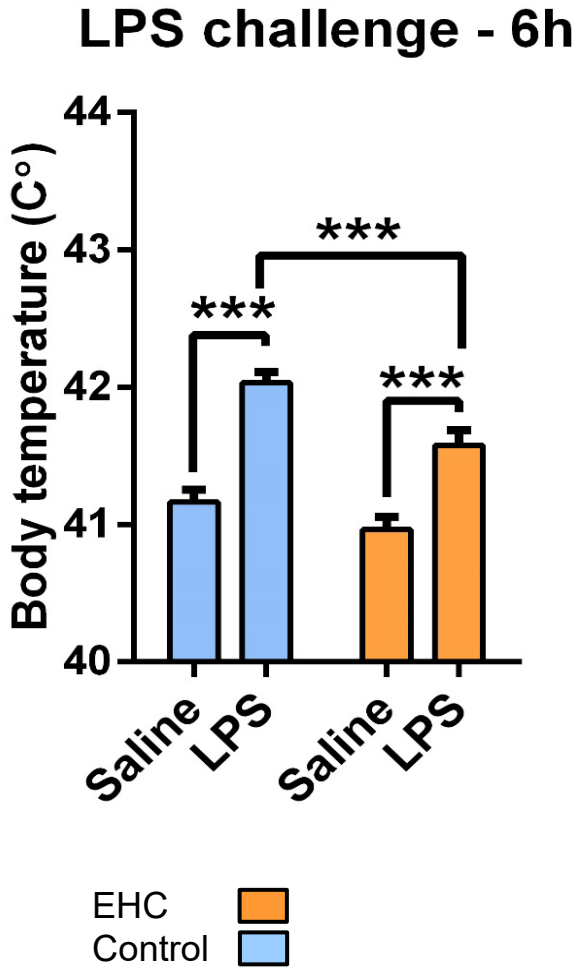
Stress cross-tolerance

- **Can embryonic heat conditioning reduce hypothalamic inflammation?**
- **Are there epigenetics mechanisms involved in this long term effect?**



The inflammatory challenge effect

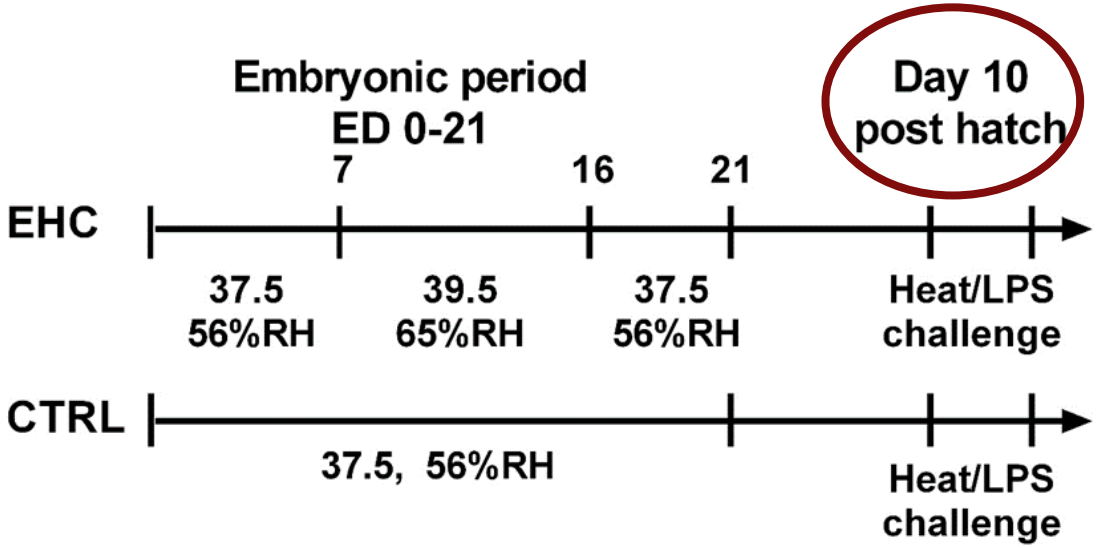




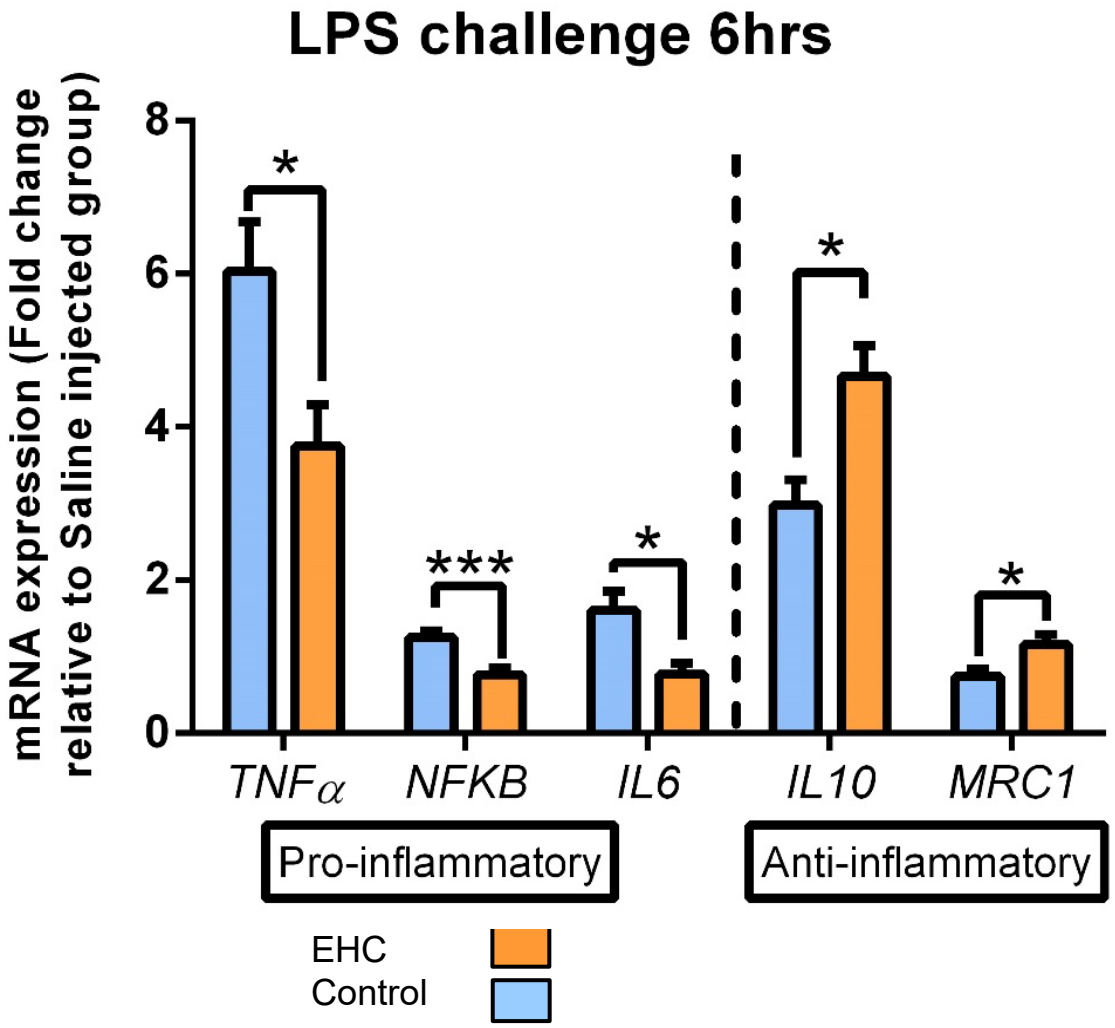
Inflammatory activation?

