

Impossible is nothing...



Qydezia Barrett

# Kabuki syndrome: A potentially treatable cause of intellectual disability.

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Hans Tómas Björnsson MD PhD



East Coast Kabuki Conference  
June 2015

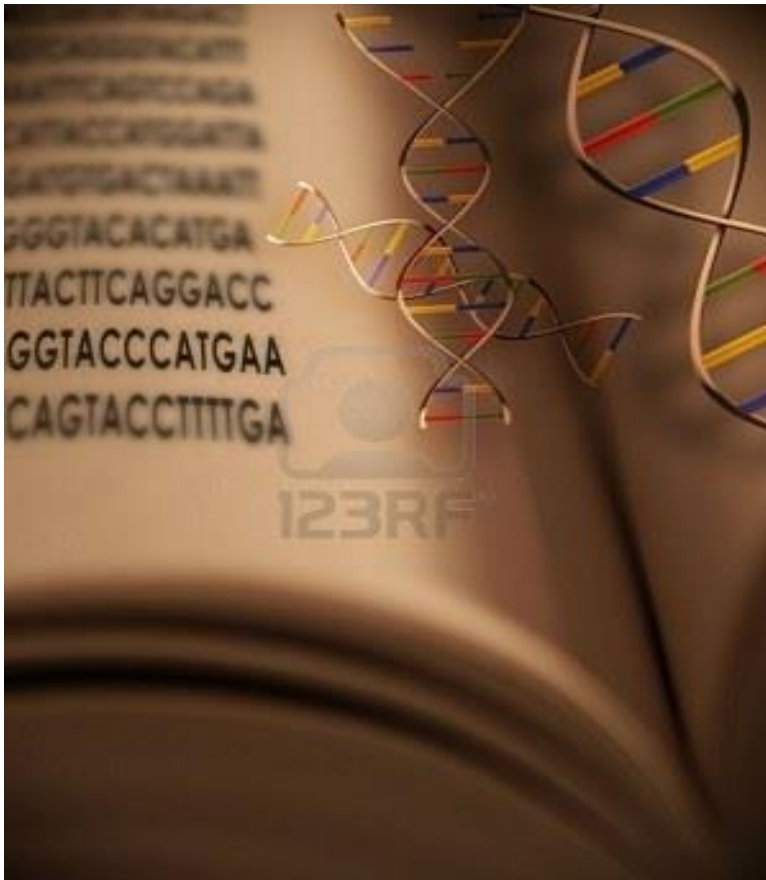
# What is DNA?

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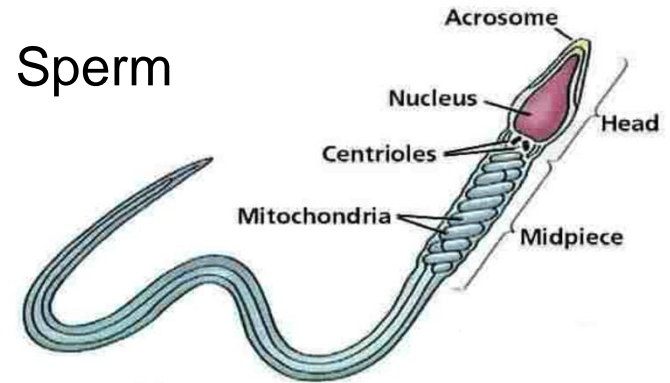
- DNA is the language of life (how the cells know what to do);
- The entire content of DNA = “Book of life” = the human genome;
- If the human genome is a book, the “genes” would be words;
- We’ve had the entire “book of life” for 15 years but we don’t know how to read it yet;

# Same text, >300 different meanings

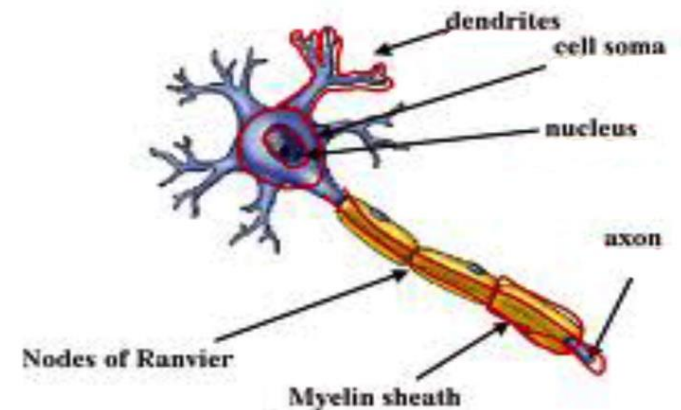
“Book of life”



Sperm



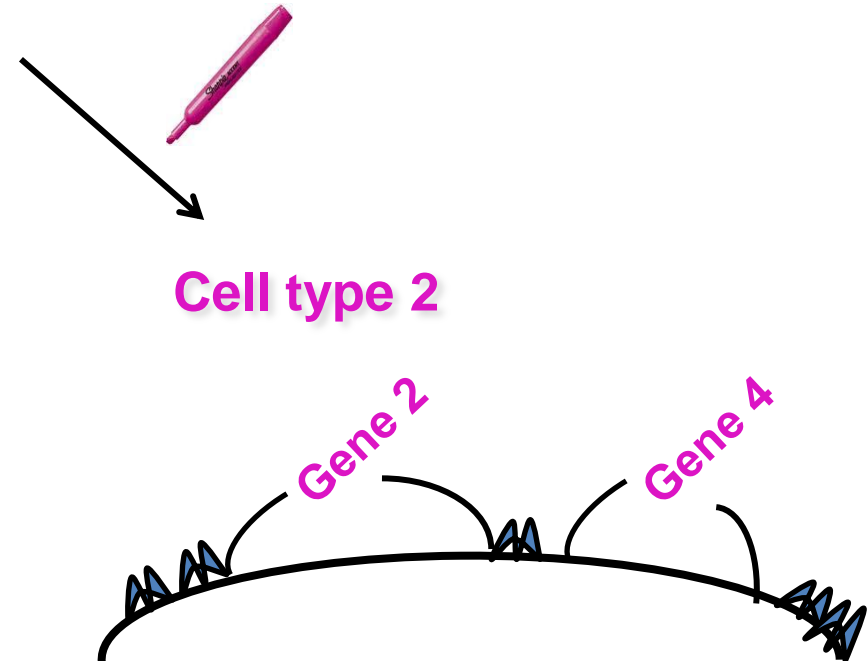
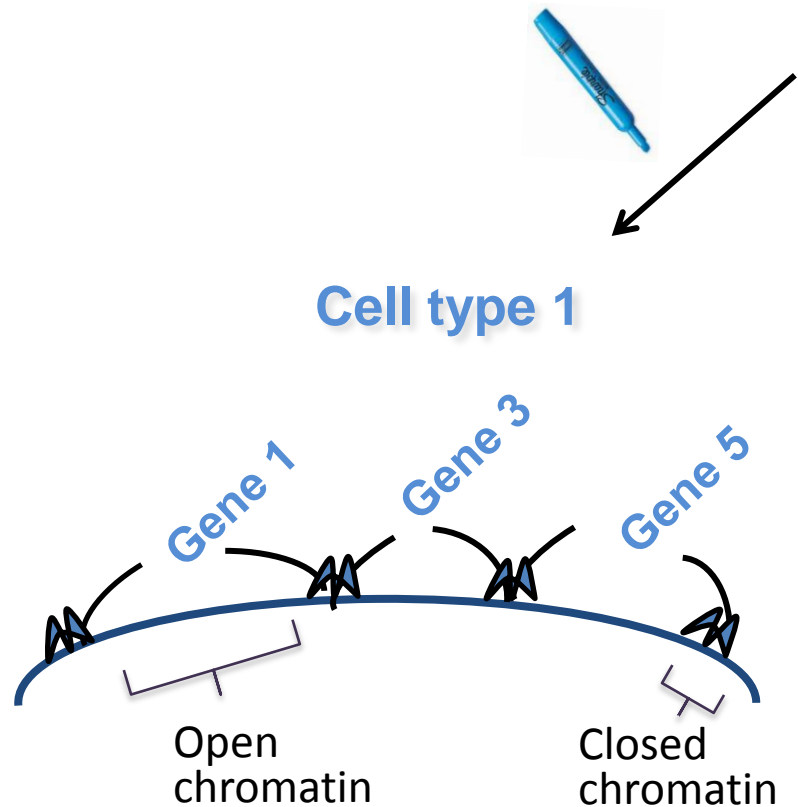
Neuron



How can you get multiple separate meanings from a single text?

# Highlighting genes for cell type specific functions

Gene 1, 2, 3, 4, 5.....genome



But how are these achieved and maintained in cells?



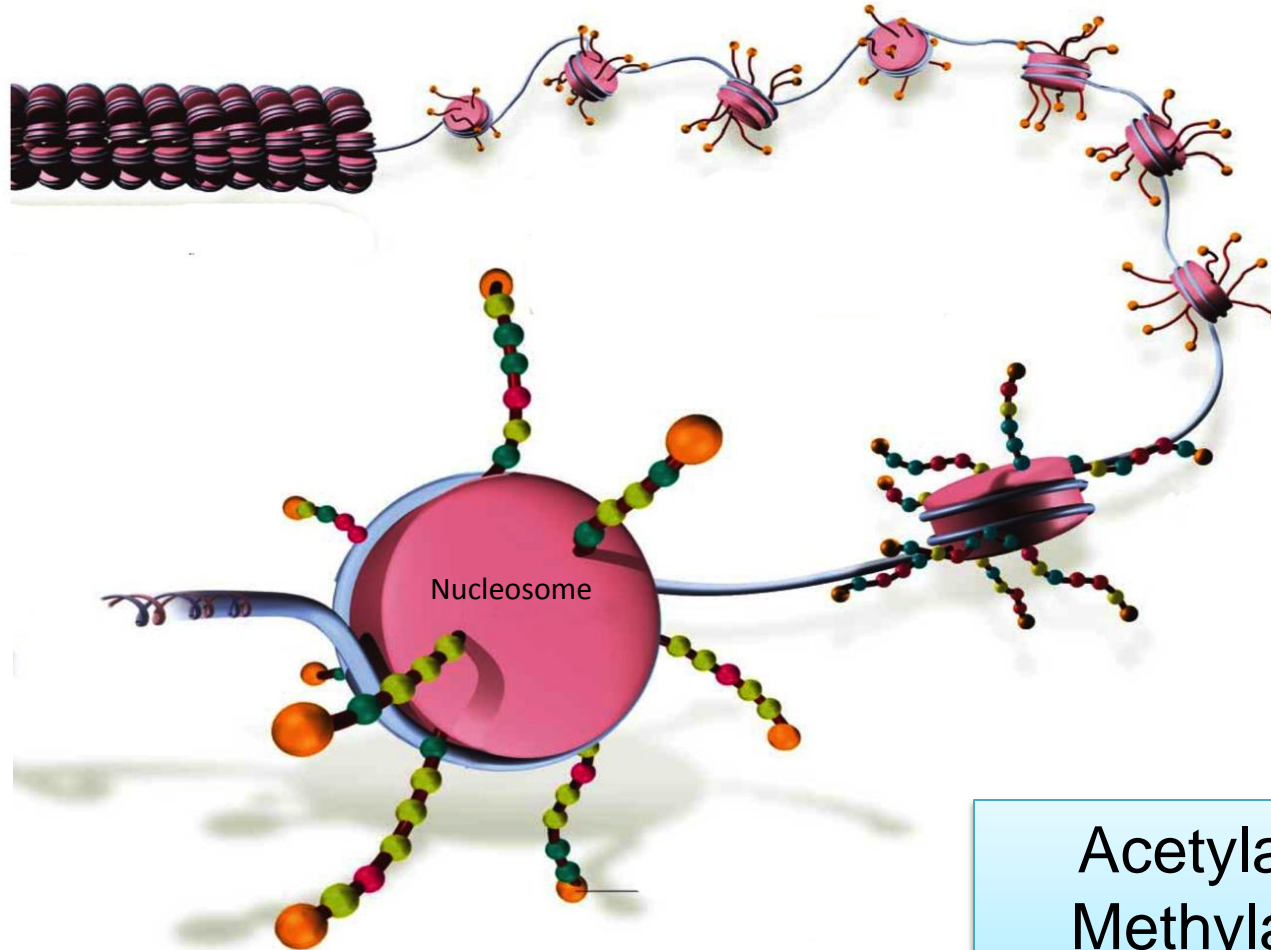
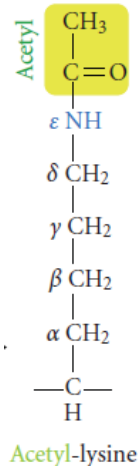
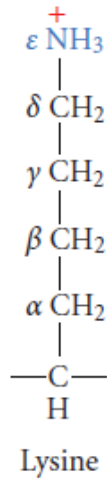
# Epigenetic machinery: the genome's “highlighter”

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- Epigenetic marks are modifications of DNA or associated proteins, other than the DNA sequence itself, that are heritable through cell division (mitosis)
- Reversible and affected by the environment.
- Add to information content of DNA
  - » DNA methylation
  - » **Histone tail modifications**

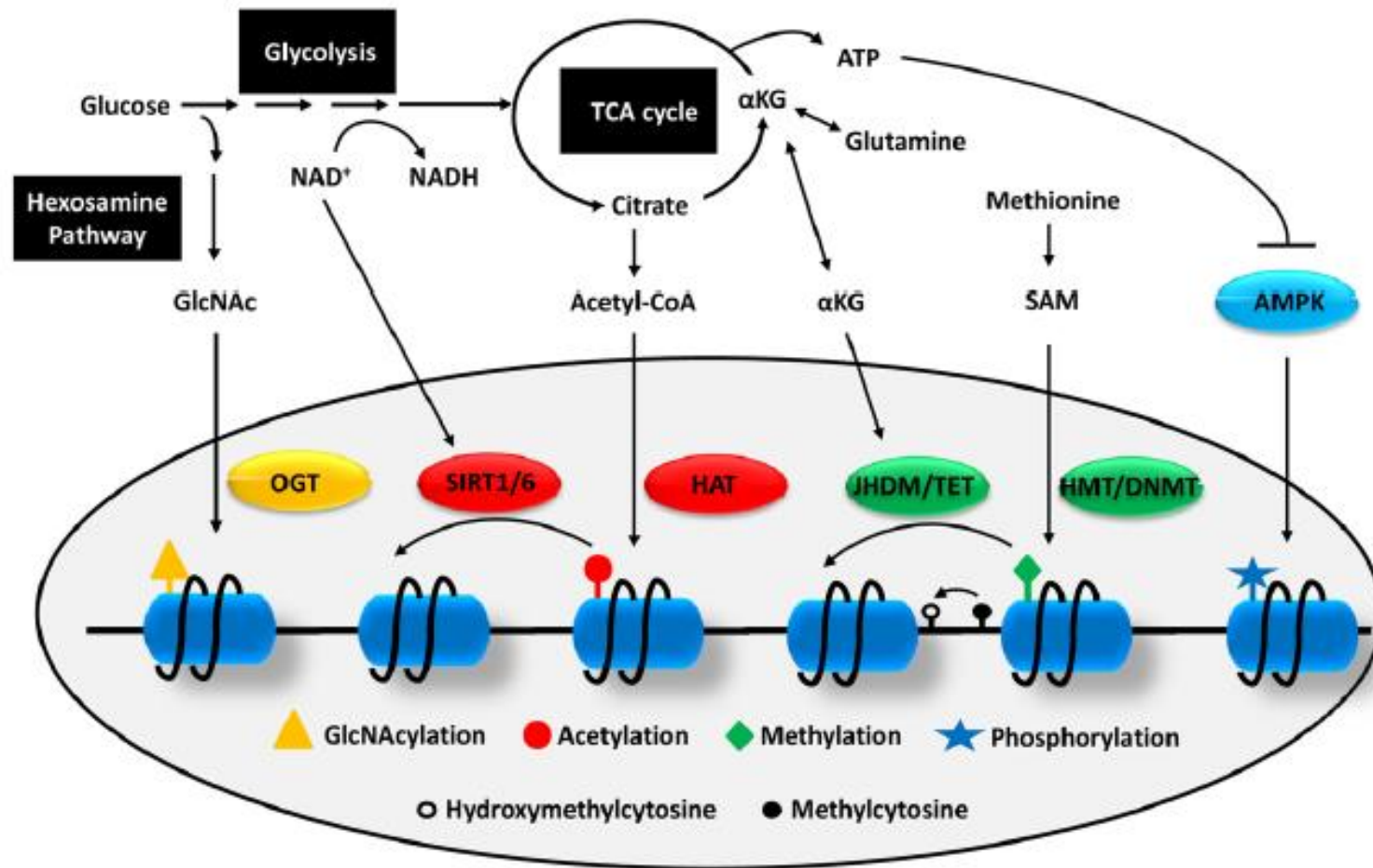


# Histone acetylation is seen in open chromatin



Acetylation,  
Methylation,  
and more.