Rosenberg et al, Frontiers in Genetics 2020

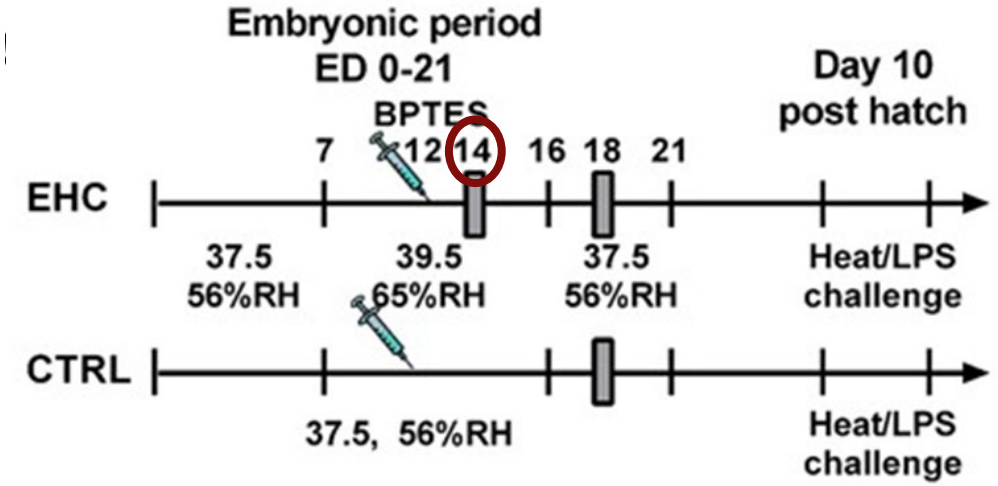
The inflammatory challenge effect

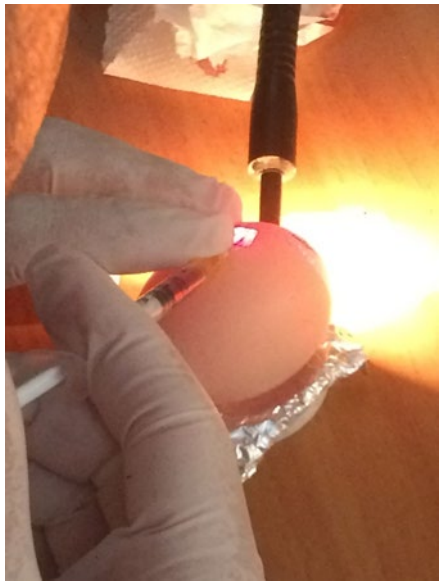


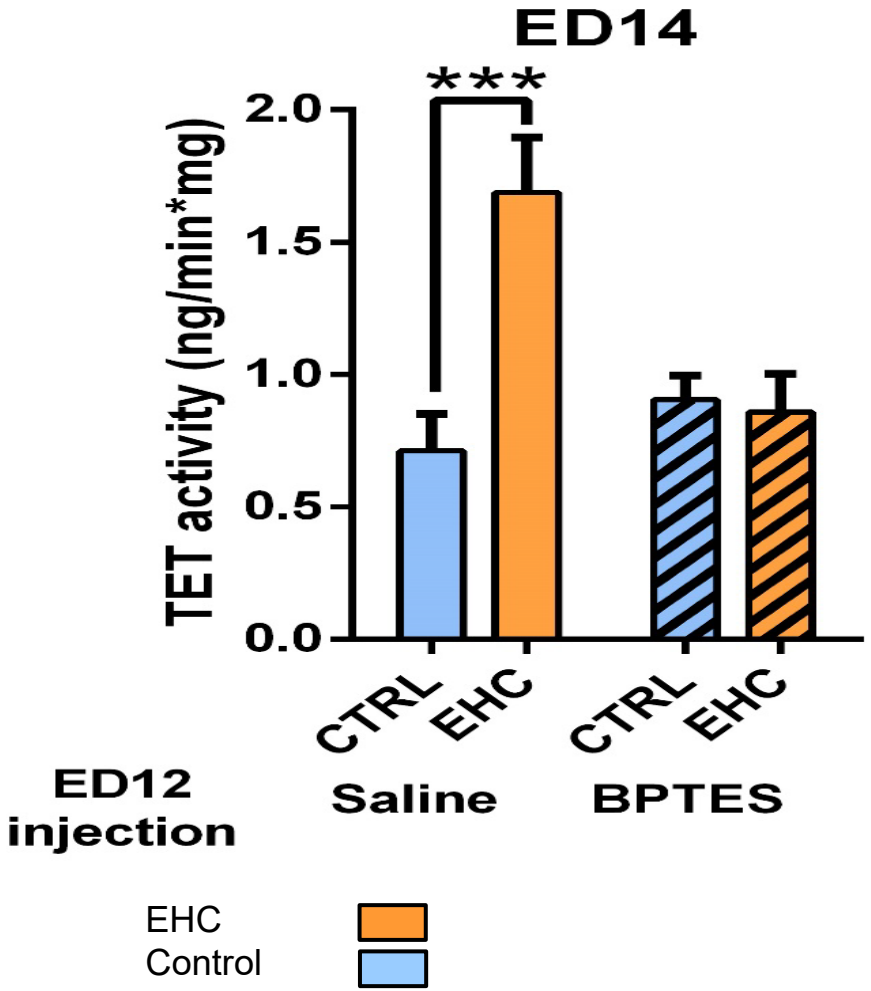
Gene expression



BPTES inhibits a-keto-glutarate and attenuates TET activity

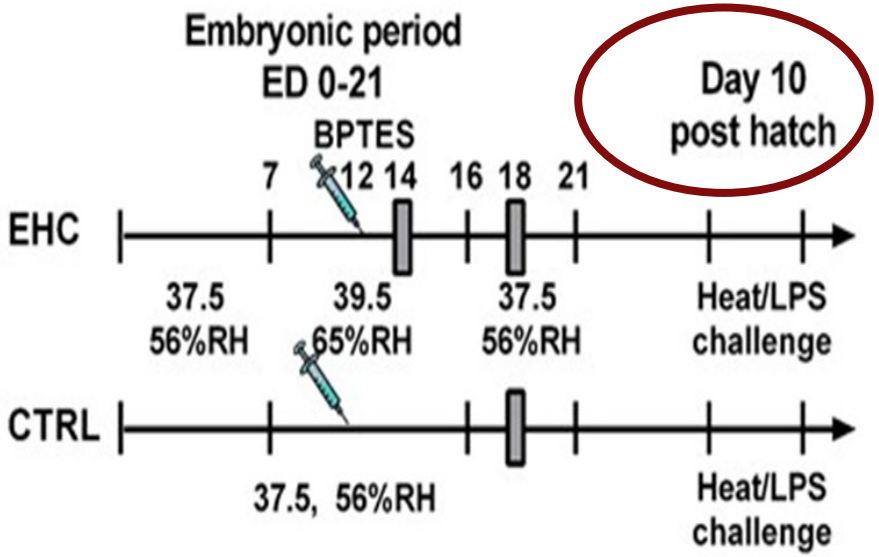


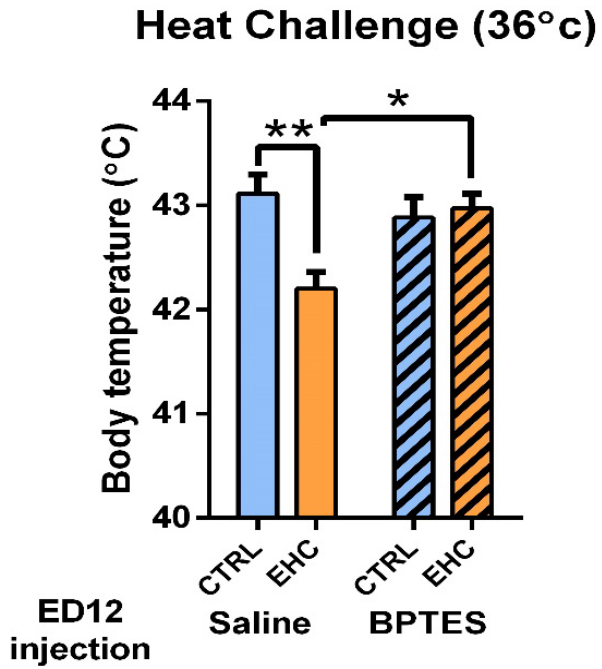


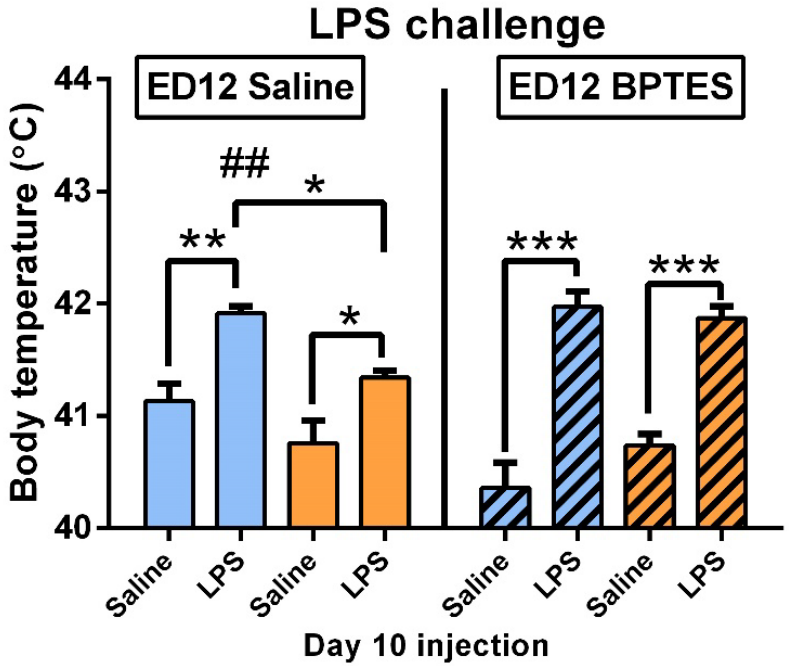


Rosenberg et al, Frontiers in genetics. 2020

Embryonic injection of BPTES inhibits both heat and inflammatory resilience





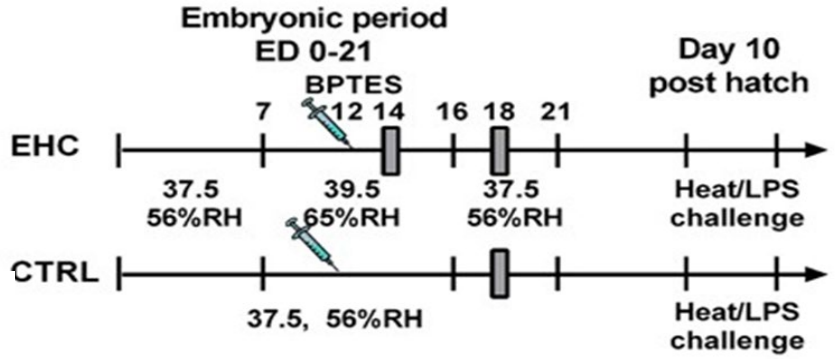


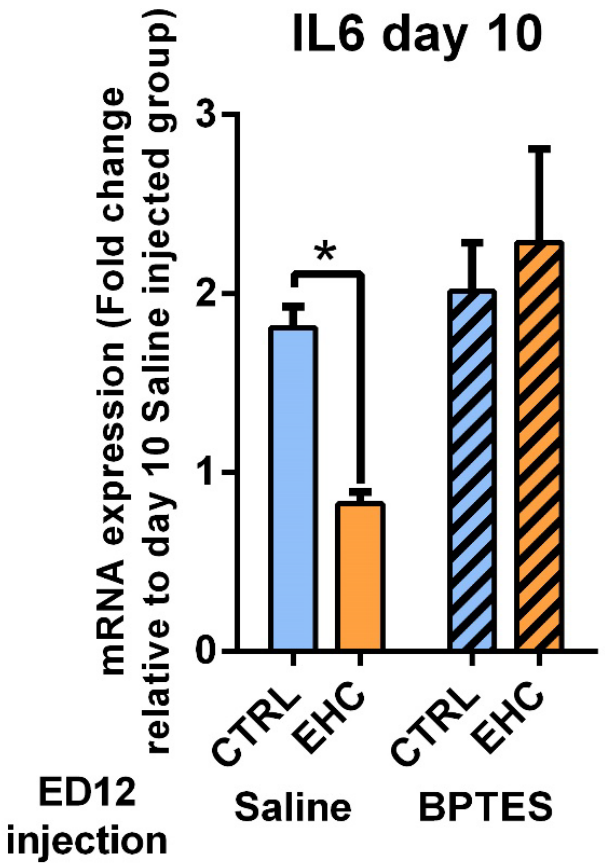
EHC
Control

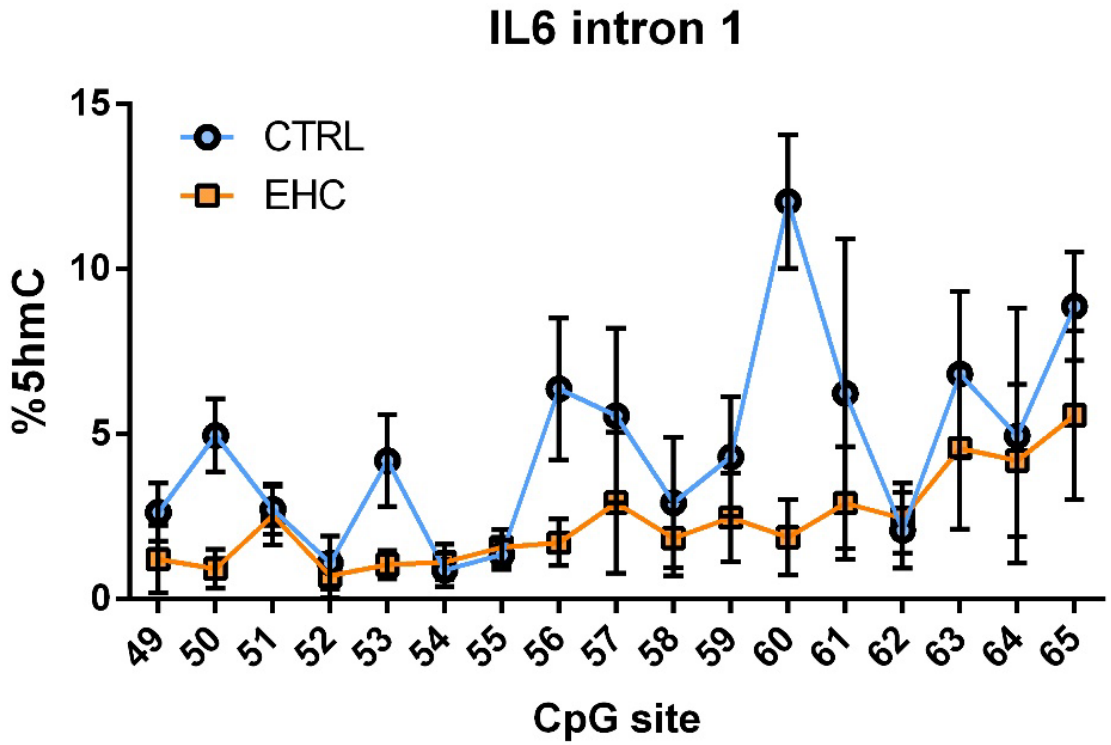
TET activity during EHC is necessary for both heat and immune resilience

Rosenberg et al, Frontiers in genetics. 2020

IL6 intron 1 presents reduced hydroxy-methylation (%5hmC)

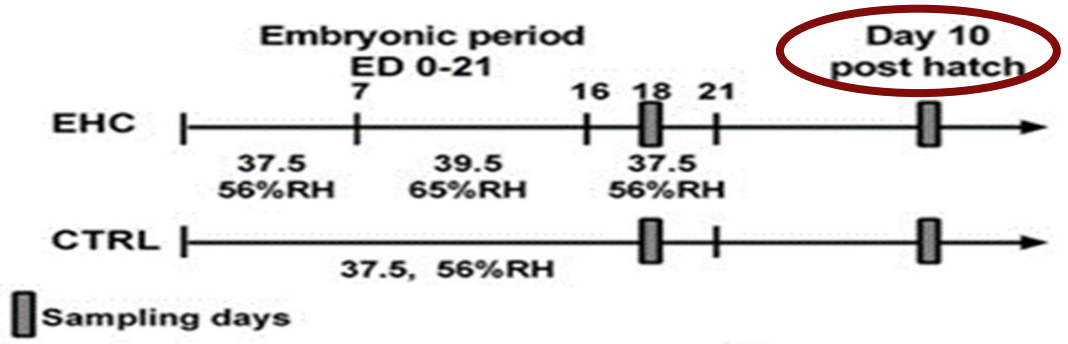


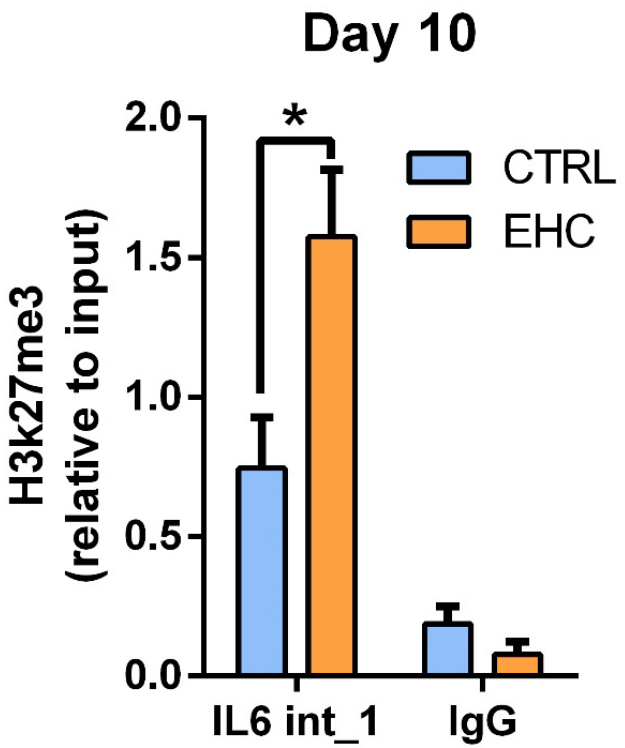


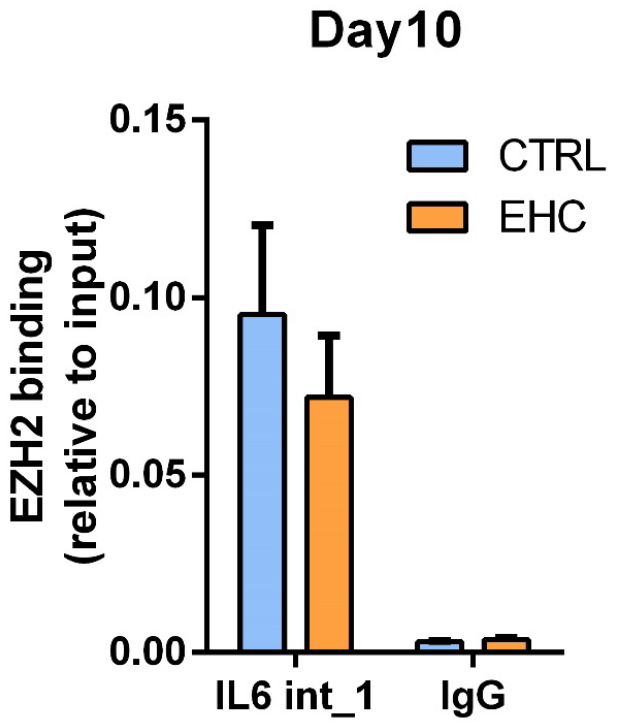


Rosenberg et al, Epigenetics 2021

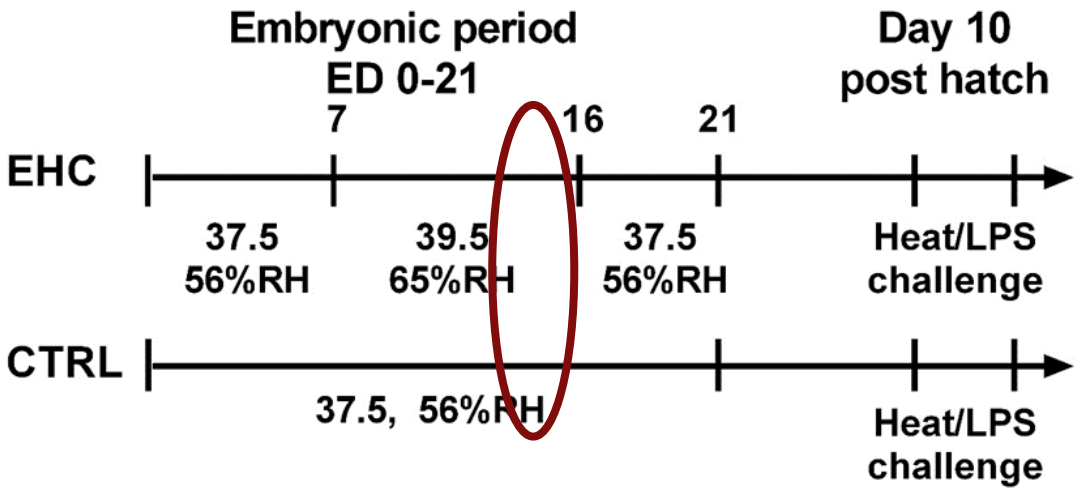
The histone methylation persists along life