# Emerging links between metabolic pathways and histone modifications





# Summary (1):

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- Epigenetic modifications are thought to help establish and maintain cell type specific identity;
- Many of the donors for epigenetic modifications are critical intermediates of cellular metabolism, linking gene expression with cellular metabolic states;

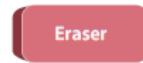
What are the components of the histone machinery?

# Histone machinery

## 1. Writers



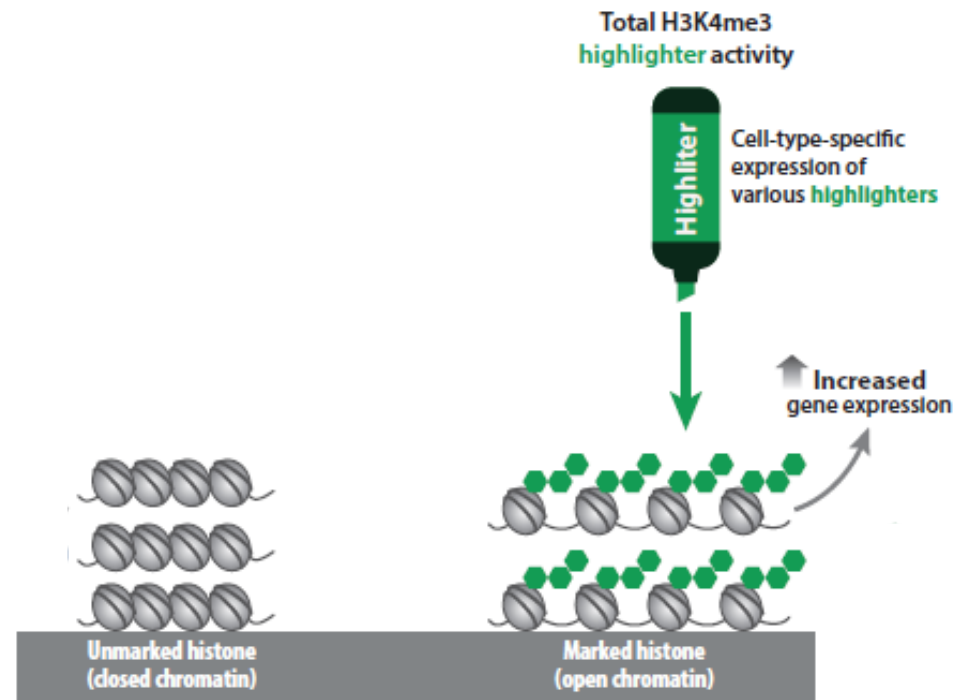
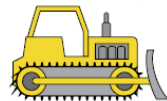
## 2. Erasers



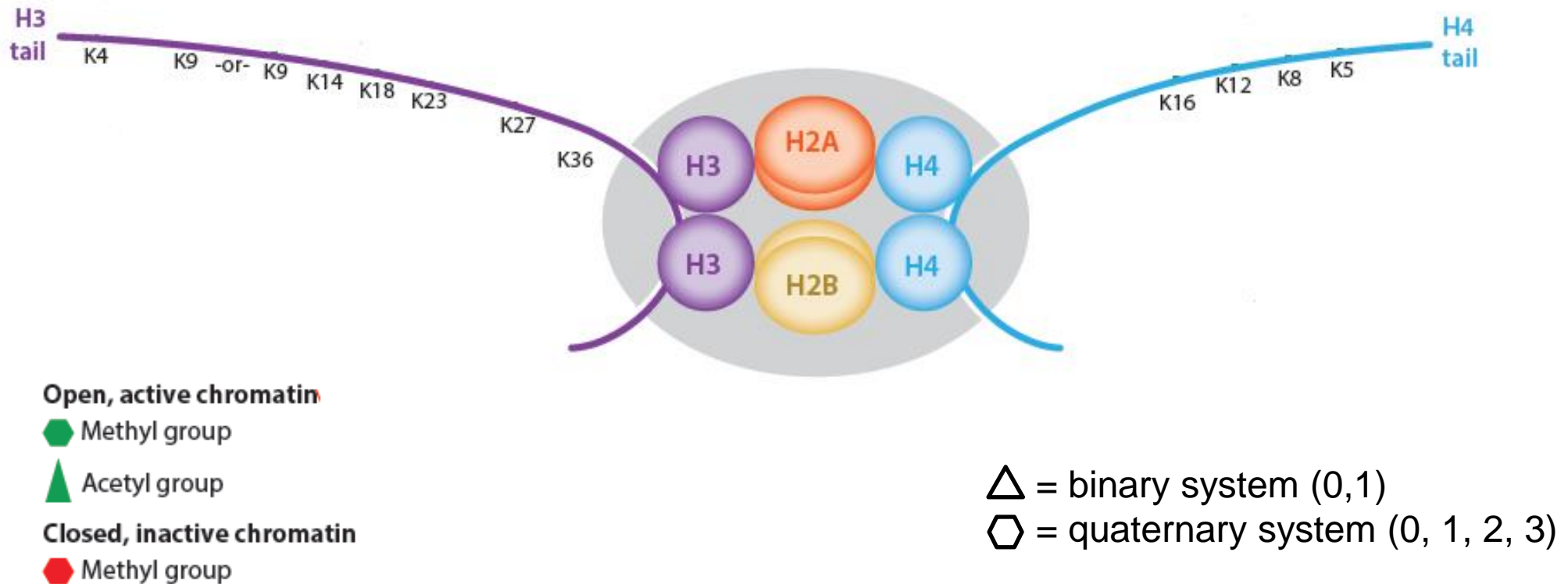
## 3. Readers



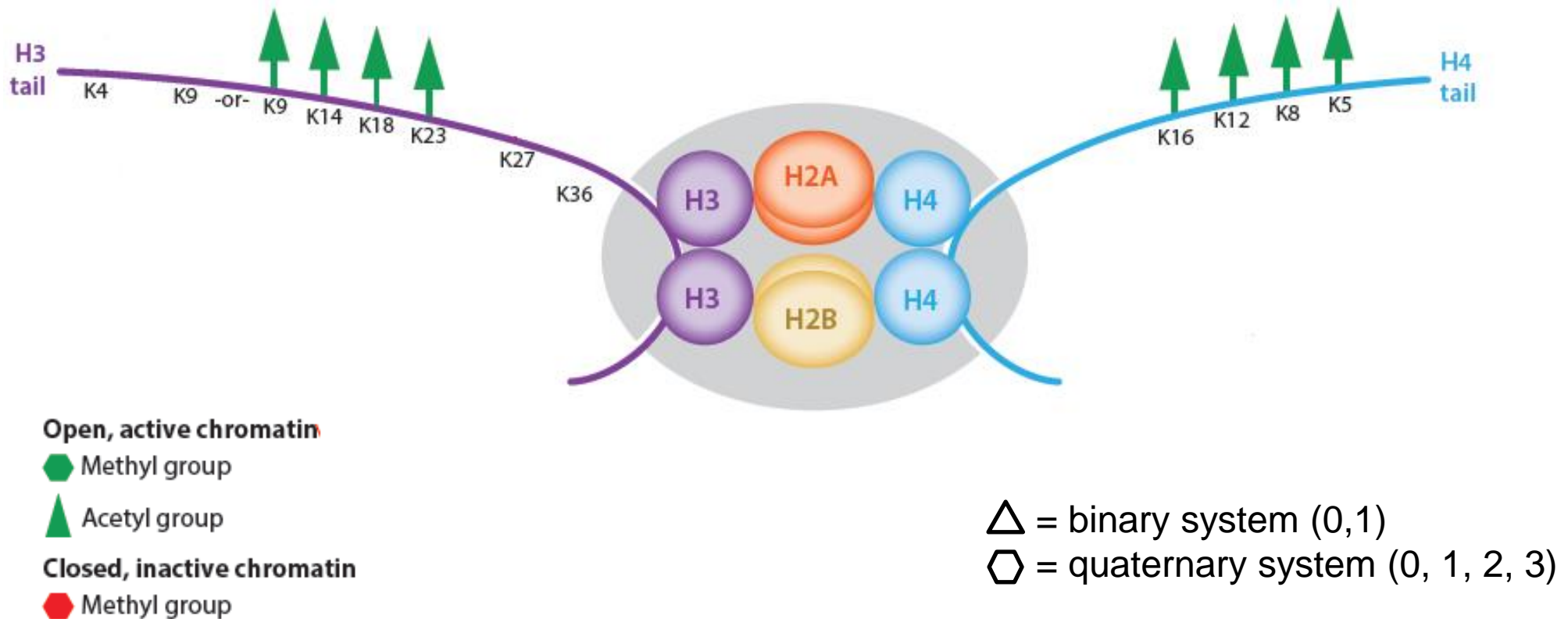
## 4. Remodeler



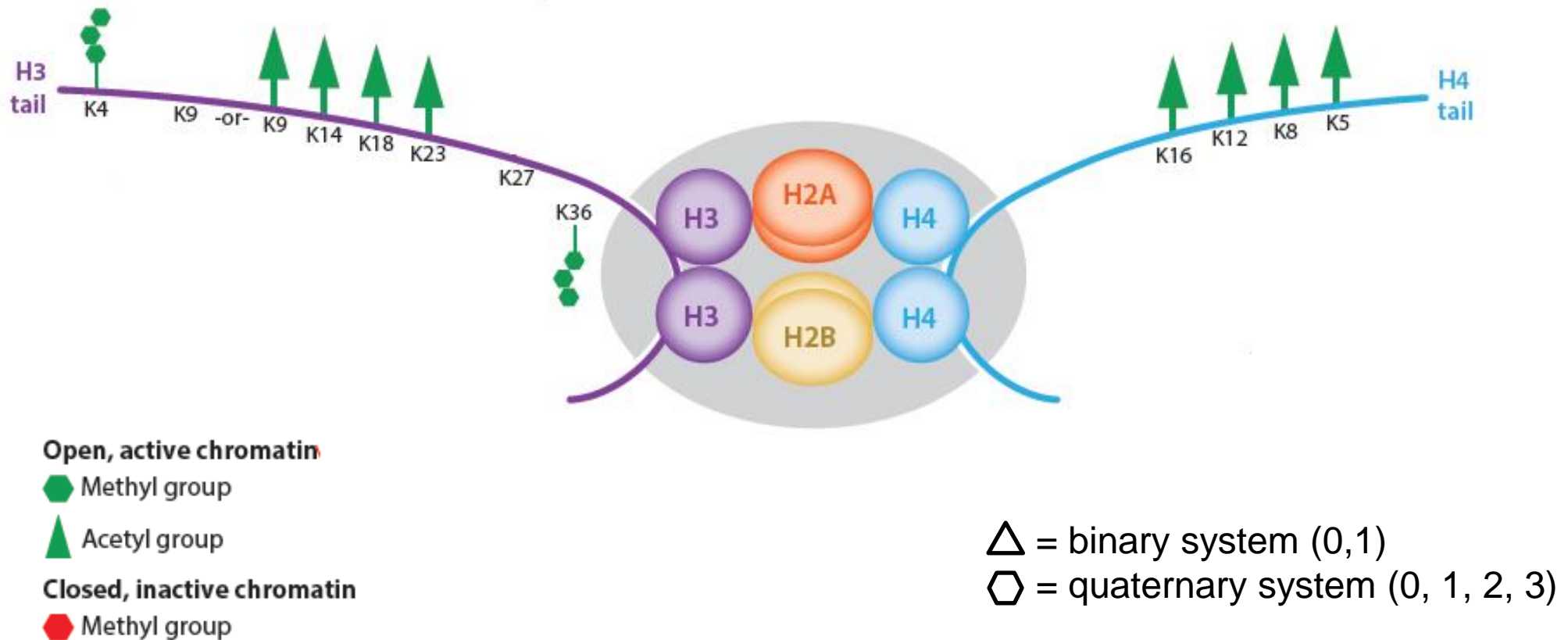
# Histone marks in open and closed chromatin



# Acetylation is an open chromatin mark

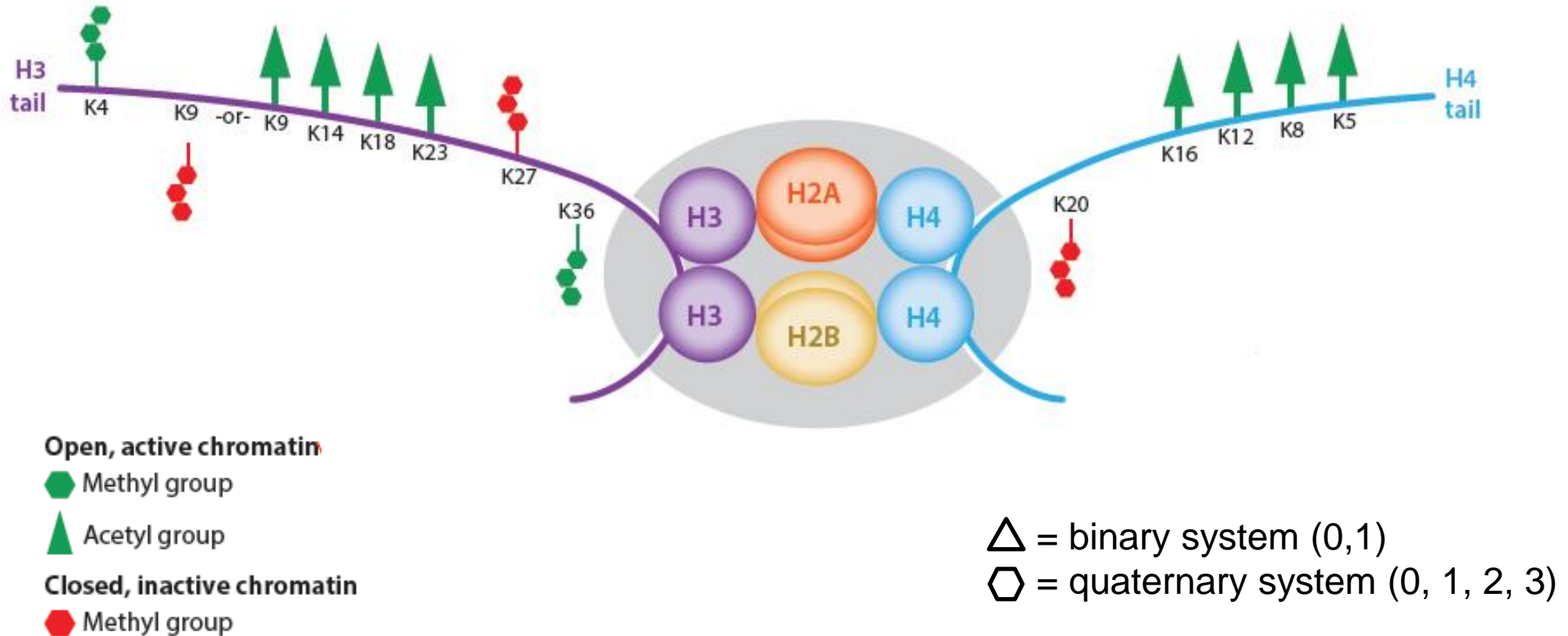


# H3K4me3 is an open chromatin mark





# H3K27me3 is a closed chromatin mark



## Summary (2):

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- The histone machinery consists of writers (highlighters), erasers, readers and remodelers;
- There are many different histone modifications, and certain combinations of marks are seen in open chromatin (H3K4me3, H4ac) and other combinations in closed chromatin (H3K9me3, H3K27me3);

But what happens when epigenetic modifications or machinery are disrupted?



The McKusick-Nathans  
Epigenetics and Chromatin Clinic

# Epigenetics and Chromatin Clinic

- Classical epigenetic disorders
  - » Beckwith Wiedemann syndrome

Mostly  
CIS

- Disorders of the DNA methylation machinery
  - » Rett syndrome
- Disorders of the histone machinery
  - » Kabuki syndrome

TRANS

Genetic disorders with epigenetic consequences

# Epigenetics & Chromatin Clinic



Johns Hopkins  
McKusick-Nathans Institute  
of Genetic Medicine

## Mission Statement

- 1) To *diagnose* and provide optimal *care* for individuals with either classical epigenetic disorders or disorders of the epigenetic machinery.
- 2) To *learn* from our patients some fundamental truths about epigenetics with the hope that this additional knowledge will lead to *therapeutic development* for our patients in the future.
- 3) To *educate* health providers, patients, and families about epigenetics and the disorders of the epigenetic machinery.