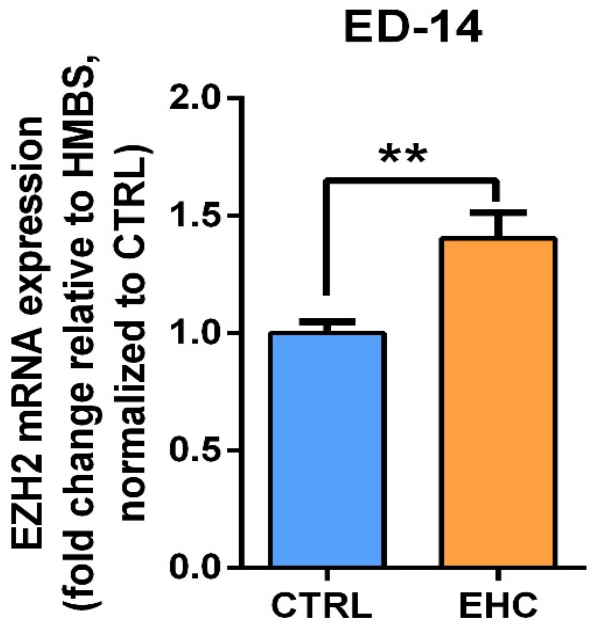


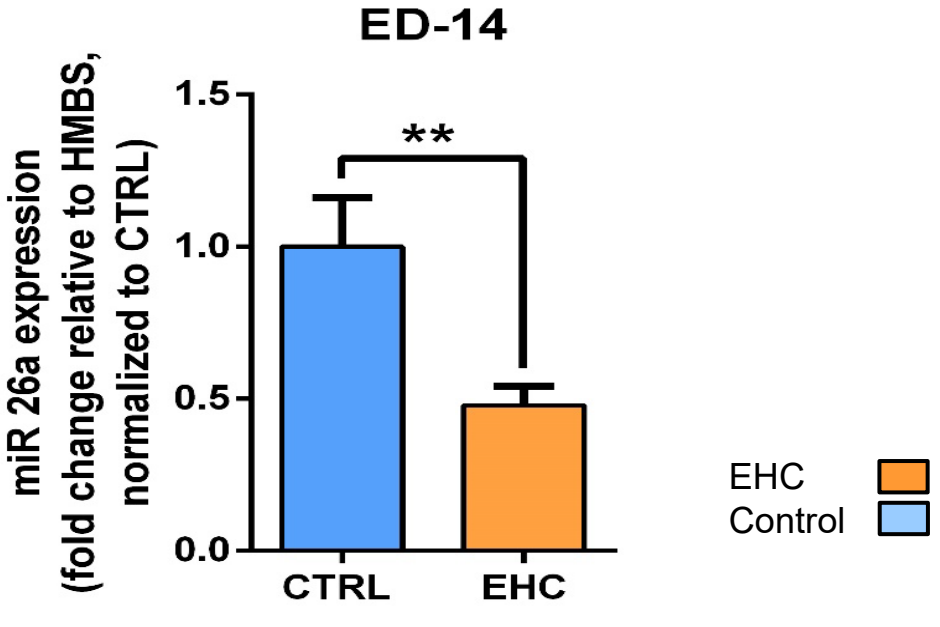




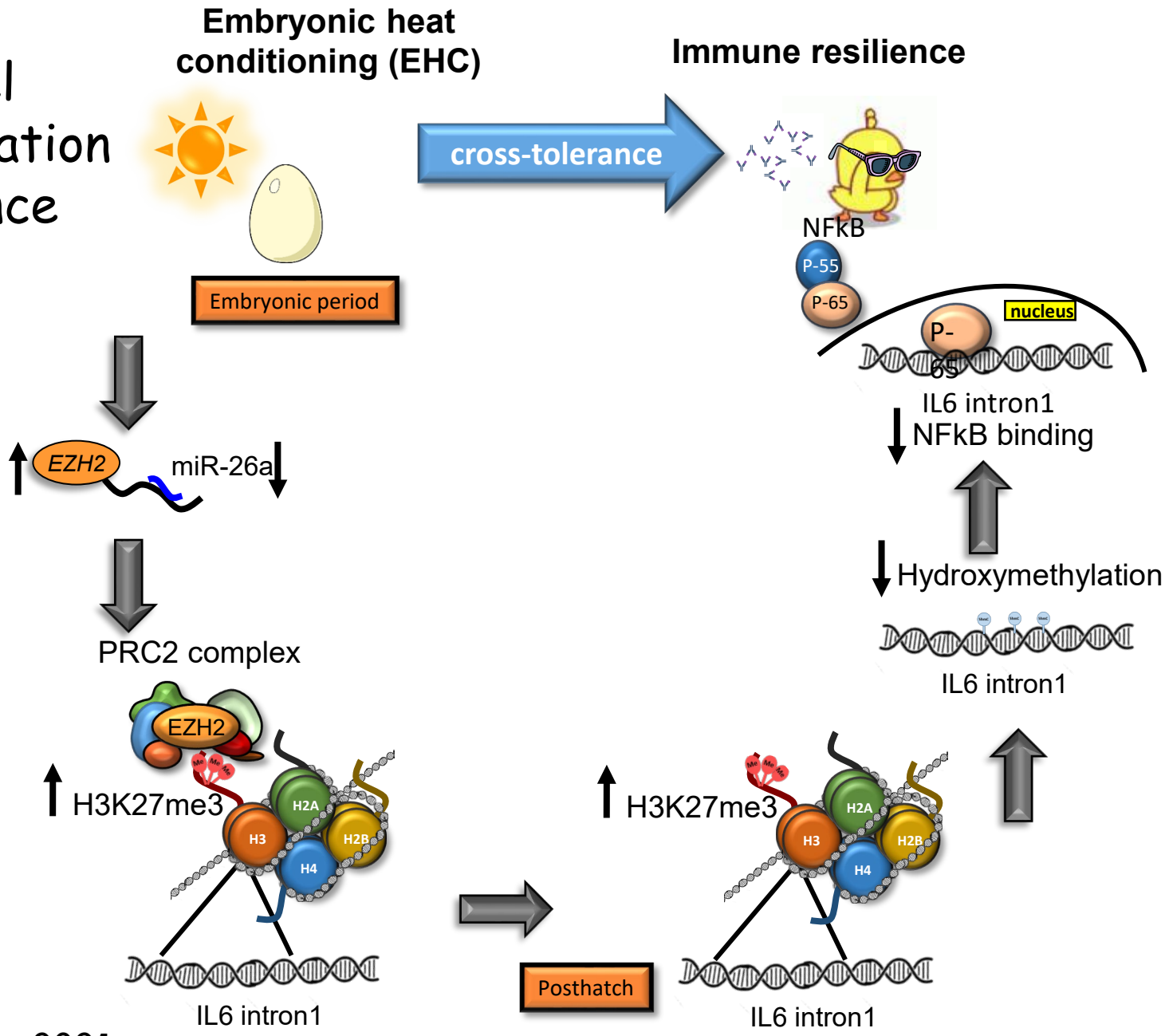
EZH2 expression during EHC is regulated by miR26a





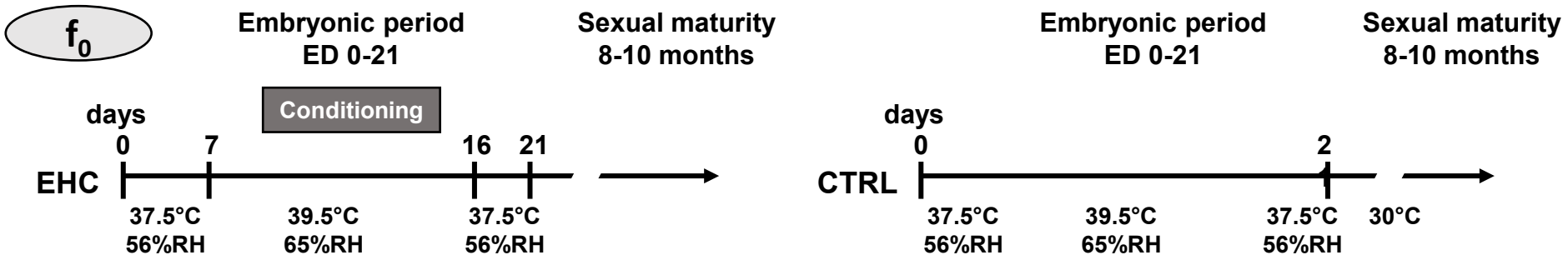


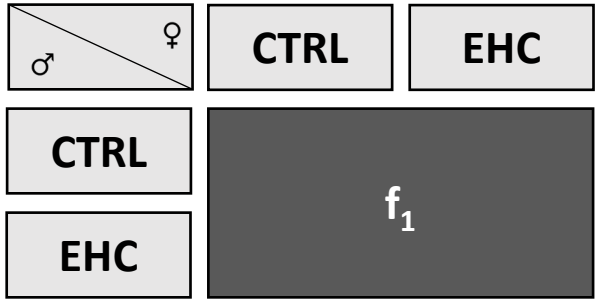
Summery Of the multilevel Epigenetic regulation Of cross tolerance

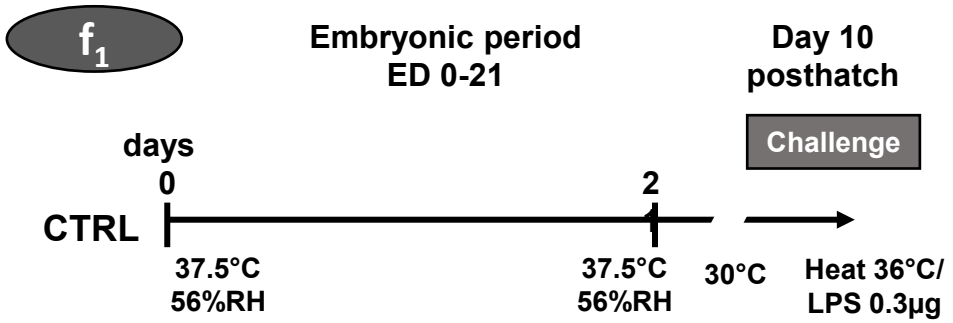


Rosenberg et al, Epigenetics 2021

Experiment scheme (intergenerational cross tolerance?)





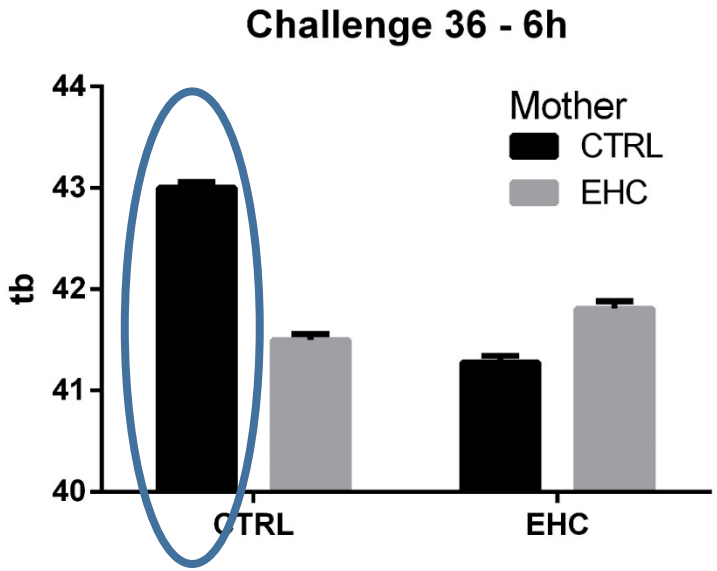


Rosenberg et al, FASEB 2022

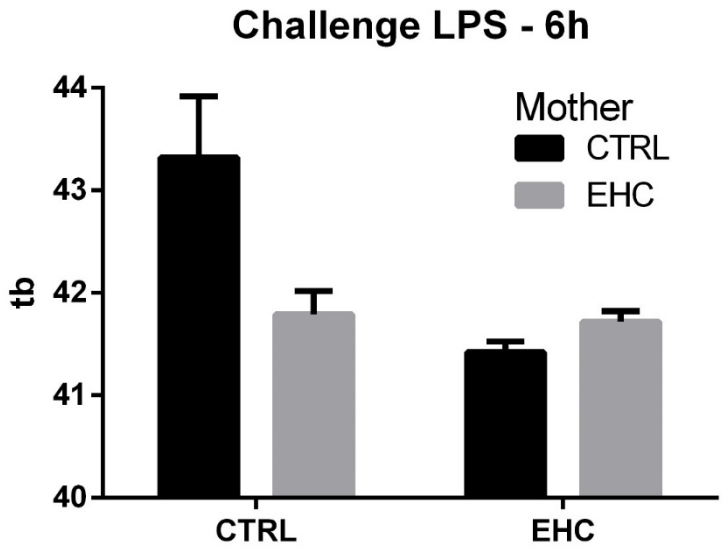
Heredity

Unconditioned f1 tb 6h into heat/LPS challenge

Body temperature (n=10)



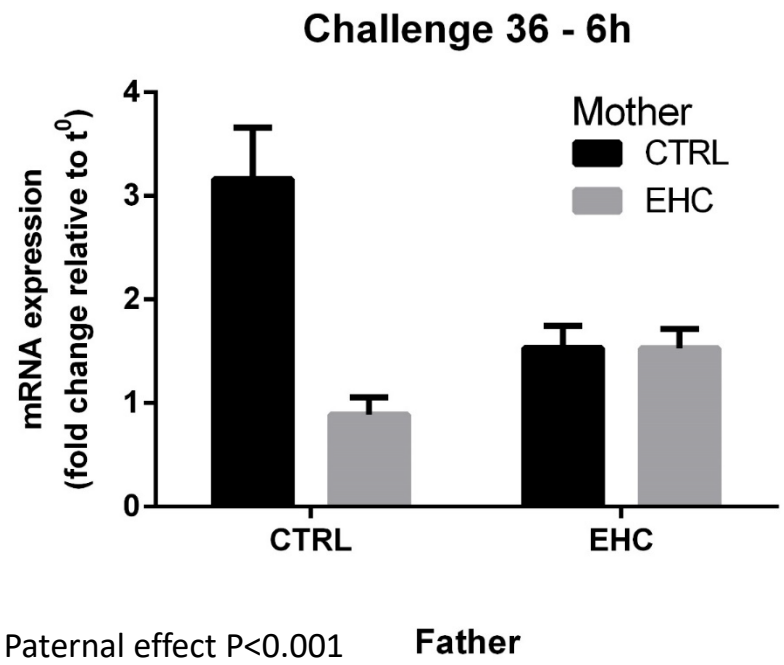
Paternal effect p<0.001
Maternal effect p<0.001
Interaction p<0.001



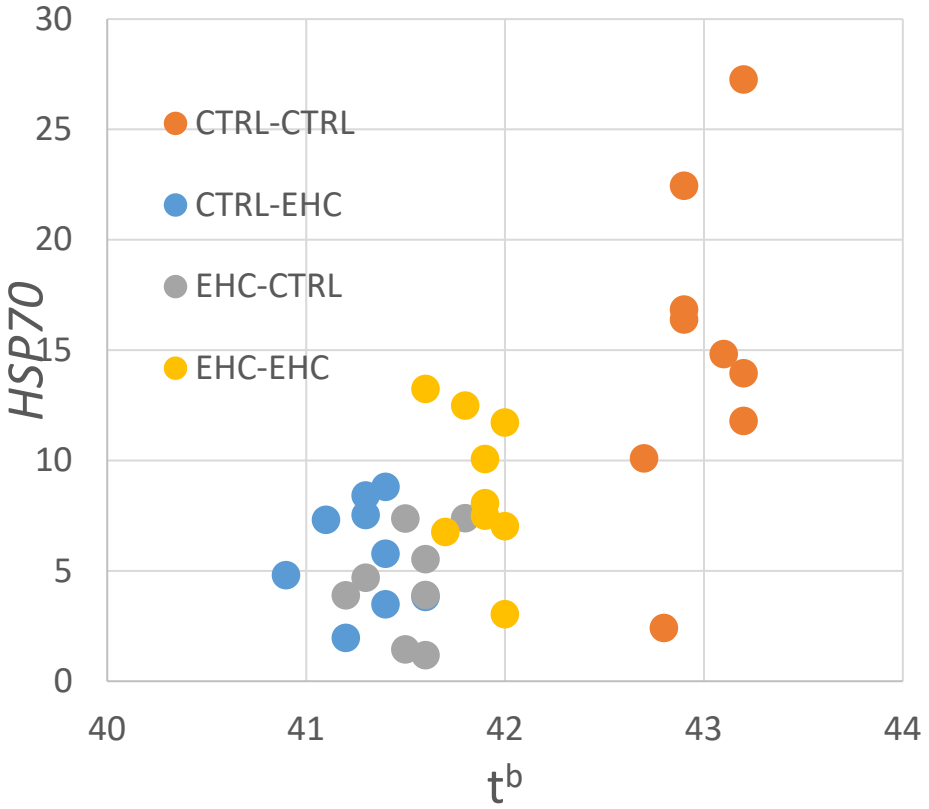
Paternal effect p=0.005
Maternal effect p=0.069
Interaction p=0.008

HSP-70 expression

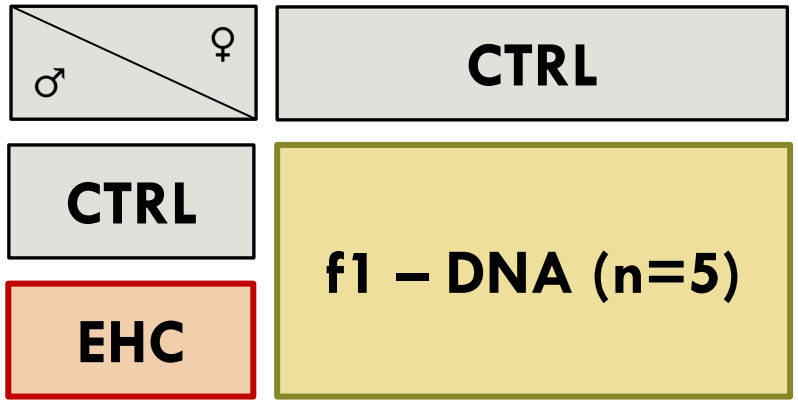
Unconditioned f1 gene expression 6h into heat challenge