## Contact Us

Epigenetics & Chromatin Clinic  
600 N. Wolfe Street  
Blalock 1008  
Baltimore, MD 21287

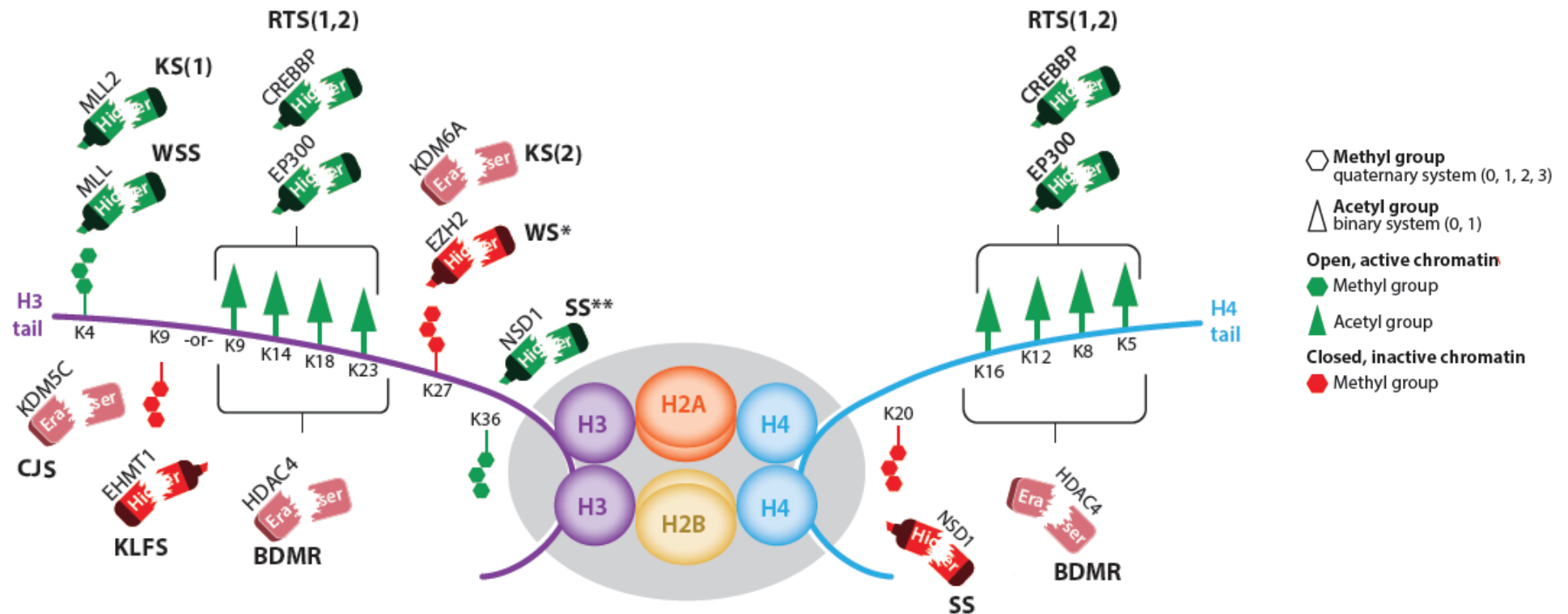
Phone: 410-955-3071  
410-955-0317  
Fax: 410-614-1733  
epigenetics@jhmi.edu

Visit our website:  
<https://igm.jhmi.edu/ecc-clinic>

## Ideas:

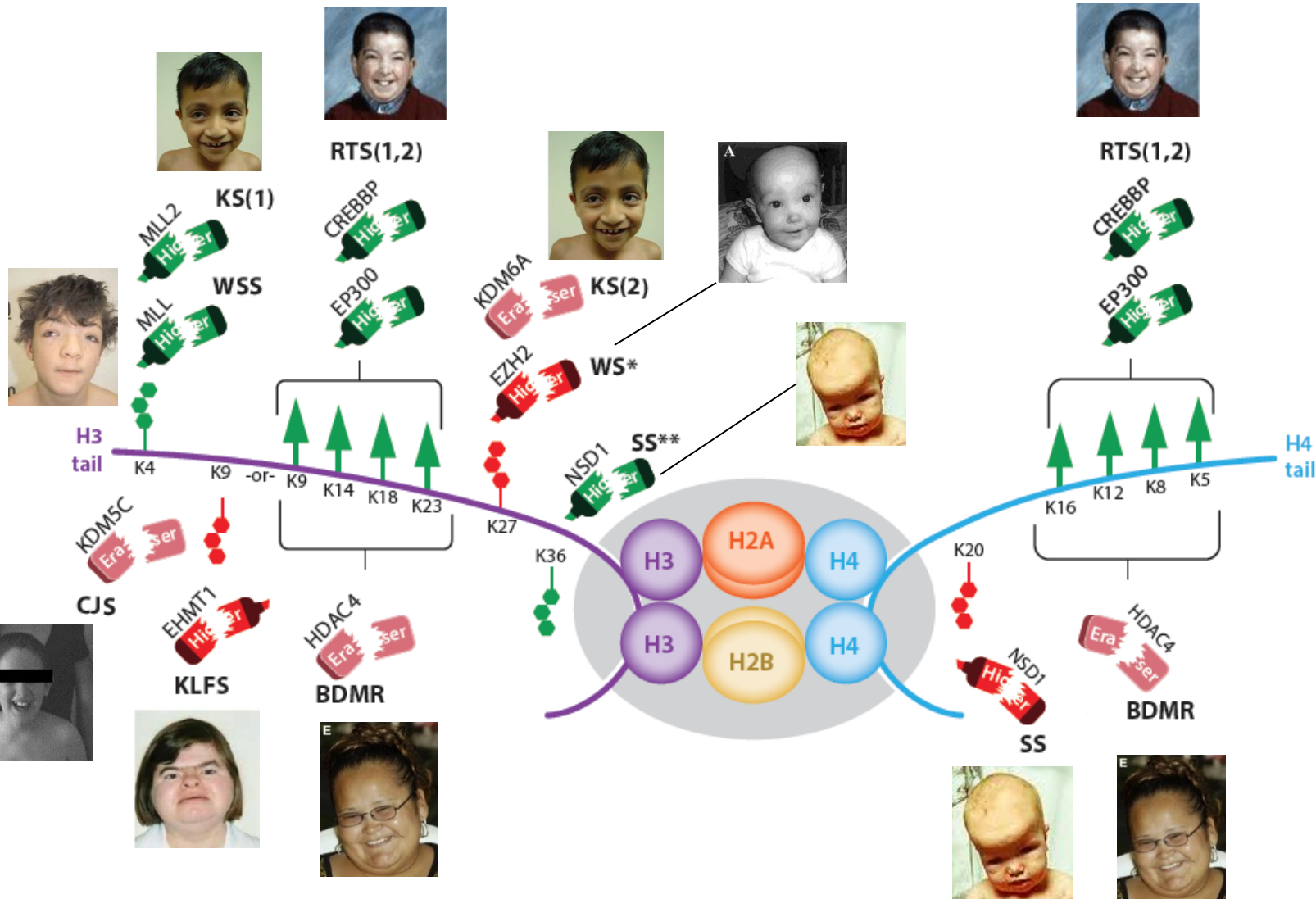
1. Build expertise;
2. Learn from patients and families;
3. Educate health professional about Kabuki syndrome and related disorders

# An ever growing number of dysfunctional histone enzymes





# An emerging cause of intellectual disability



All thought to be caused by haplo-insufficiency!

Baylor: WES with ID/DD 19% had mutation in histone machinery

## Summary (3):

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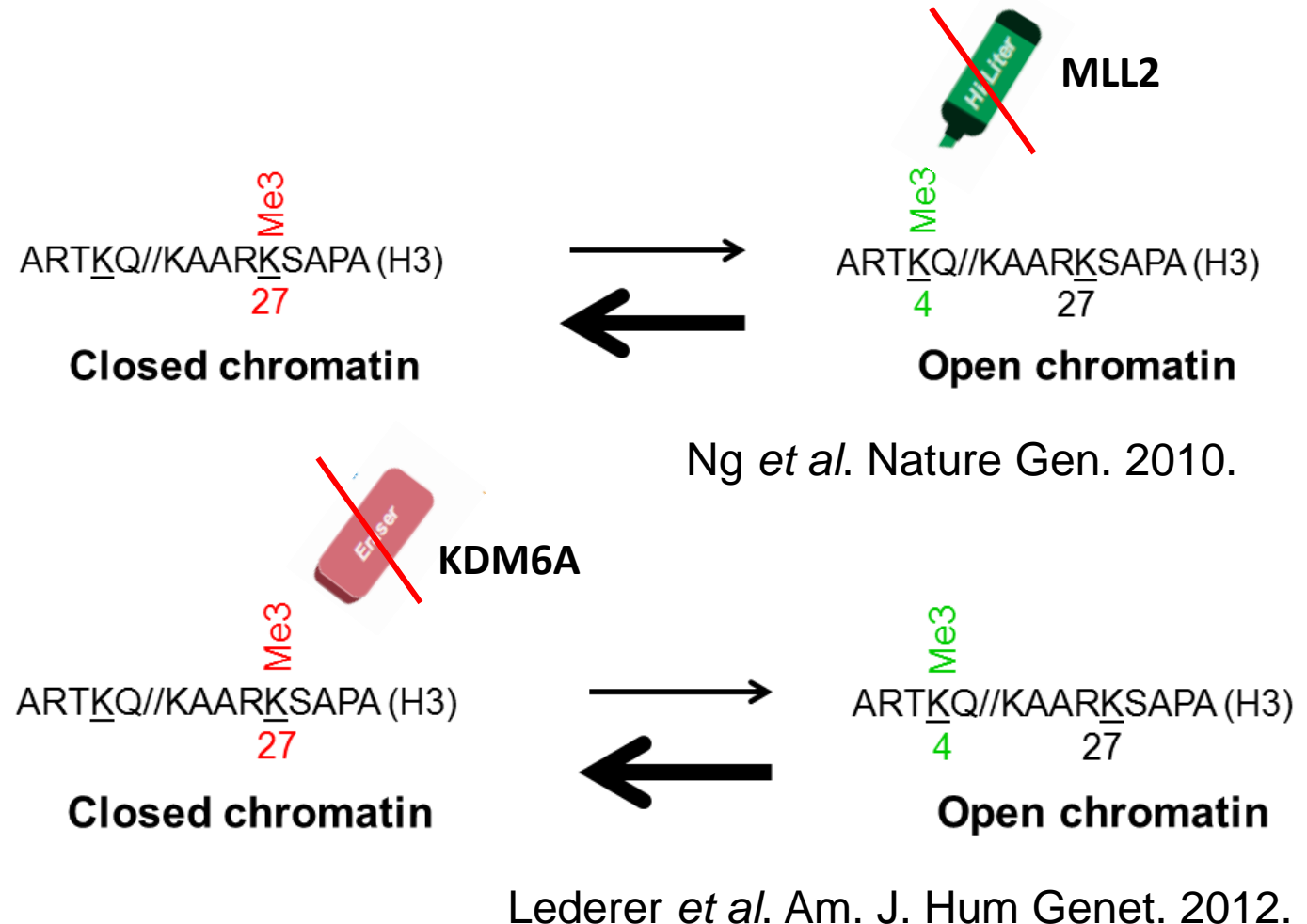
- Deficiencies of histone writers and erasers are Mendelian disorders with epigenetic consequences;
- Even though these disorders all involve enzymes, they uniformly are caused by the loss of a single allele;
- Despite known redundancy of the histone machinery, other components with overlapping function are not able to compensate for the loss of a single allele, indicating tight regulation of the levels of these enzymes and the marks they affect.

# Kabuki syndrome: An imbalance of open and closed chromatin?

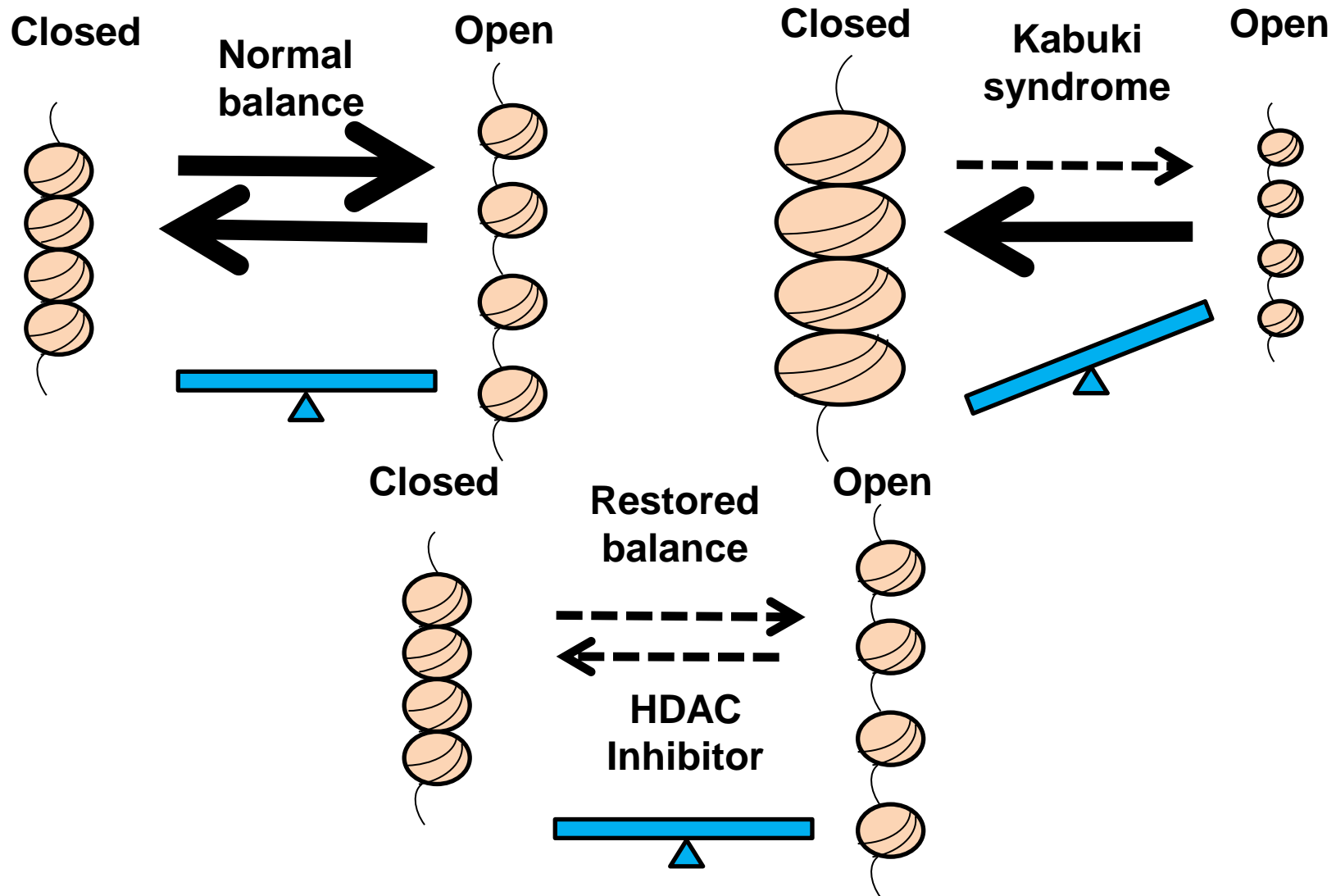
Disorder of histone methylation
Autosomal dominant/ X linked (escapes)
Mutations in <i>MLL2</i> or <i>KDM6A</i> ( <i>UTX</i> )
Variable intellectual disability, postnatal growth retardation
1/30,000



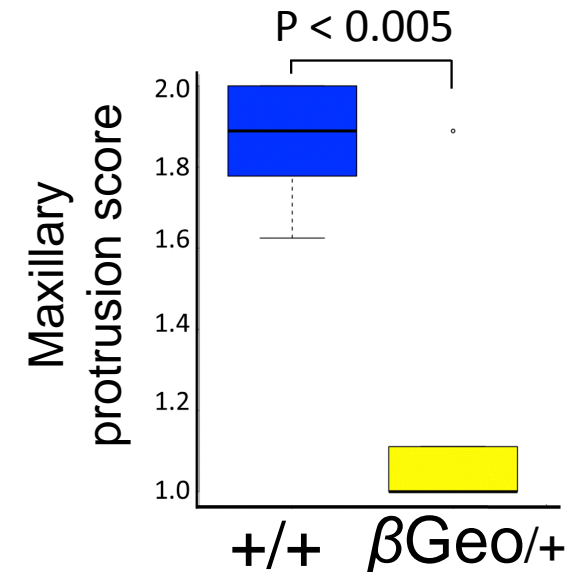
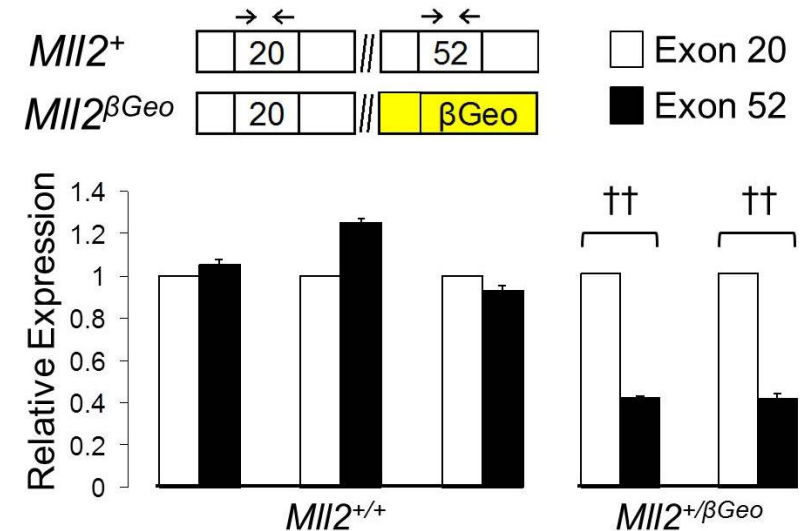
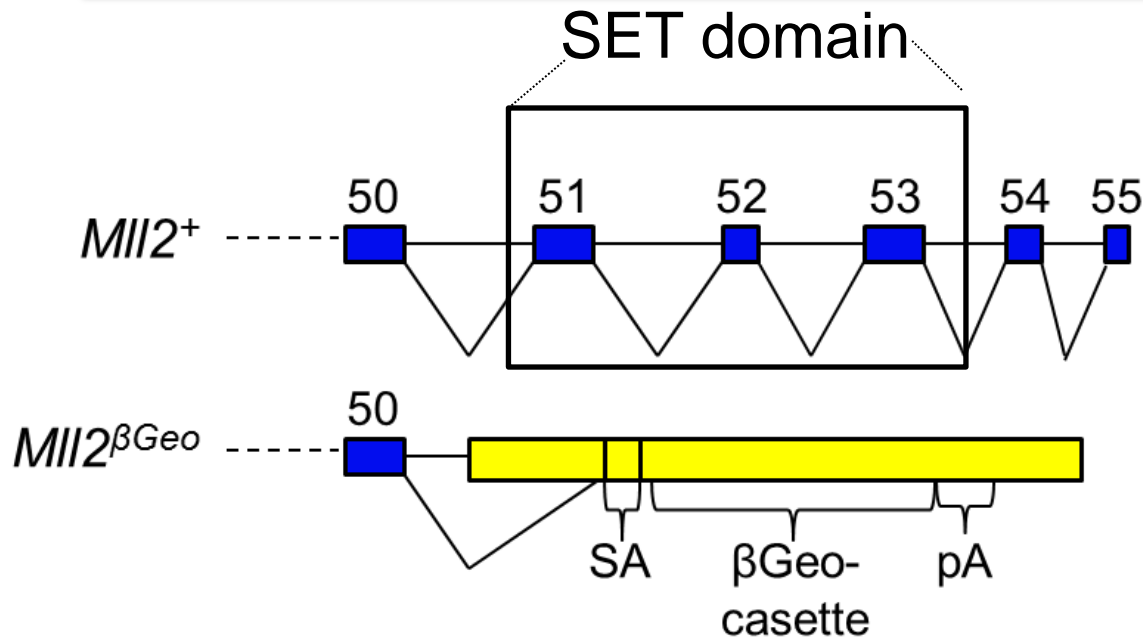
Adam MP *et al.* Clin. Genet. 2005



# Kabuki syndrome (KS): A treatable cause of intellectual disability?

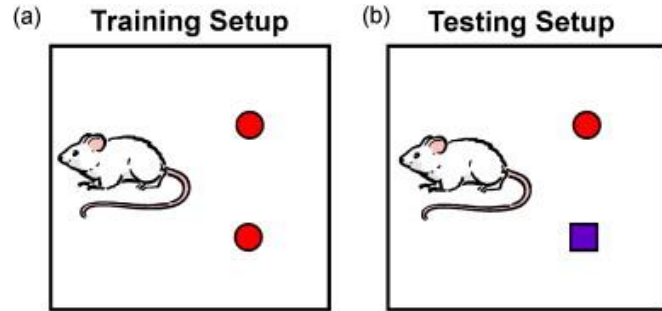


# $Mll2^{+/\beta Geo}$ mice: Knock-out of the SET methyltransferase domain from $Mll2$

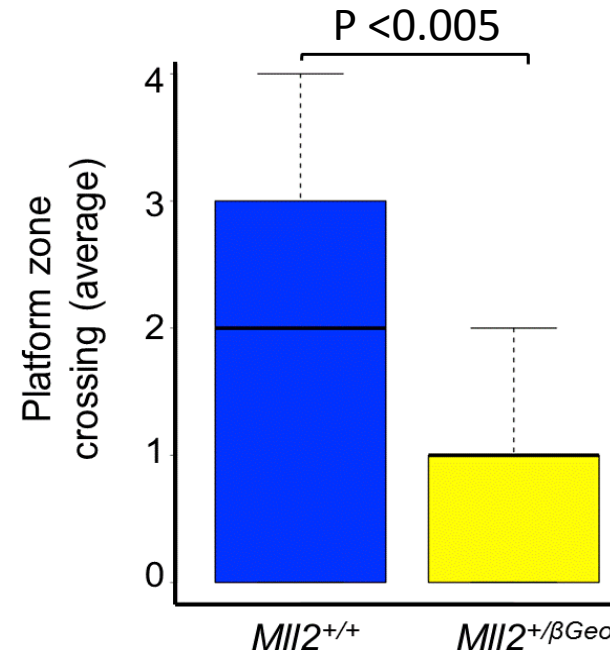
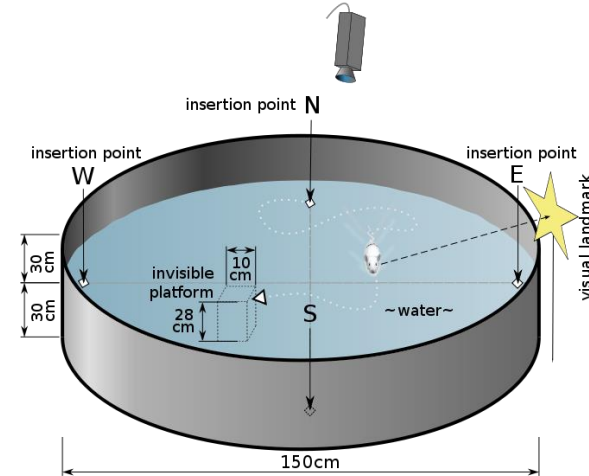
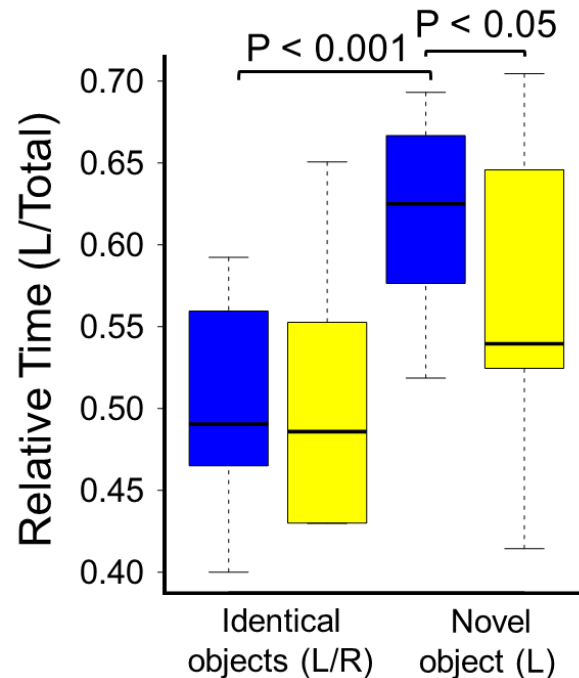




# $Mii2^{+/\beta Geo}$ mice: hippocampal memory defects in NOR and MWM testing



Taglialatela et al. Behav. Brain. Res. 2009

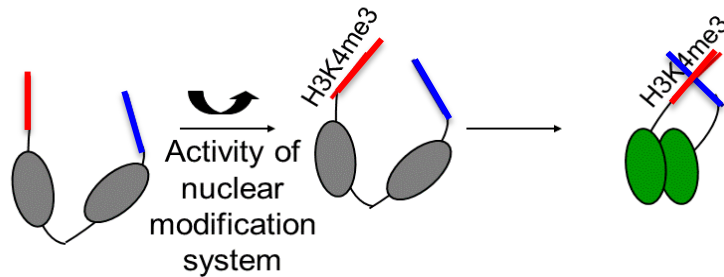


# $Mll2^{+/\beta Geo}$ mice: decreased H3K4me3 activity

H3K4me3 indicator

H3 (40 AA)	C-EGFP	L	N-EGFP	TAFIII BD	3xNLS
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/K4, K9, K27/



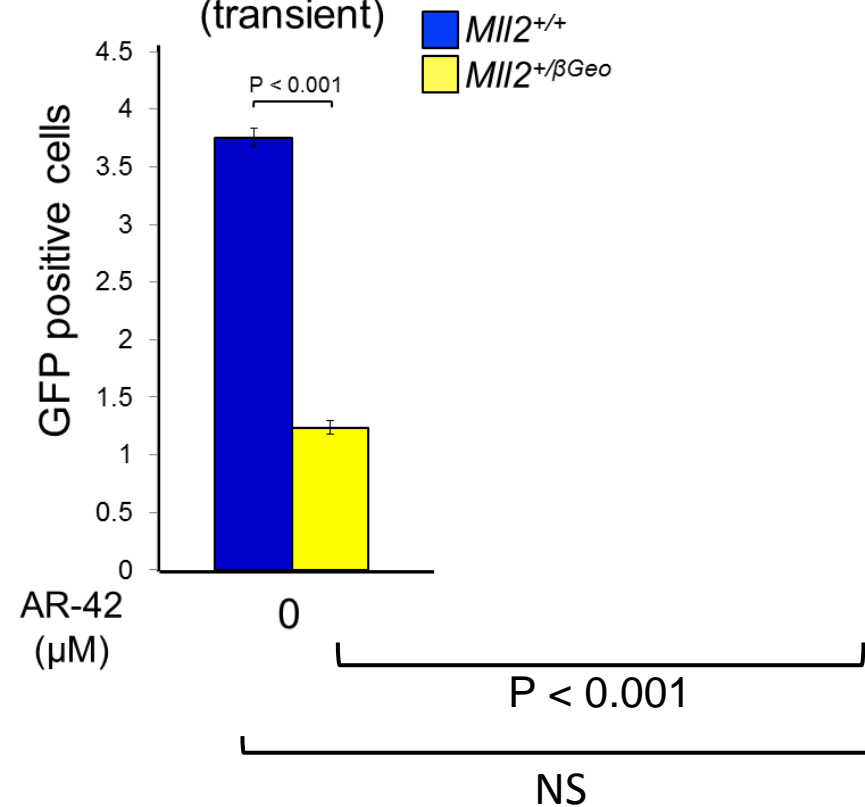
Histone tail

Histone reader

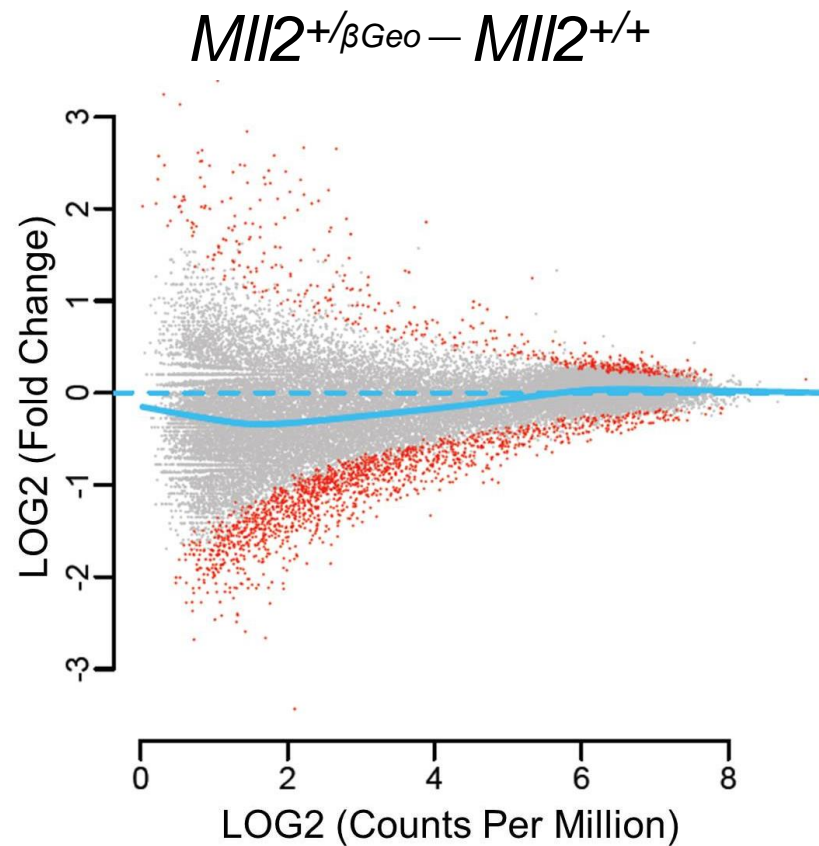
GFP subunits

Reconstituted GFP with fluorescence

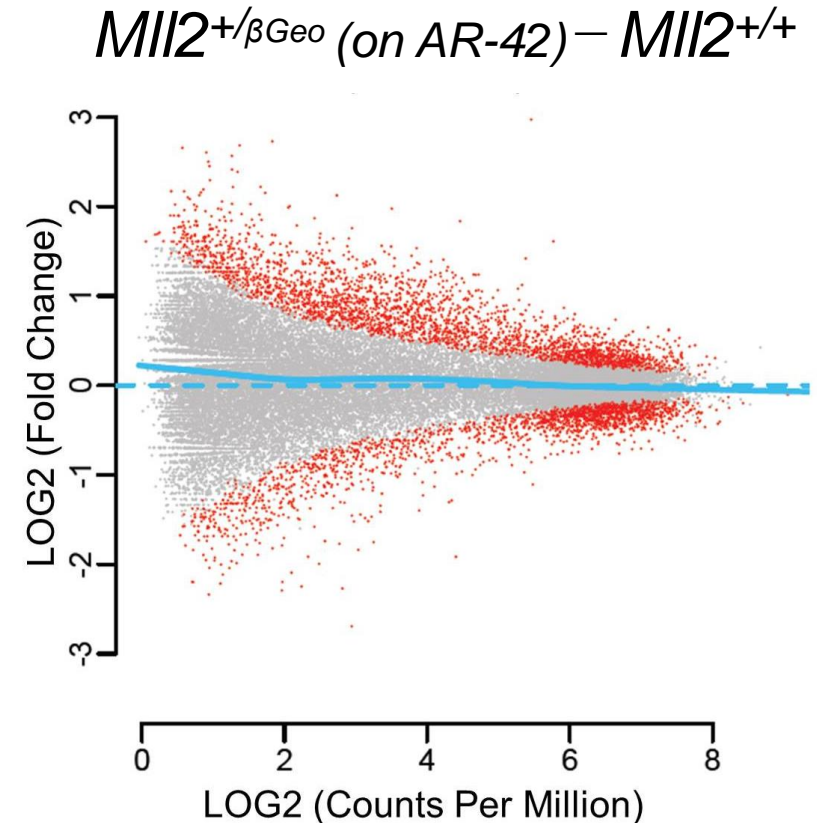
H3K4 Methylation Indicator,  
Mouse embryonic fibroblasts  
(transient)