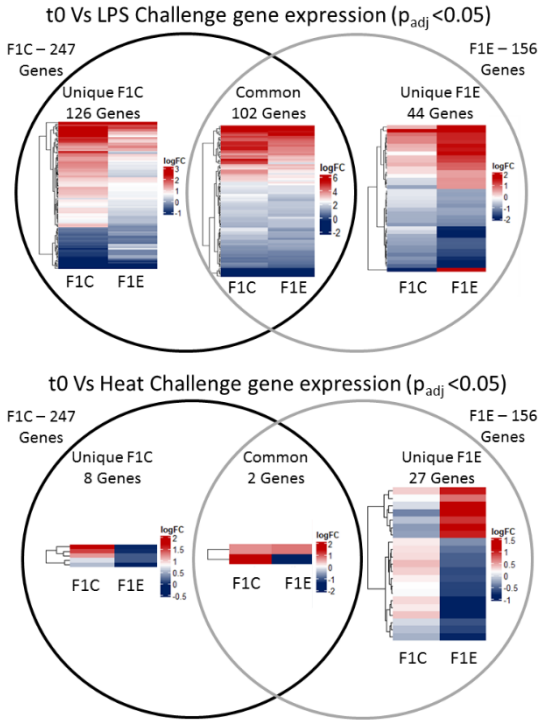


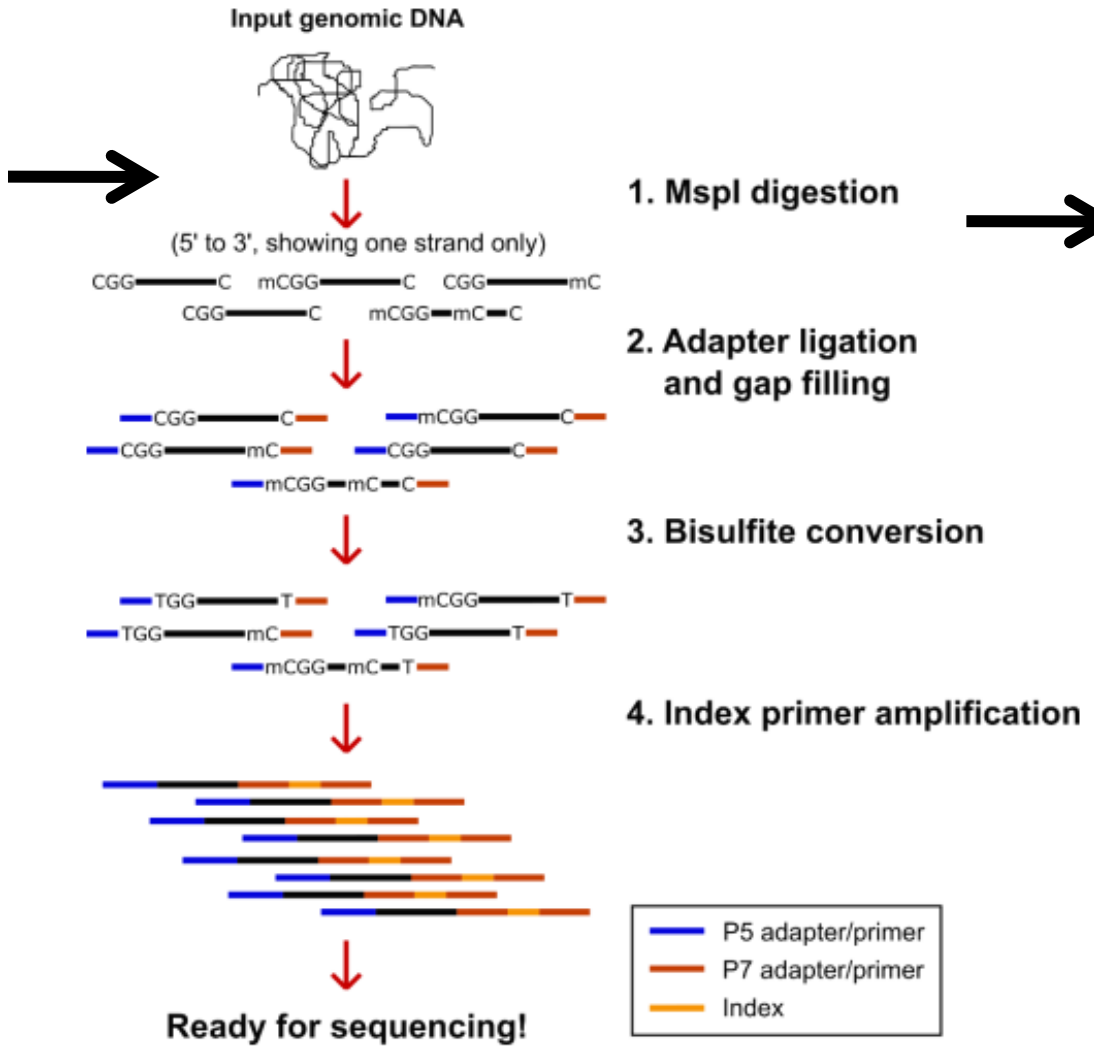
Paternal effect P<0.001
Maternal effect p=0.014
Interaction p<0.001



RRBS of F1 samples from CTRL mothers & CTRL/EHC fathers

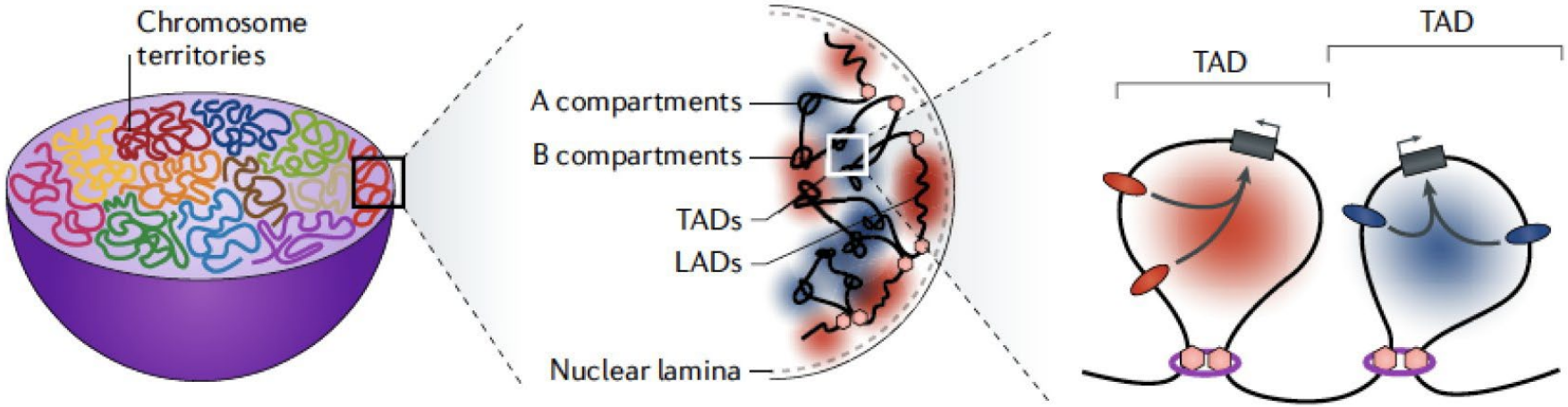






- NovaSeq SP 200-cycles kit
- 800M reads
- 20% PhiX

Rosenberg et al, FASEB 2022

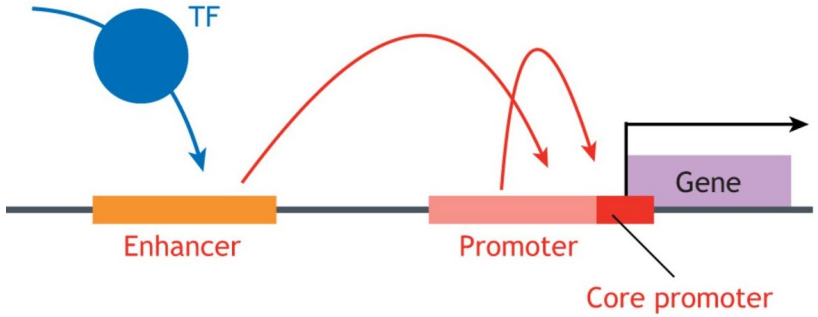


1. Chromosomes Territories

2. Chromosome Compartments

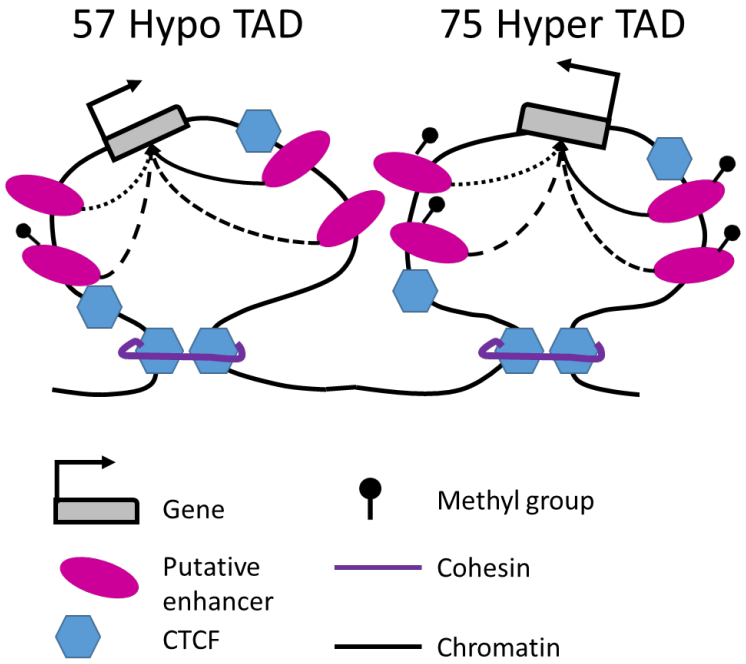
3. Topologically associating domains (TAD)

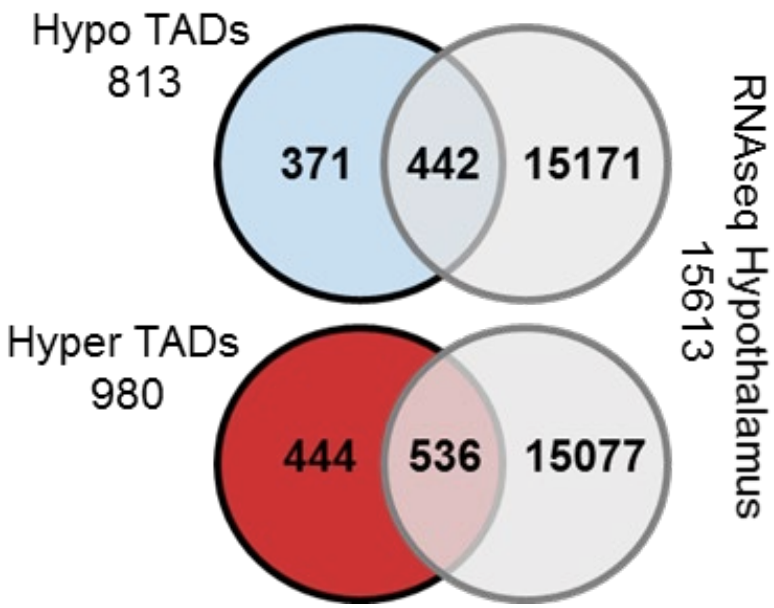
4. Promoter – Enhancer interactions



Malte, S. et al. Nat. Rev. Genetics. (2018).

DMS were enriched for regulatory areas and CTCF

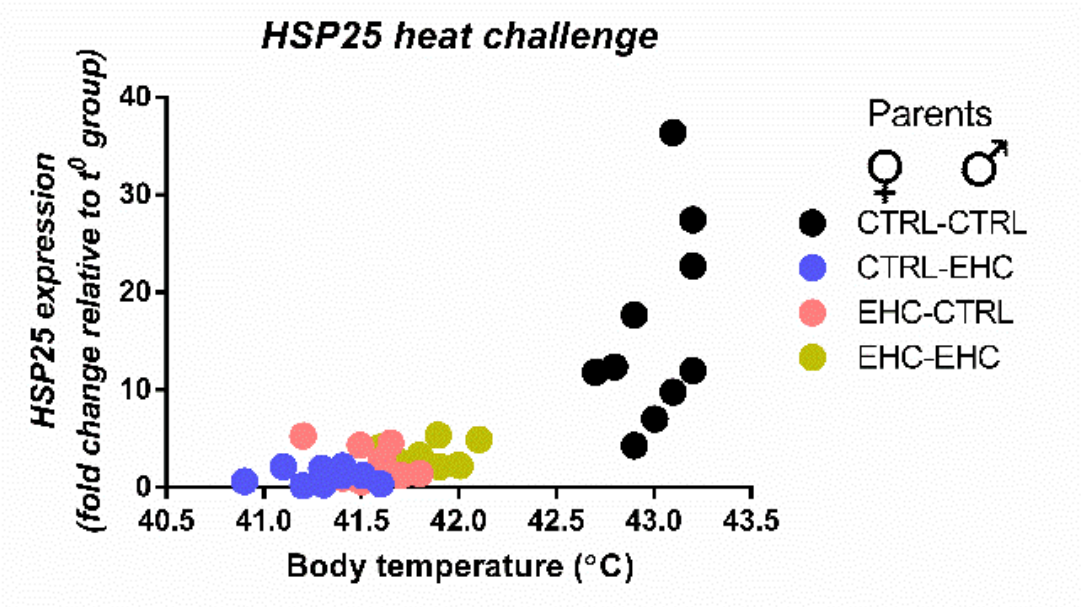


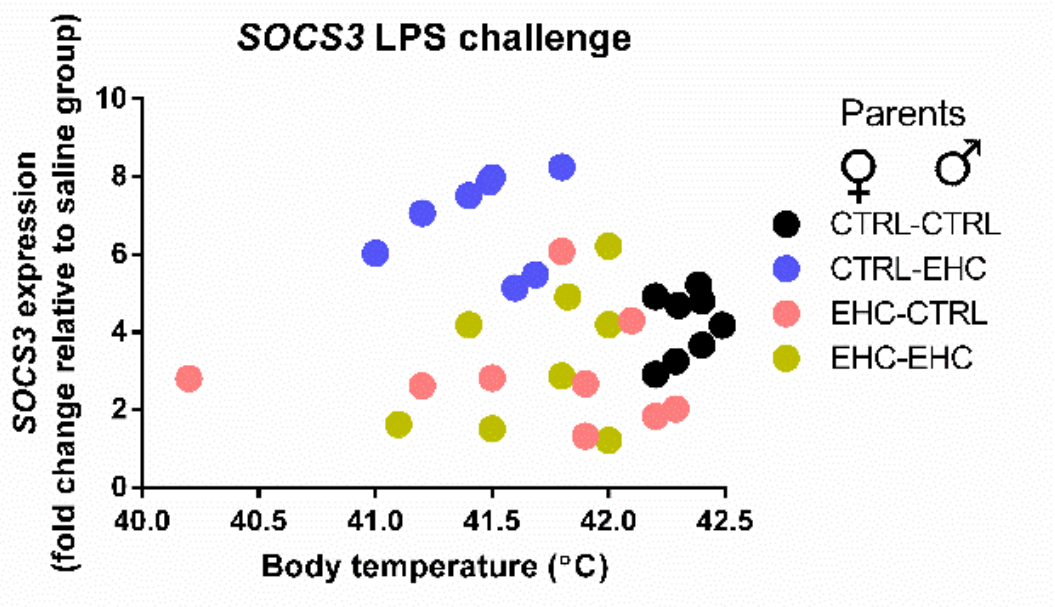


Thus, to better understand the functional role of DMS on introns and intergenic regions, we parsed methylated sites into TADs.