# H3K4me3 deficiency is improved *in vivo* with HDACi AR-42



No drug



AR-42

## Summary (4):

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- The two types of Kabuki syndrome suggest that an imbalance between open and closed chromatin states may play a role;
- Our mouse model of Kabuki syndrome has overlapping phenotypic features with patients with Kabuki syndrome as well as hippocampal memory defects, a phenotype that can be monitored during therapeutic trials.
- *Mll2*<sup>+/ $\beta$ Geo</sup> mice have a deficiency of both H3K4me3 activity and genome-wide H3K4me3 levels and either can be manipulated with histone deacetylase inhibition;

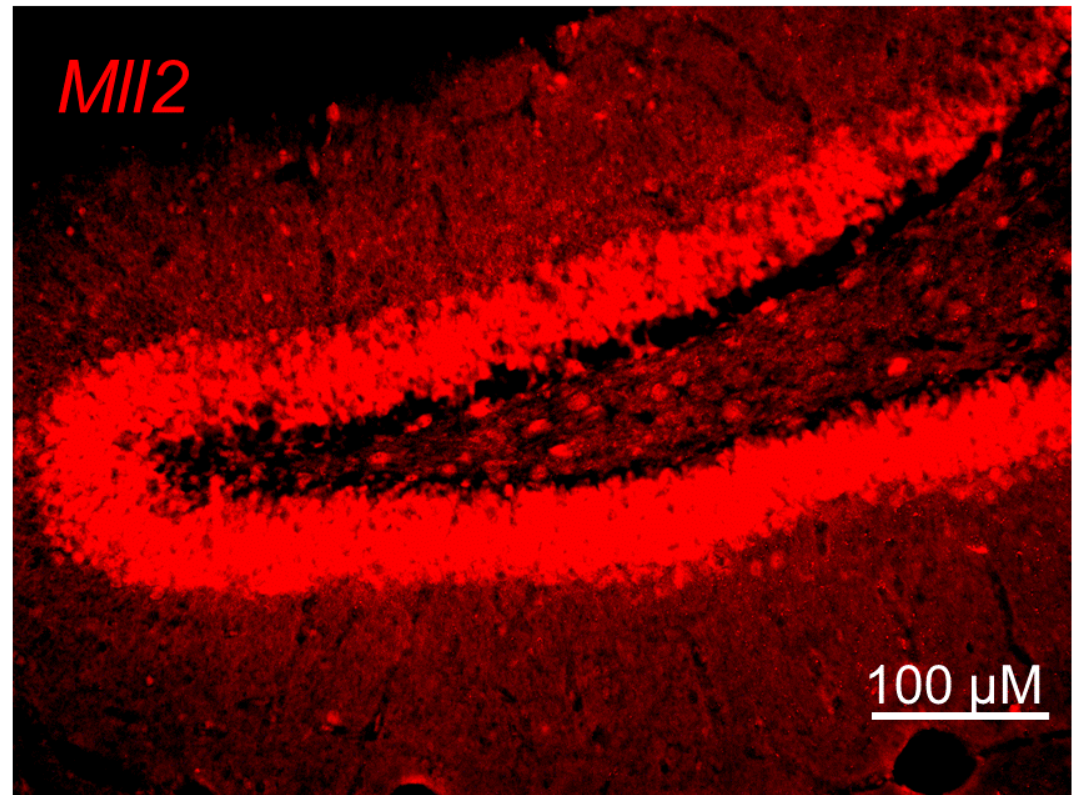
# *Mll2* is highly expressed in Granule Cell Layer (GCL) of the dentate gyrus

Dentate gyrus is part of hippocampus;

Strongest expression in Granule Cell Layer (GCL);

Adult neurogenesis occurs in subgranular zone (SGZ);

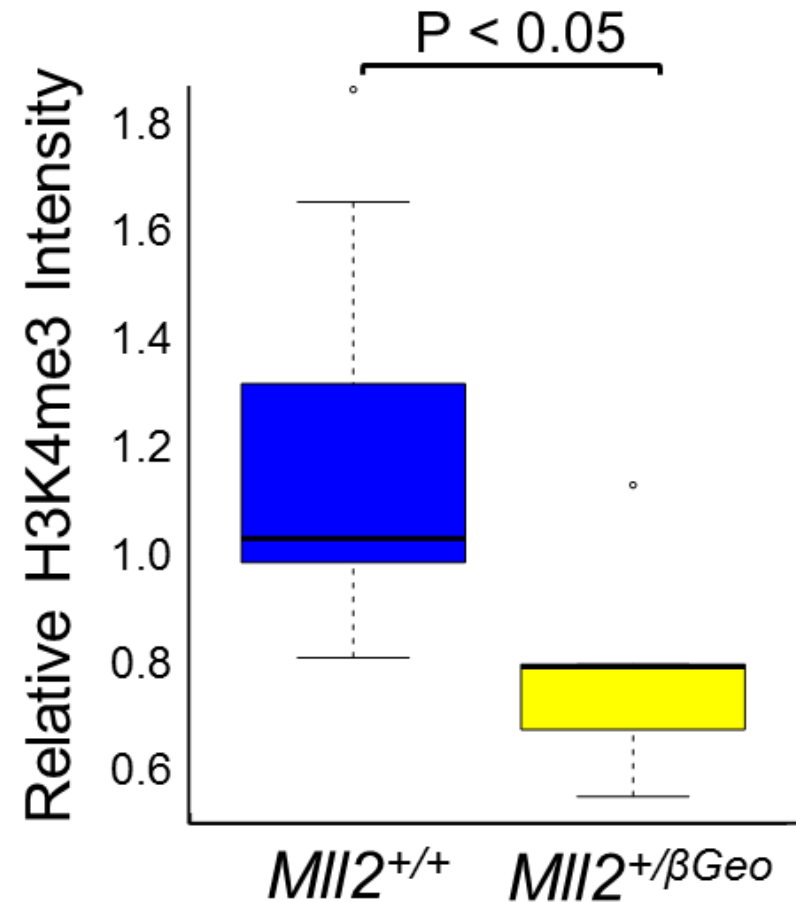
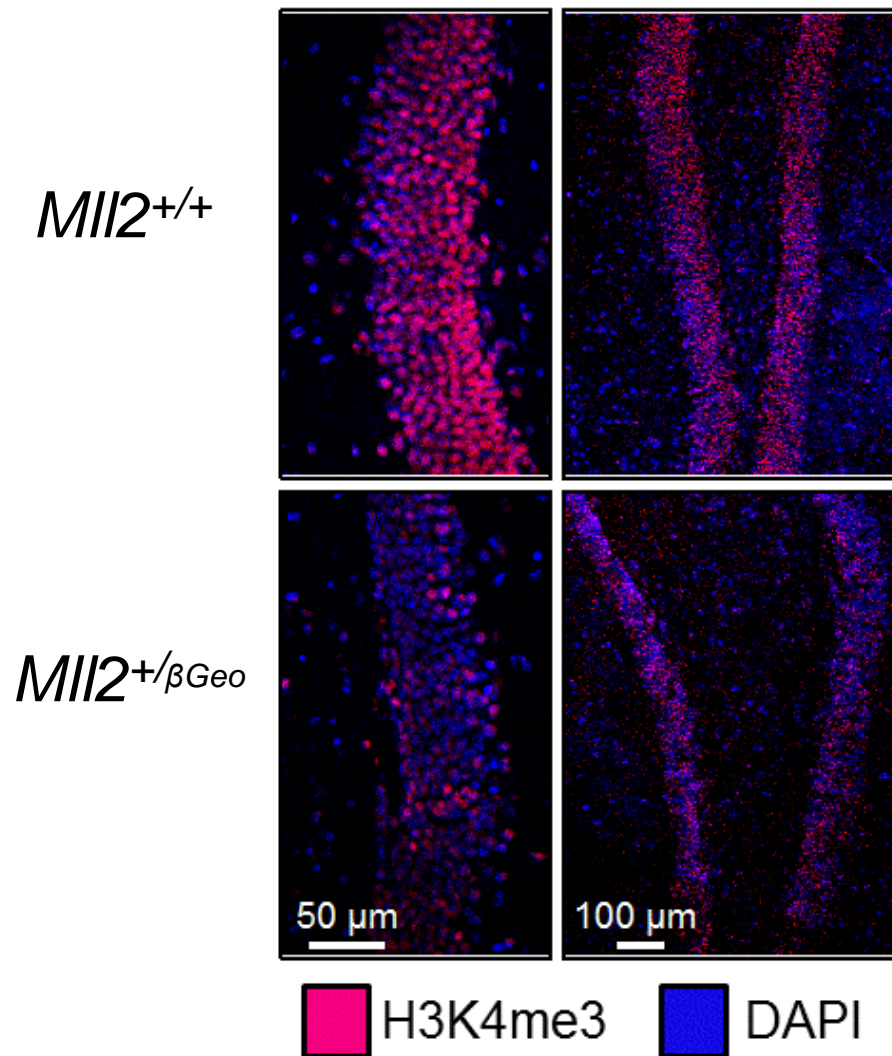
Defects of adult neurogenesis lead to hippocampal memory defects.



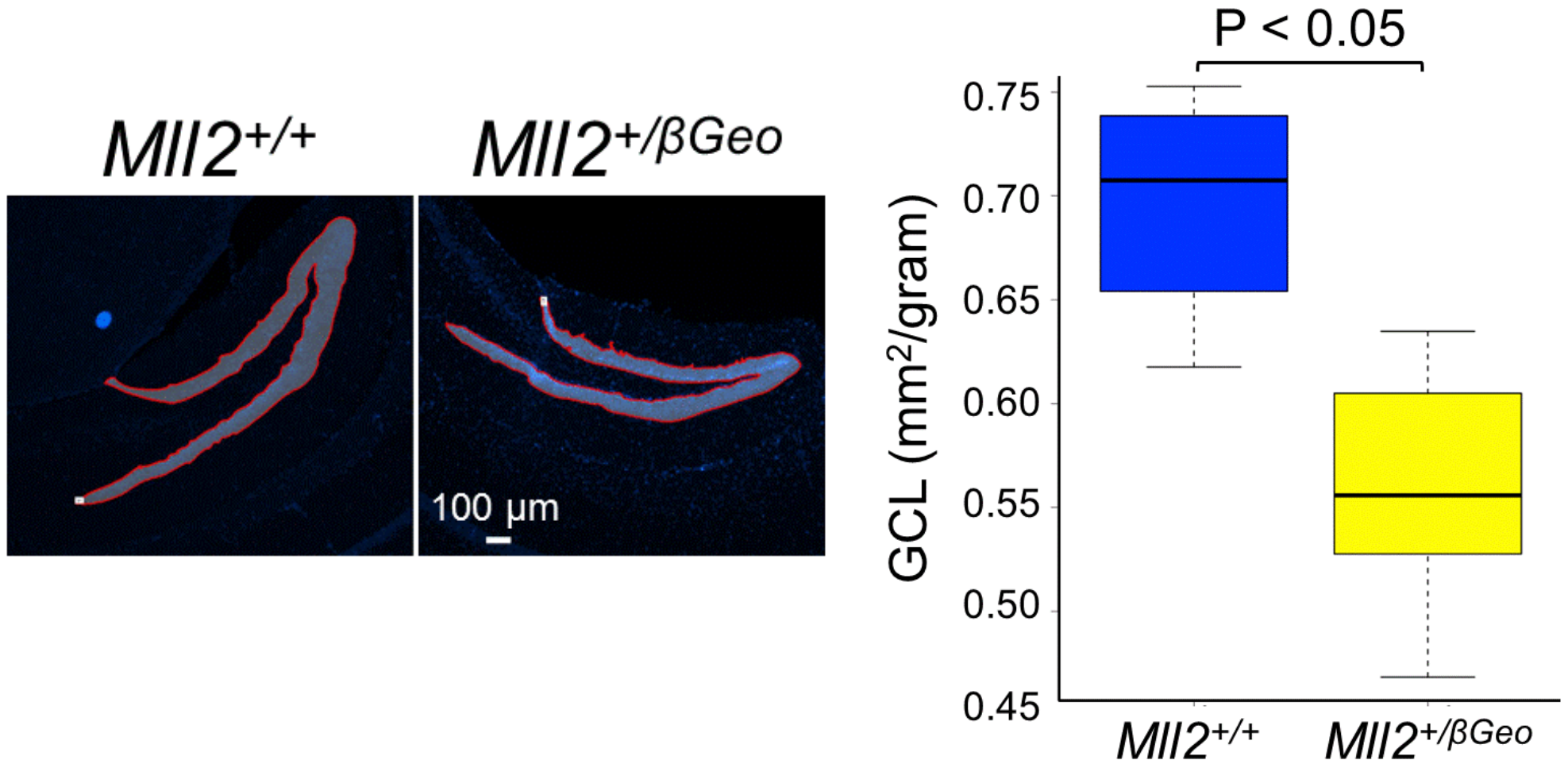
Immunofluorescence against *Mll2* ( WT)



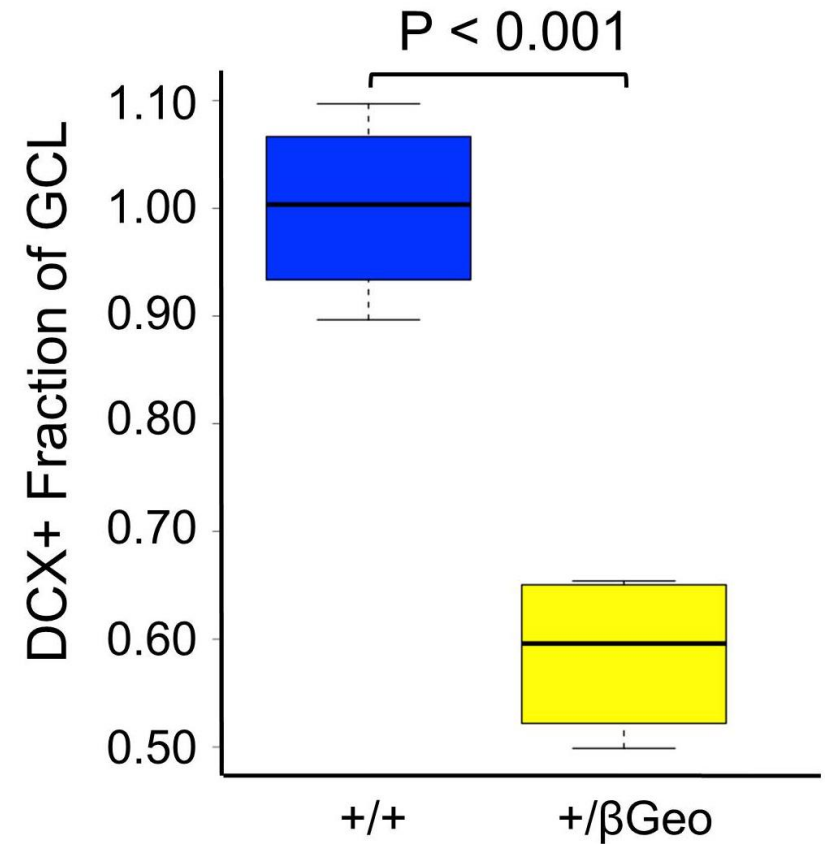
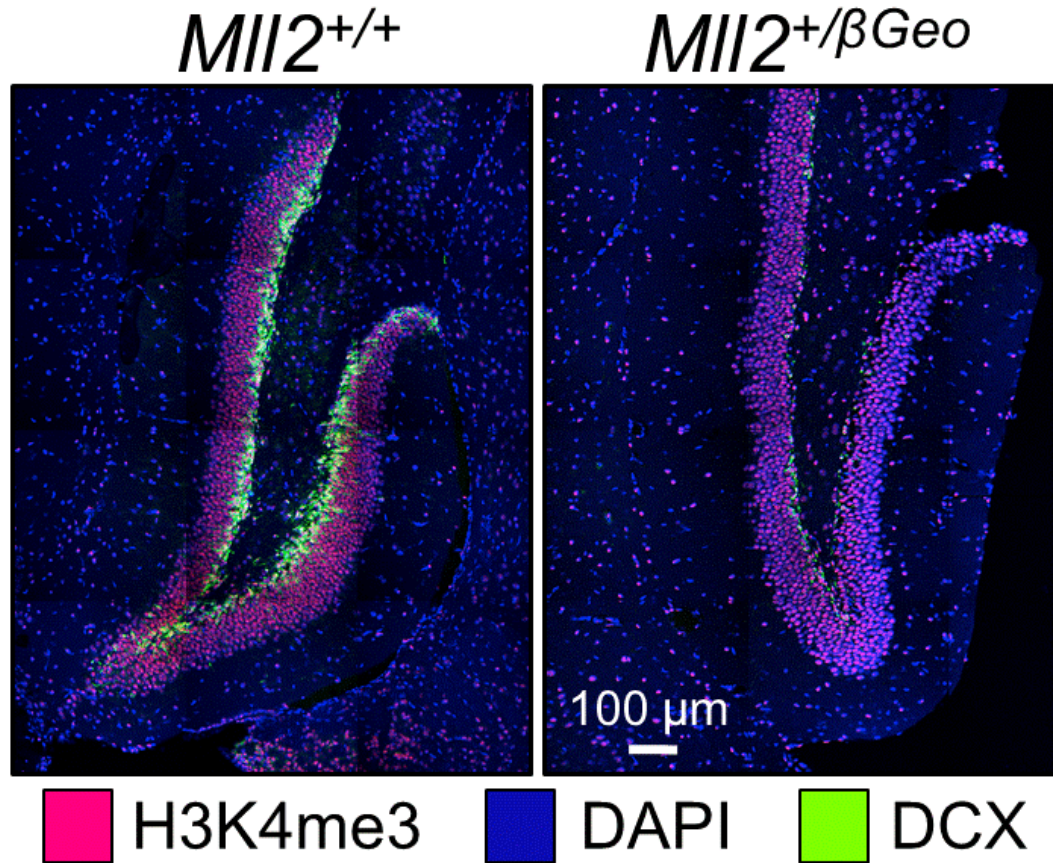
# $MII2^{+/\beta Geo}$ mice have decreased H3K4me3 in the GCL of the dentate gyrus



# $MII2^{+/\beta Geo}$ mice have a thinner GCL layer

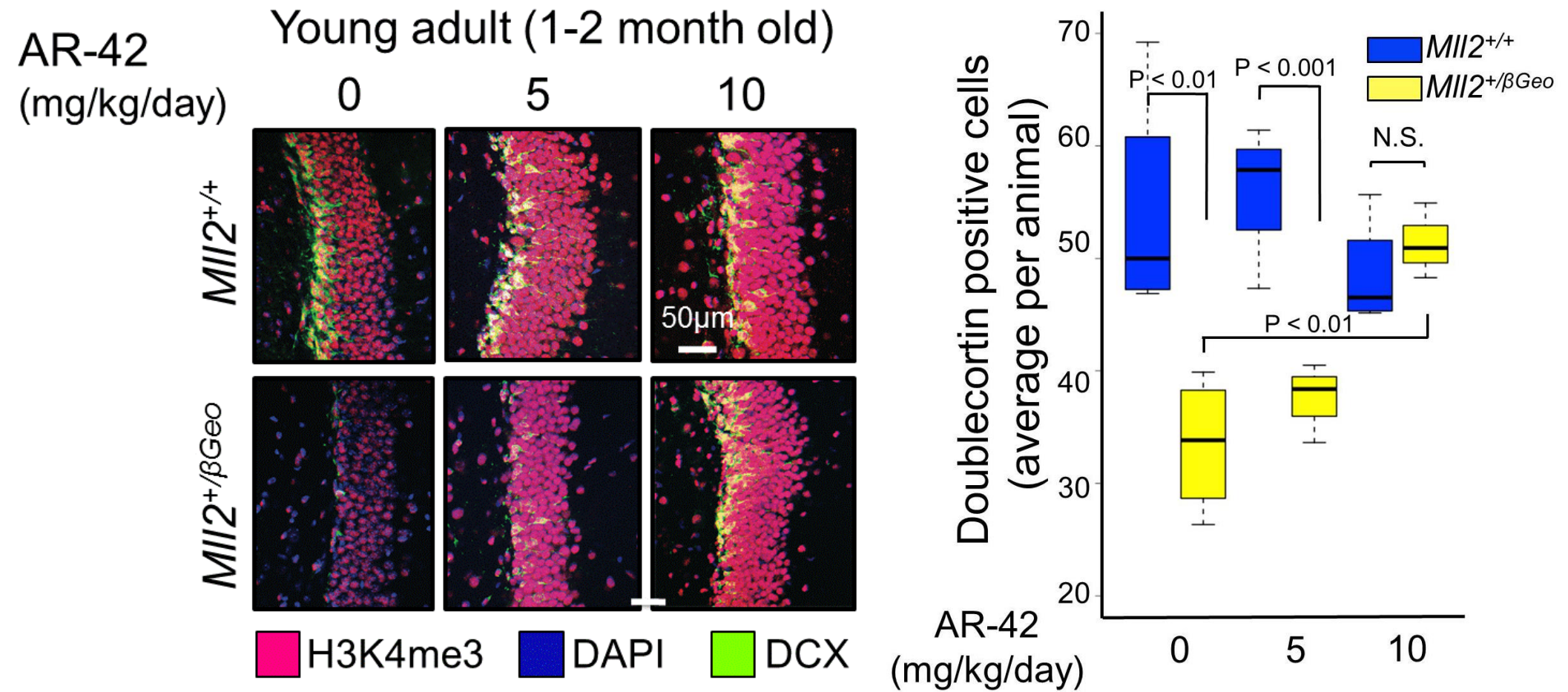


# $Mll2^{+/\beta Geo}$ mice have a deficiency of neurogenesis



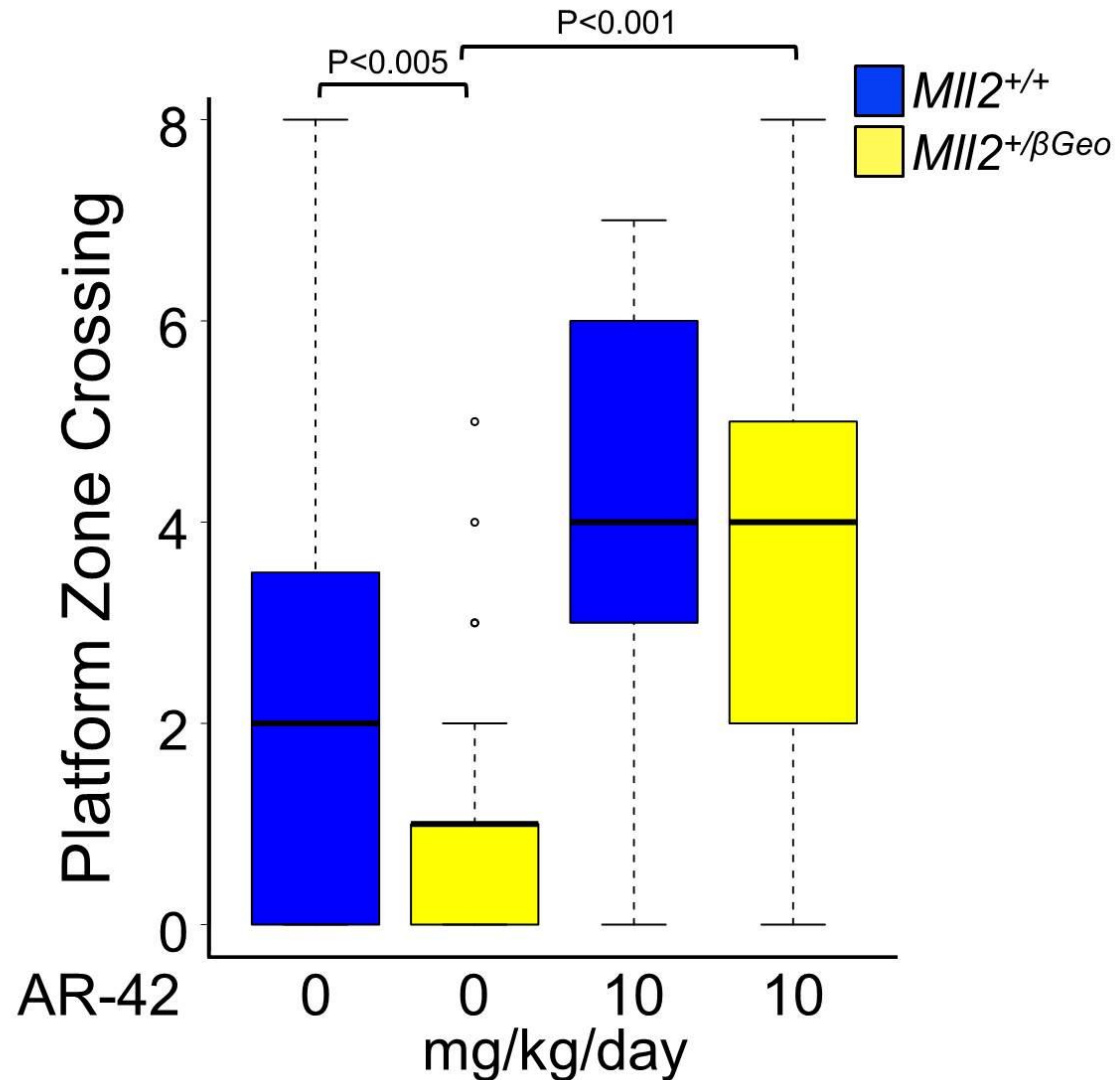


# Impaired neurogenesis in $Mll2^{+/\beta Geo}$ mice is improved with 2 weeks of HDAC inhibitor AR-42



Five month old cohort

# Hippocampal memory defect in $Mll2^{+/\beta Geo}$ mice is improved with 2 weeks of HDAC inhibitor AR-42



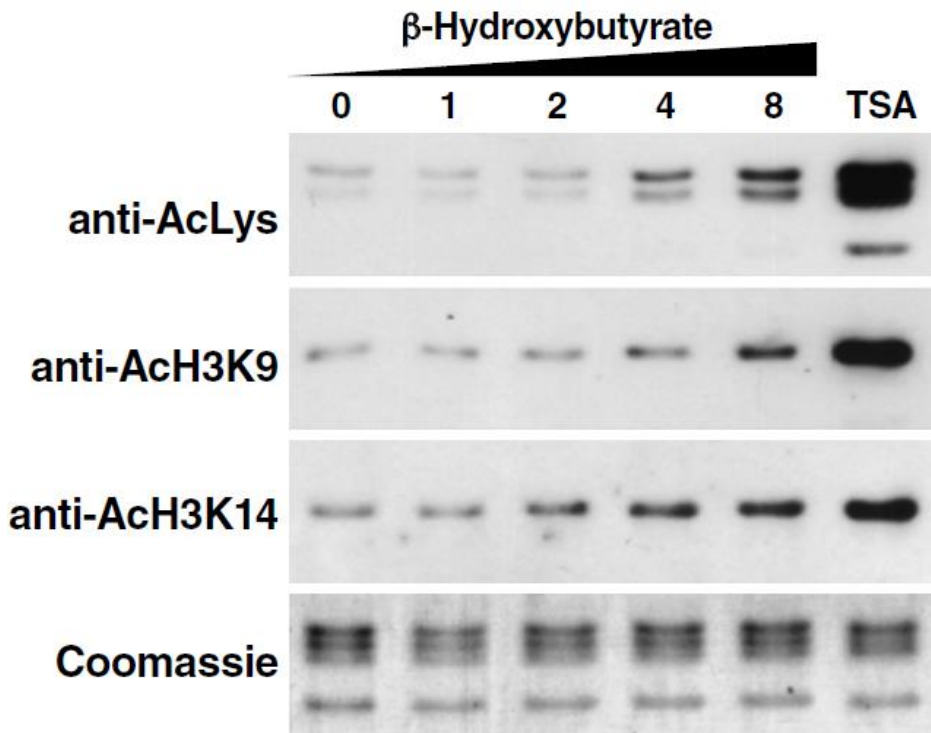
## Summary (5):

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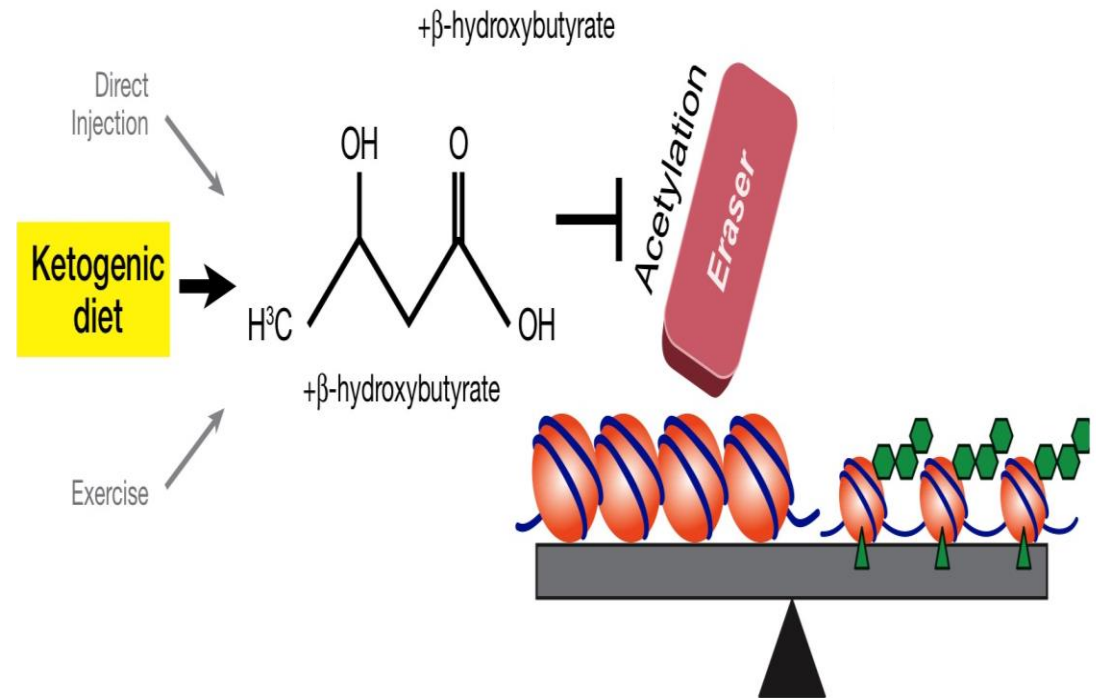
- A mouse model of KS reveals defective H3K4me3 in the GCL layer of dentate gyrus, thinning of this cell layer caused by impaired neurogenesis;
- Defects in the dentate gyrus can be reversed using drugs that target the epigenetic machinery, suggesting that the intellectual disability seen in KS (and perhaps other disorders of epigenome homeostasis) may be treatable;

Moving a cancer therapeutic drug to kids with ID will be a challenge. Alternative options?