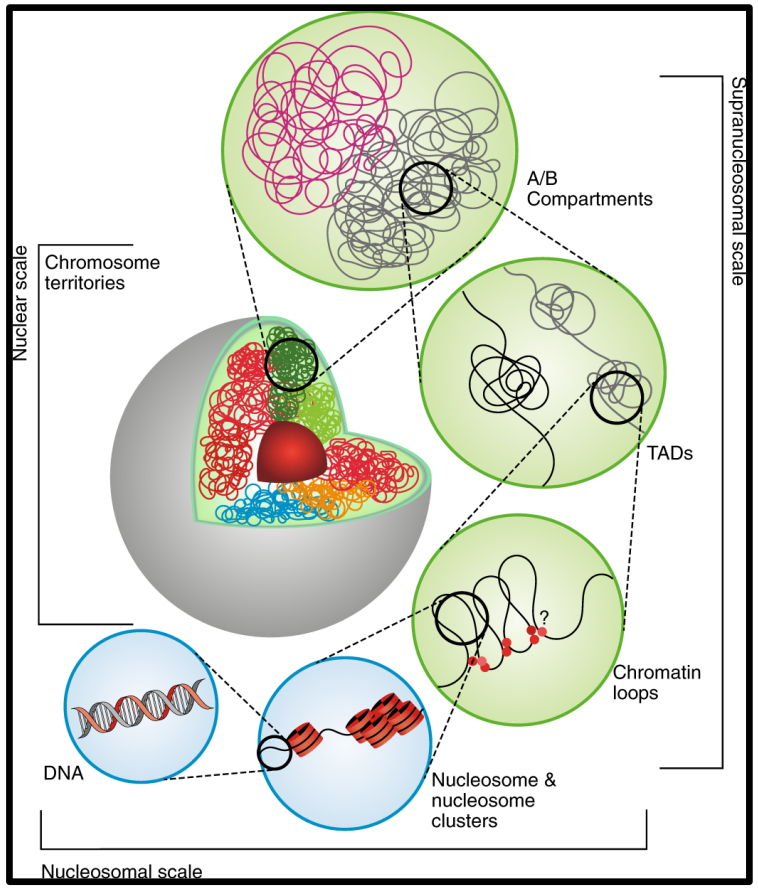
FOCUSING ON HSP25 AND SOCS3

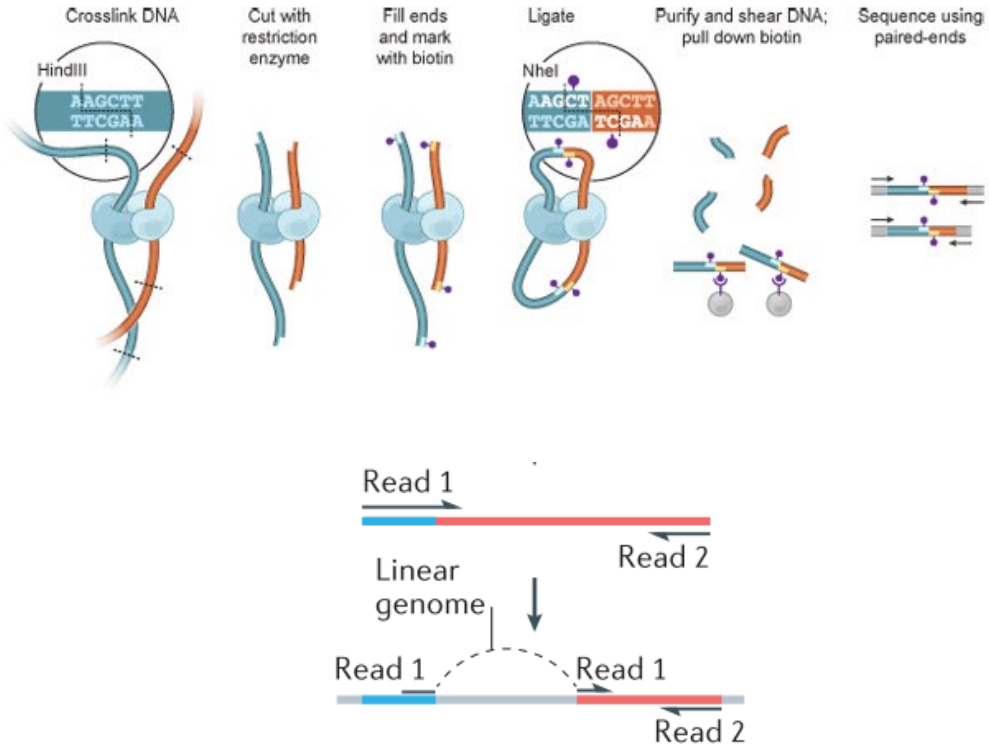
- Hypo and Hyper DMS
- Hypo and Hyper TADs
- Expression during challenge distinguishes between groups





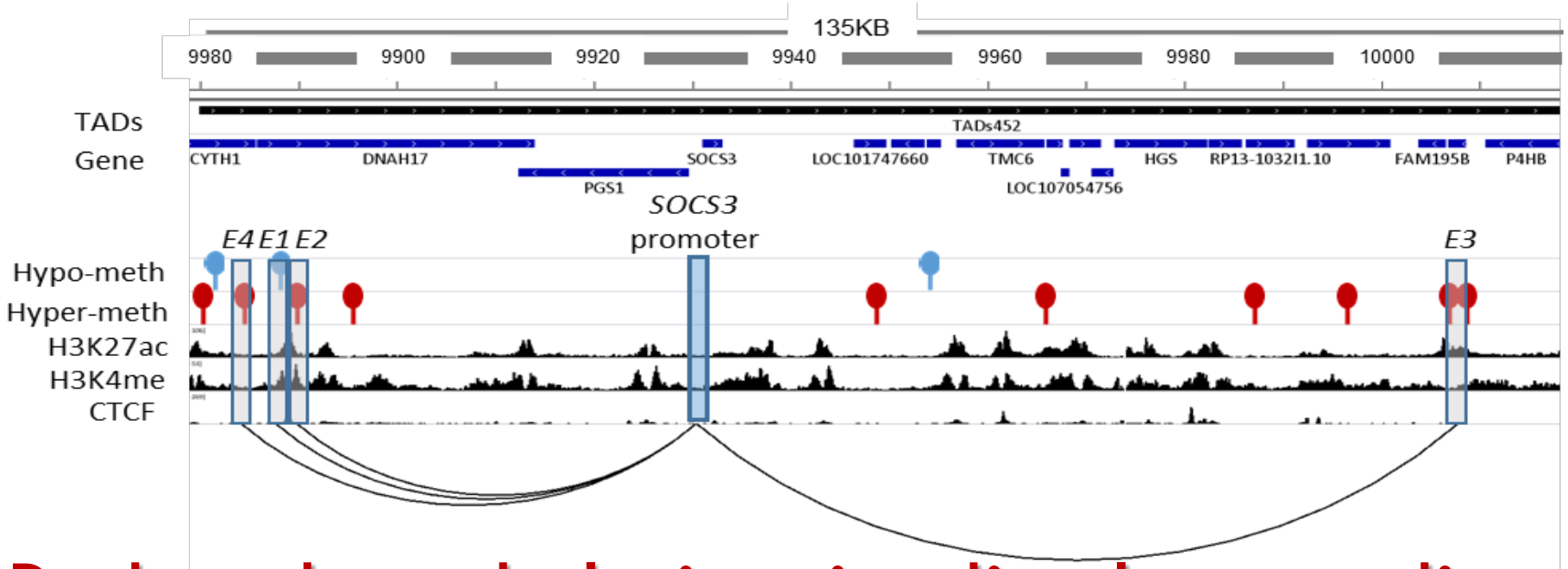
Chromosome Conformation Capture (3C): a technique to map 3D -chromatin contacts on a genome-wide scale





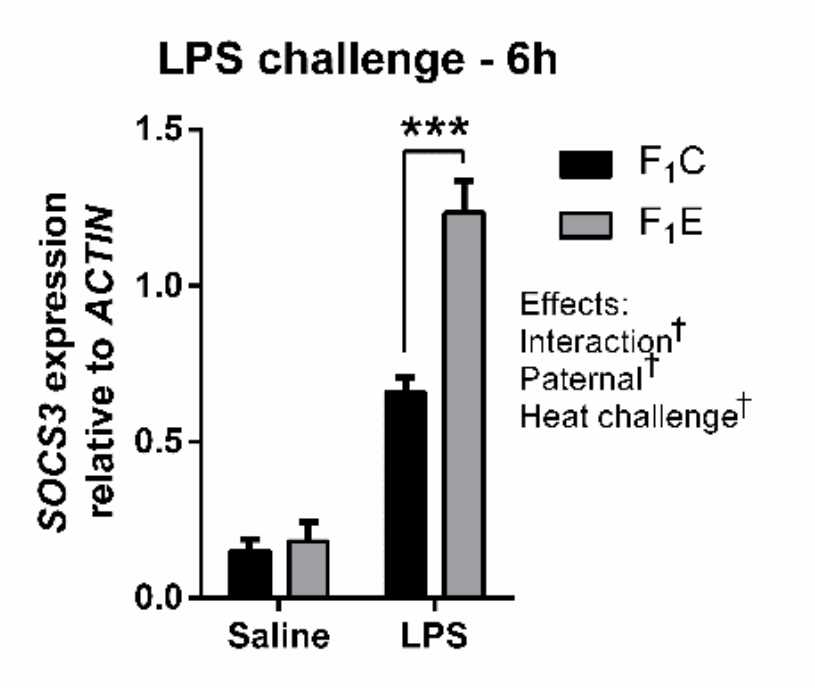
Lieberman-Aiden Erez. et al. Science (2009).
Watson, L. A. et al Curr. Opin. Neurobiol. (2017).

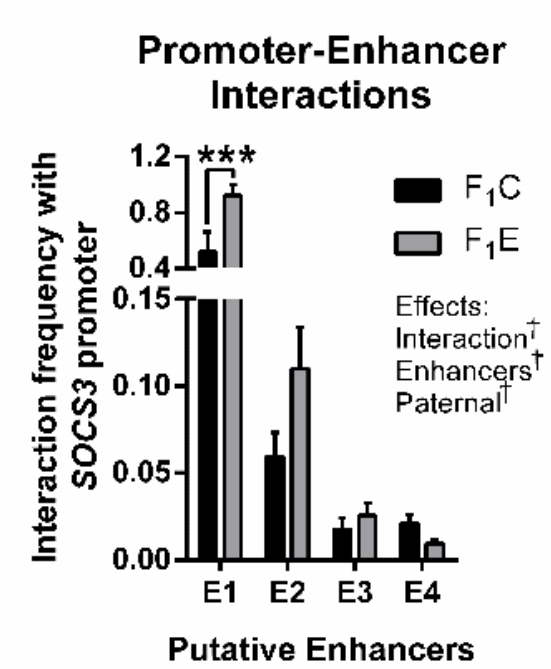
EHC SOCS3 regulatory element 1 (RE1) is hypo methylated, presents increased CTCF binding.

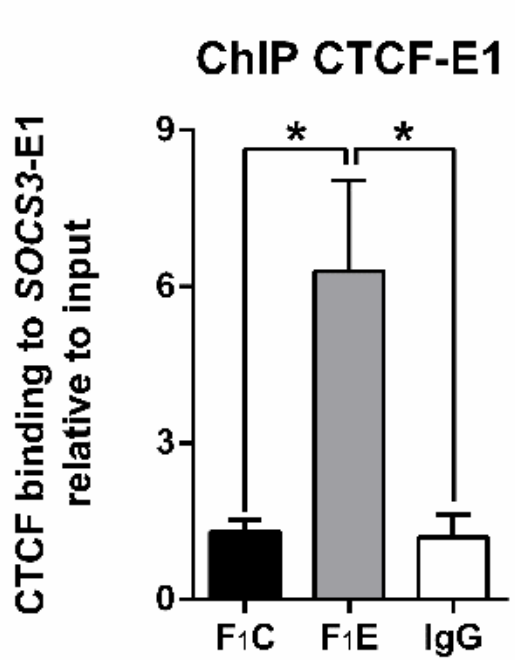


Reduced methylation in distal noncoding region along with increased expression

EHC SOCS3 REGULATORY ELEMENT 1 (RE1) is hypo methylated, presents increased CTCF binding.







*Take home message

- We do not understand epigenetics!
- In this stage we can not encipher the code
- Epigenetic is regulating gene expression by a cross talk between its different components/layers
- The epigenetic regulation is inherited

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