# Beta-hydroxybutyrate as a therapeutic agent for Kabuki syndrome?



Shimazu et al. Science. 2013



Ketogenic used for many therapeutic applications including seizures

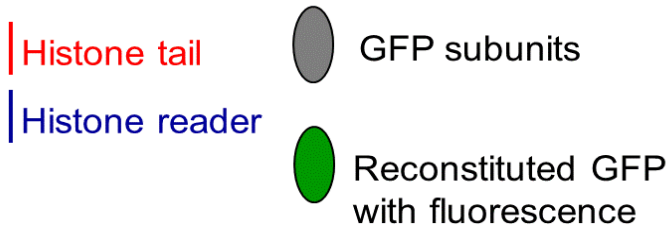
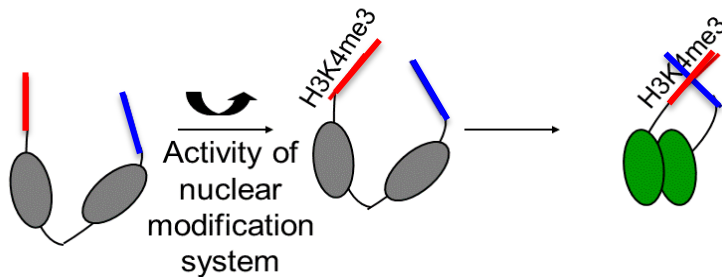


# *In vitro* beta-hydroxybutyrate increases histone acetylation (less potent than AR-42)

H4Ac indicator



K5, K8, K12, K16, K20



# Mice tolerate a modified ketogenic diet for months without any ill effect

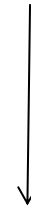
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- Ingredients : Lard, Butter, Corn Oil, Casein, Cellulose, Mineral Mix, Vitamin Mix, Dextrose (trace)



**Ketogenic Diet AIN-76A-Modified, High Fat, Paste**

6:1 fat to protein ratio

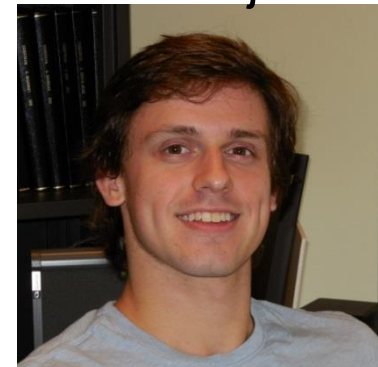


4:1 fat to protein ratio

(Dr. Hilary Vernon)

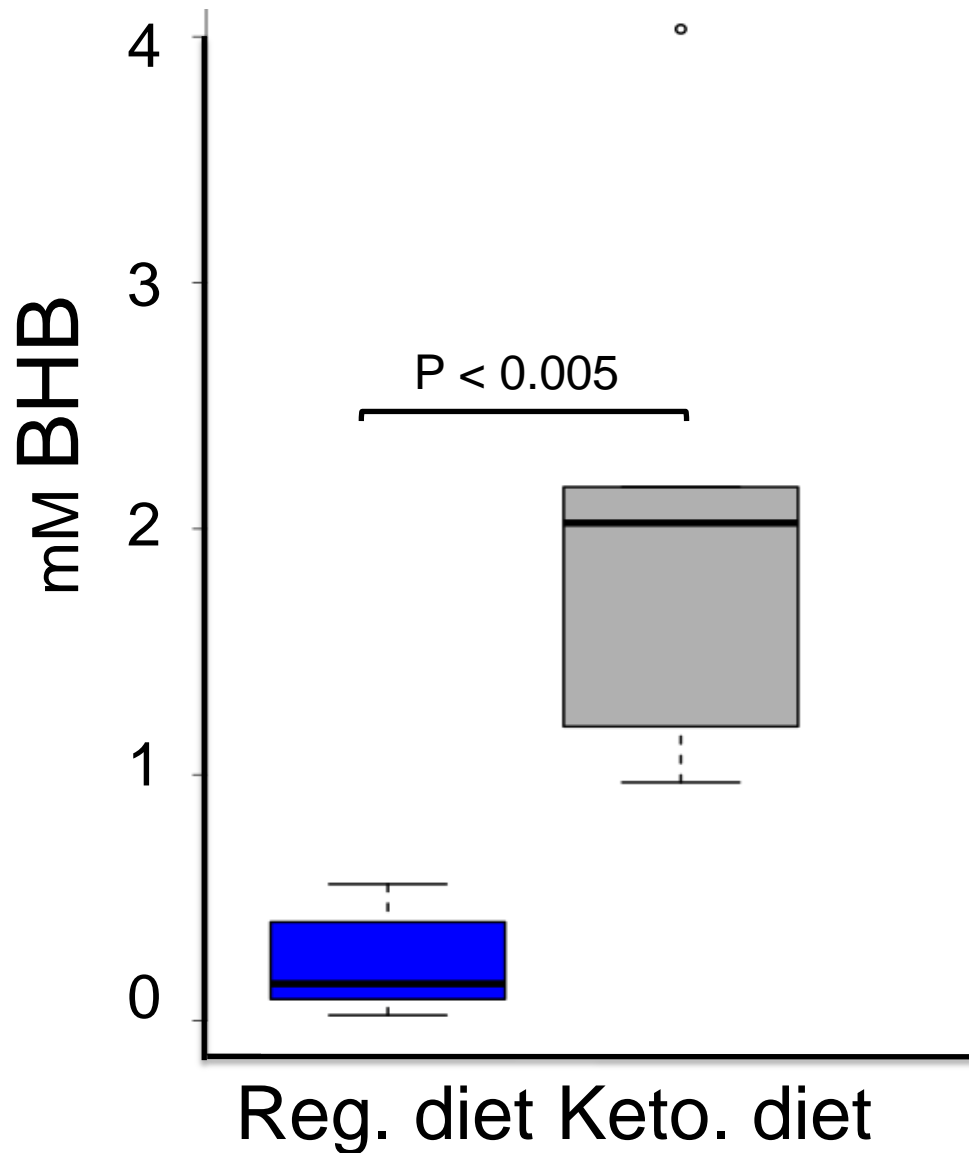


**Joel Benjamin**

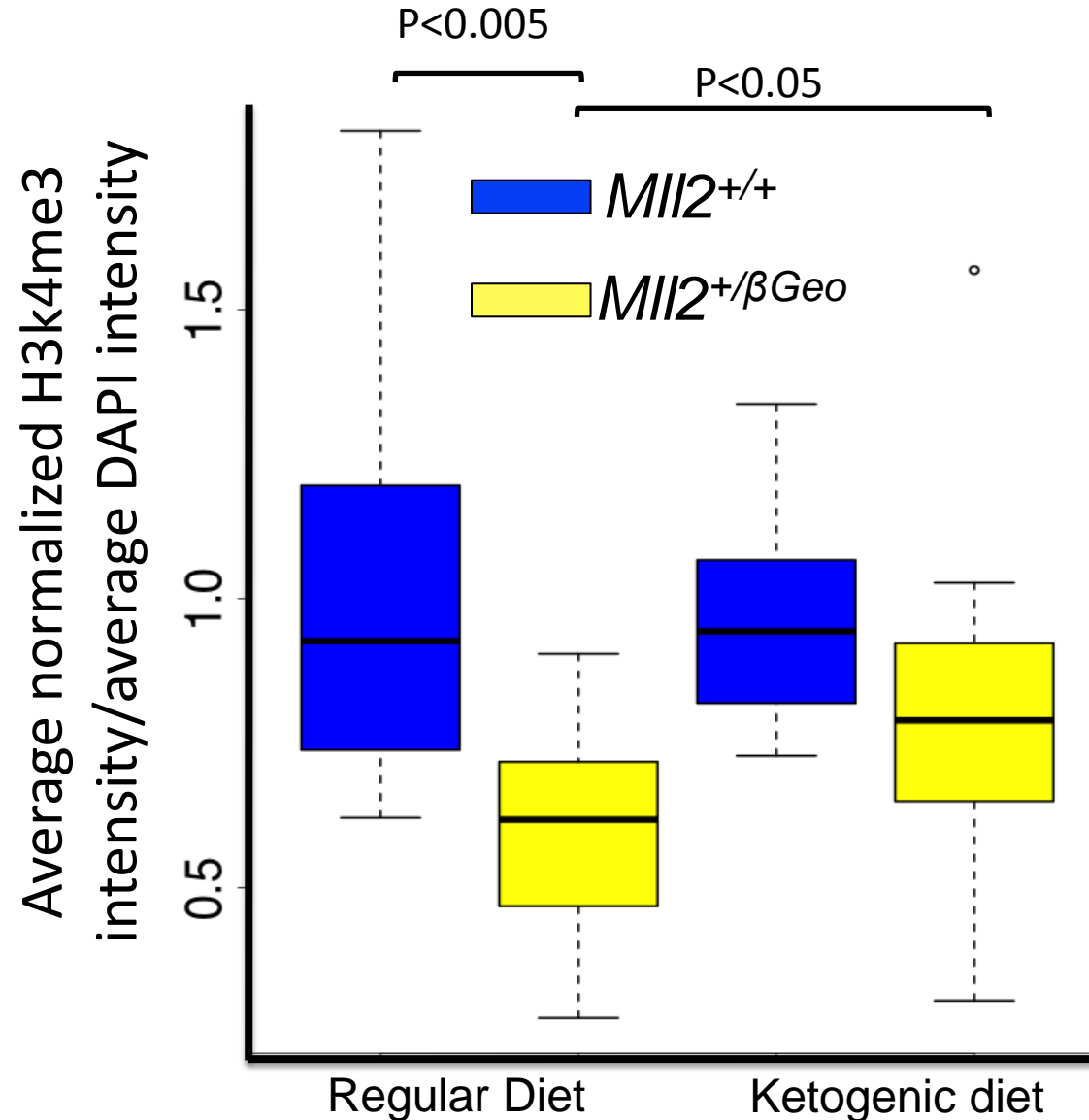
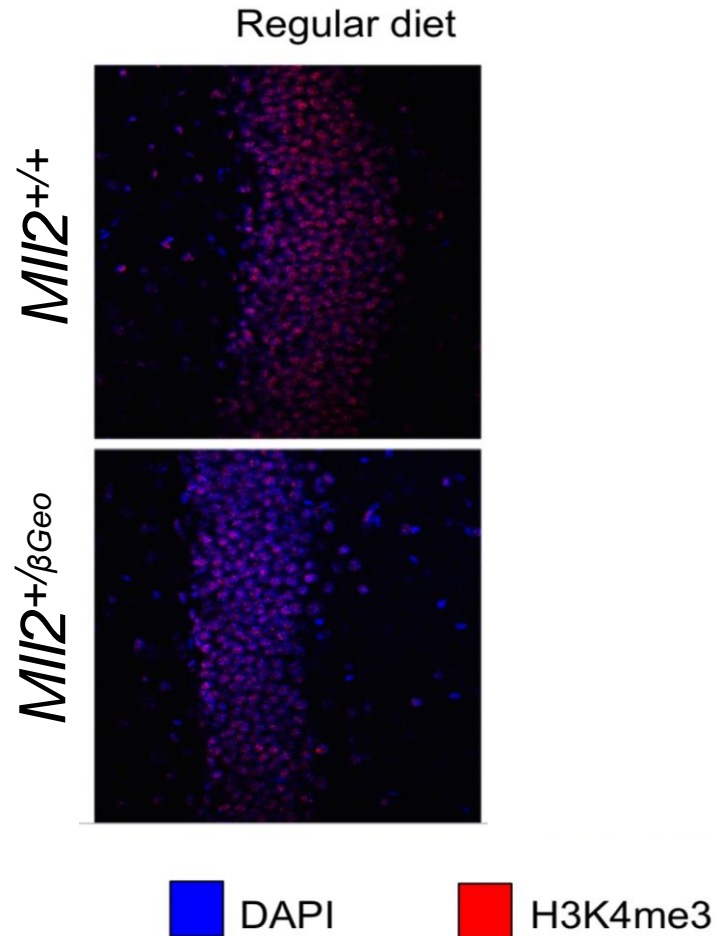


**Giovanni Carosso**

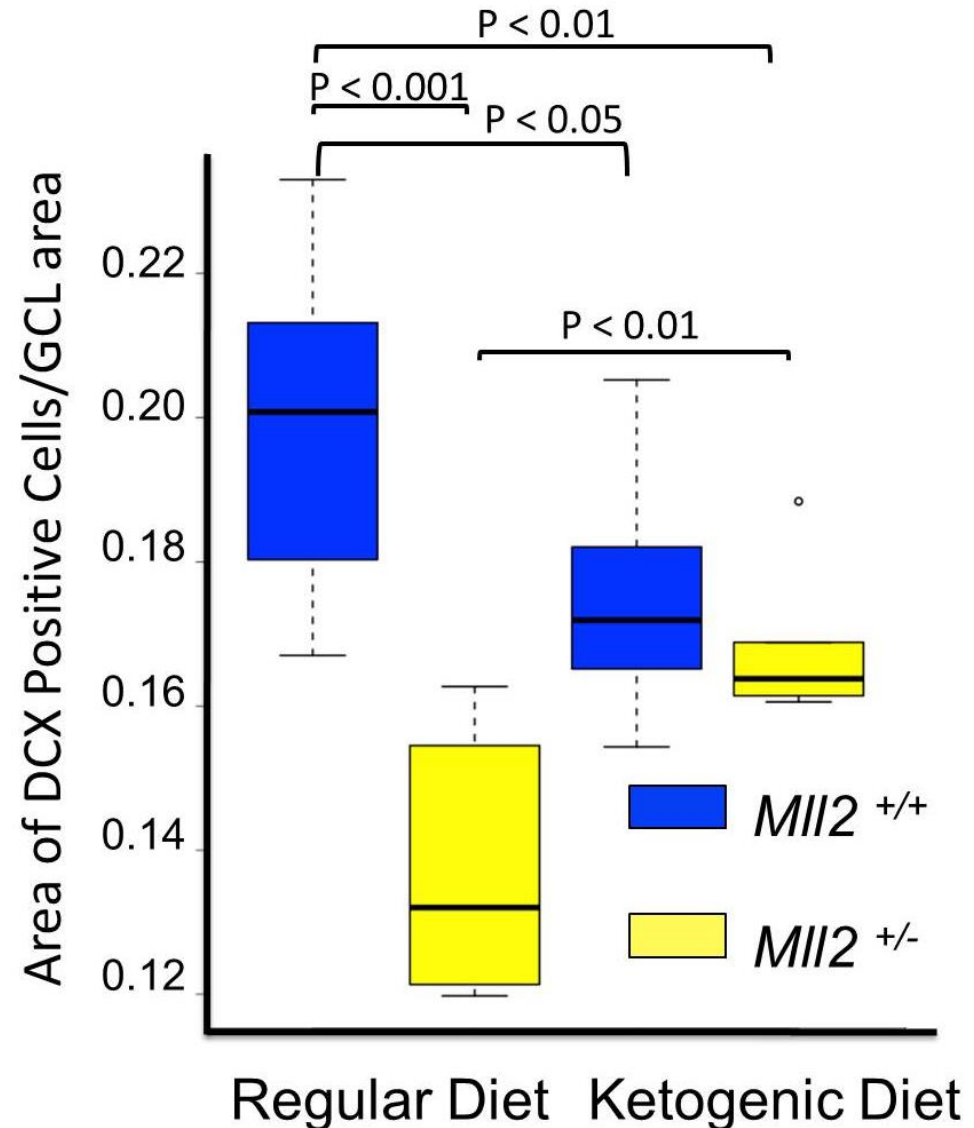
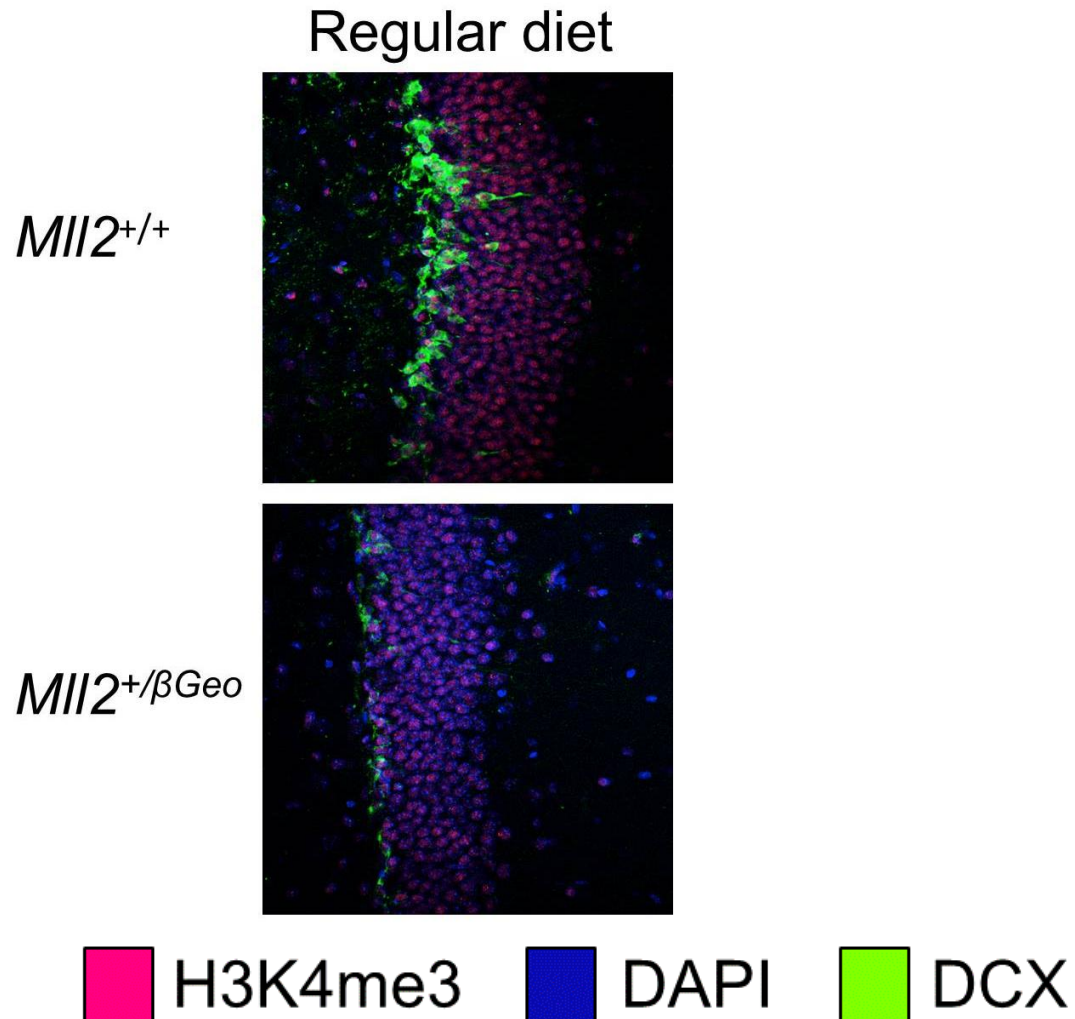
# BHB increases on a ketogenic diet in both $Mll2^{+/+}$ and $Mll2^{+/\beta Geo}$ mice



# H3K4me3 deficiency in GCL of dentate gyrus improves on a ketogenic diet



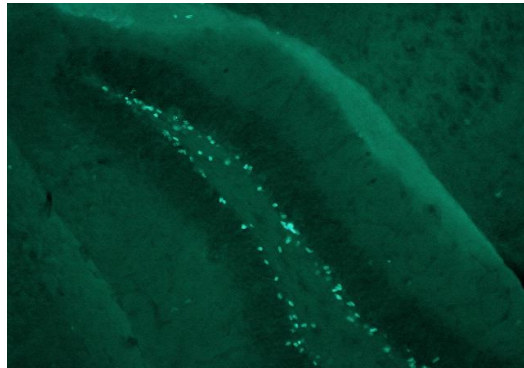
# A deficiency of neurogenesis seen in $Mll2^{+/\beta Geo}$ mice improves on ketogenic diet



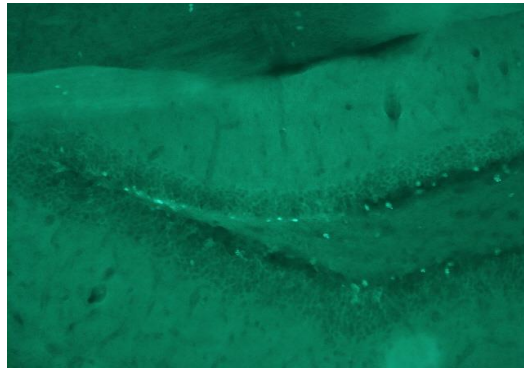
# A deficiency of neurogenesis seen in $Mll2^{+/\beta Geo}$ mice improves on ketogenic diet

Reg.  
Diet

$Mll2^{+/+}$

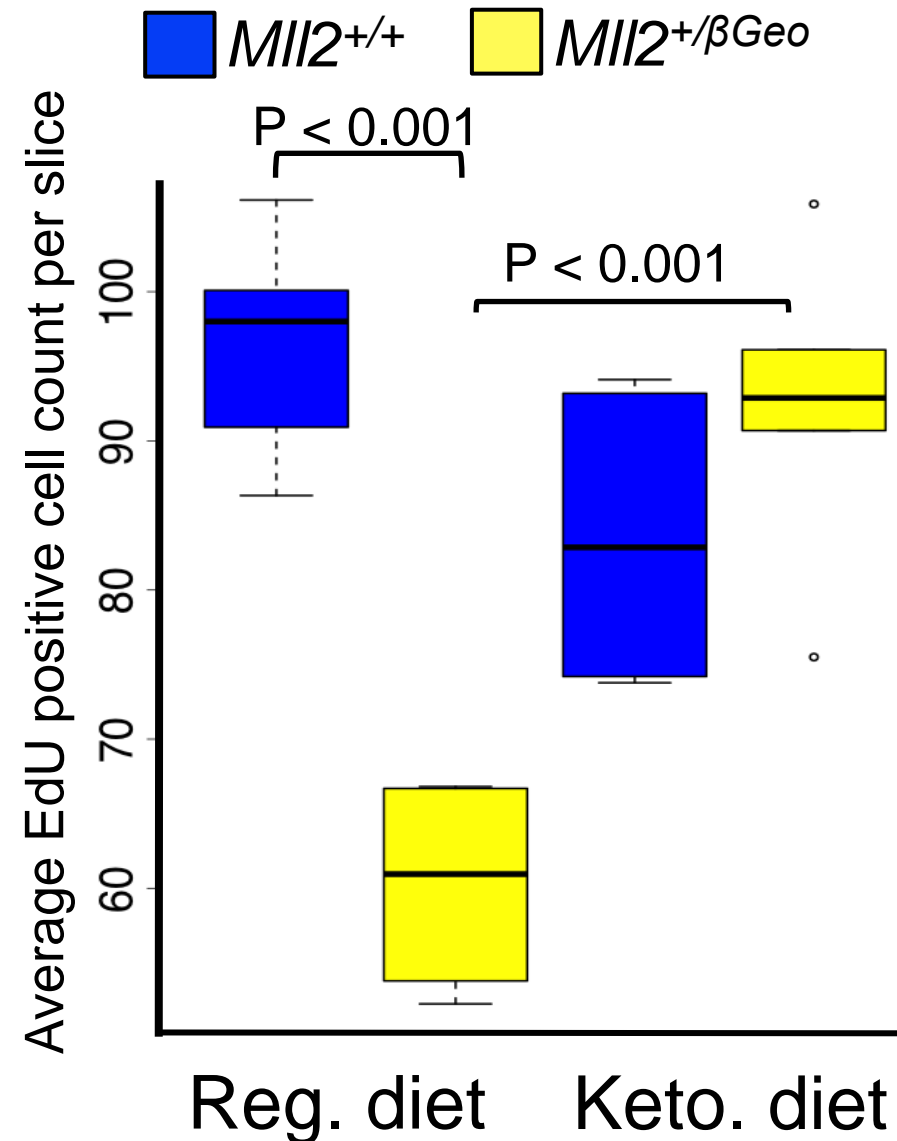


$Mll2^{+/\beta Geo}$

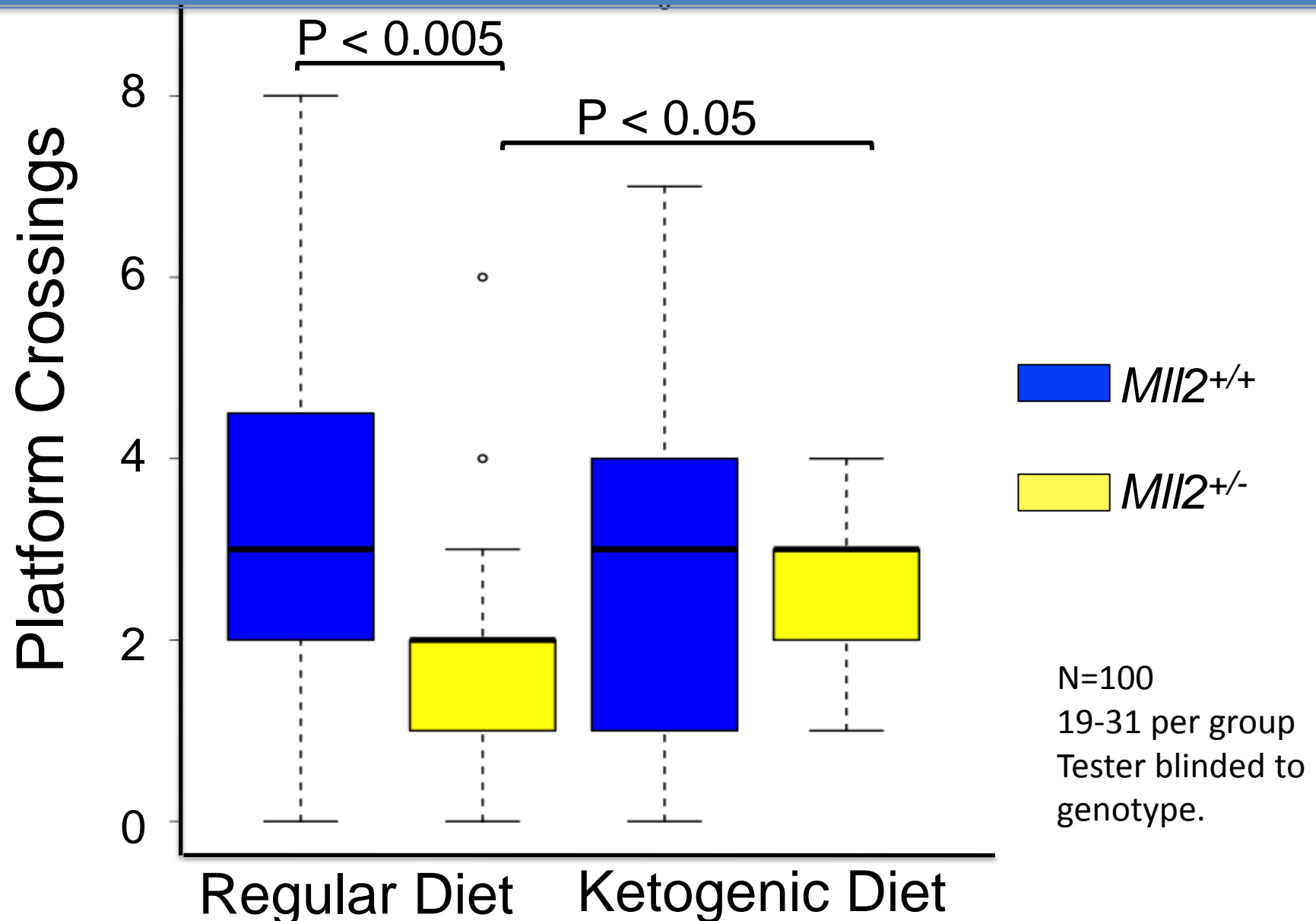


EdU positive cells 

7 day period (proliferation)



# Hippocampal memory defect improves on a ketogenic diet





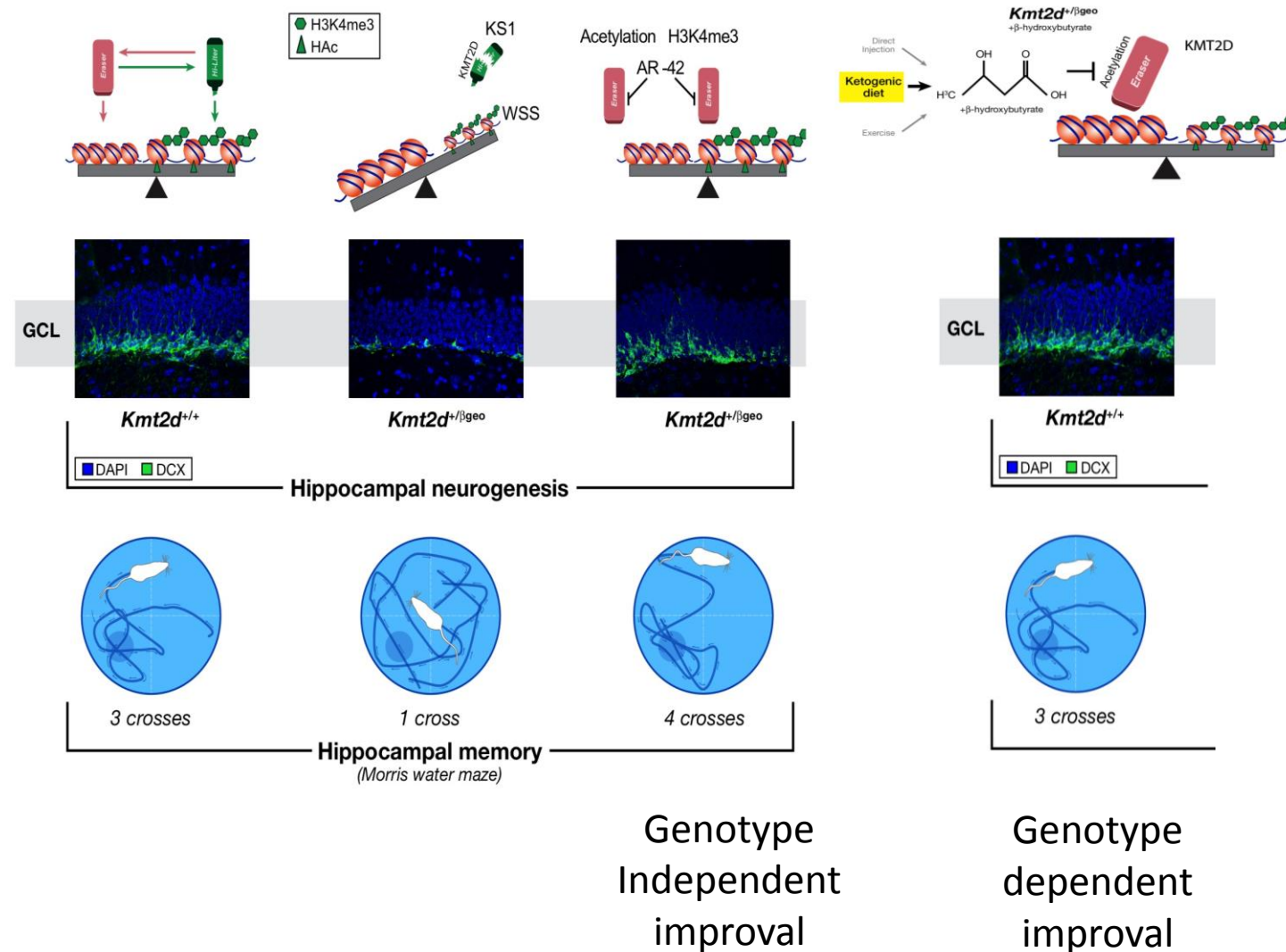
## Summary (6):

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- A modified ketogenic diet can increase beta-hydroxybutyrate in our mice;
- A modified ketogenic diet increases open chromatin marks (H3Ac, H3K4me3) and normalized gene expression in relevant neurons;
- Defects of neurogenesis in dentate gyrus and hippocampal memory defects in mouse model of Kabuki syndrome normalize after 2 weeks of ketogenic diet;

# Is Kabuki syndrome a treatable cause of intellectual disability?

- Proof-of-principle
- AR-42
- MKD
- Neurogenesis



# Q1: Do our findings apply to humans?

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- If the neurogenesis defect in Kabuki syndrome is a major component of the ID one should be able to demonstrate this studying patients (specific tasks linked to dentate gyrus);
- Recruited Jacquie Weissman MD, and Rebecca Vaurio PhD from the KKI. Jacquie is a neurobehavioral fellow who is conducting a prospective study at JHH/KKI to ask what tasks are abnormal in kids with KS (30-40 minutes for test);
- This will also help us understand strengths/weaknesses in general, form a baseline for any clinical trial and perhaps give some general educational recommendations.

## Q2: What could we potentially treat?

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### What we may be possibly able to target with a medical treatment

- Memory defect to some extent (if proven in patients);
- Hypotonia (early in life);
- Immune dysregulation (immunodeficiency, and/or autoimmune phenomena);

### What we will never be able to target with a medical treatment

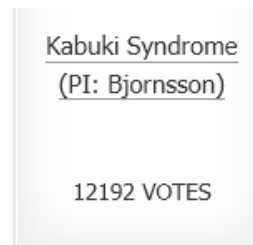
- Anatomical defects (heart, kidneys, ortho, ophtho, fingerpads);
- Facial features (eyes, ears, and nose);

Remember: these results need to be verified in other labs and in humans before we can get to optimistic

# How can we work together?

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- Participate in research: samples, studies, results (Jacqui Weissman study), Send us MRI's;
- Fundraise: sometimes a small amount of money can help a lab get preliminary data so can get NIH funding: thank you to all the families attending today fundraising for our lab;
- Organize meetings (great for patients and family interaction, great for providers to interact), great to push the research (East Coast Gathering organized by Dana Levinson);
- Lobby for Kabuki syndrome, help increase awareness of Kabuki syndrome among public, among lawmakers, among funders;



*BeHeard Research Challenge by the Rare Genomics Institute to help raise awareness about Kabuki syndrome (2013). 12,000 votes!!!*

# Acknowledgements

## **Bjornsson lab**

- Joel Benjamin (mice, diet)
- Li Zhang (ChIP)
- Giovanni Carosso (diet)
- Genay Pilarowski (microarray)
- Hyun-Seo Cho (Microscopy)

## **Dietz lab**

- Hal Dietz;
- Yi-Chun Chen, Sara Cooke, Liz Gerber, Juan, Elena and others;

Questions/comments:  
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## **Department of Biostatistics**

- Kasper D. Hansen (ChIP-Seq)

## **Department of Psychiatry**

- Mikhail Pletnikov (behavior)
- Michelle Potter (neurogenesis)

## **Epigenetics and Chromatin Clinic**

- Jill Fahrner
- Carolyn Applegate



**Funding:** YIRG from SOGBD  
AAP; Early Independence  
Award from NIH common fund;

H.T.B is also a paid consultant to Third Rock Ventures, LLC.  
This arrangement has been reviewed and approved  
by the JHU in accordance with its conflict of interest policies.