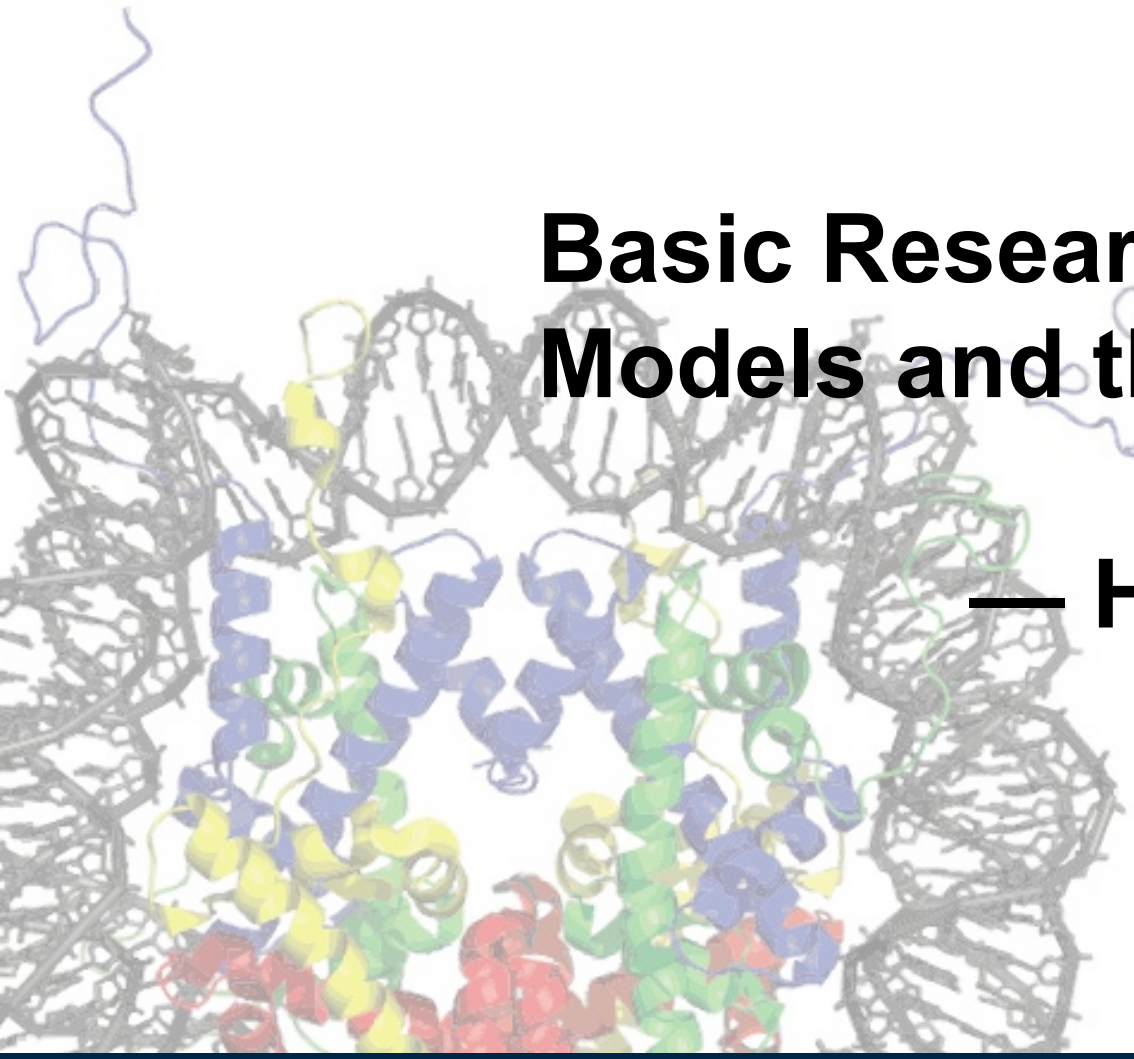
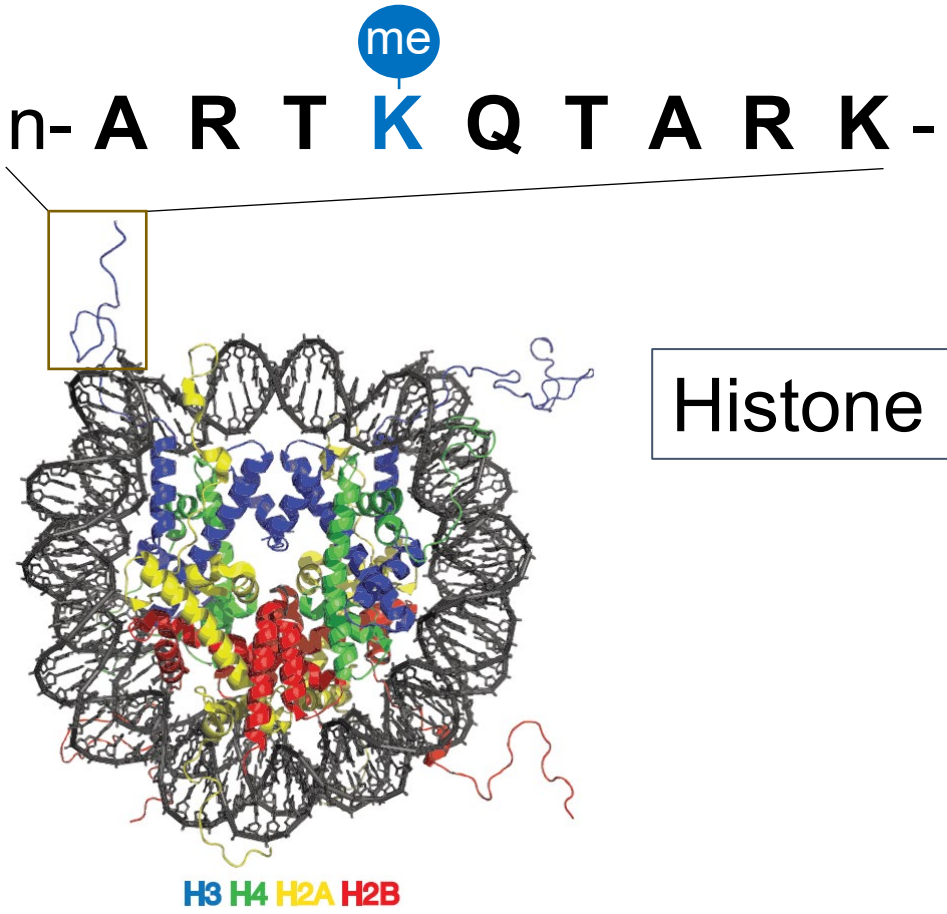


Basic Research: The Power of Animal Models and their Promise in Rare Disease

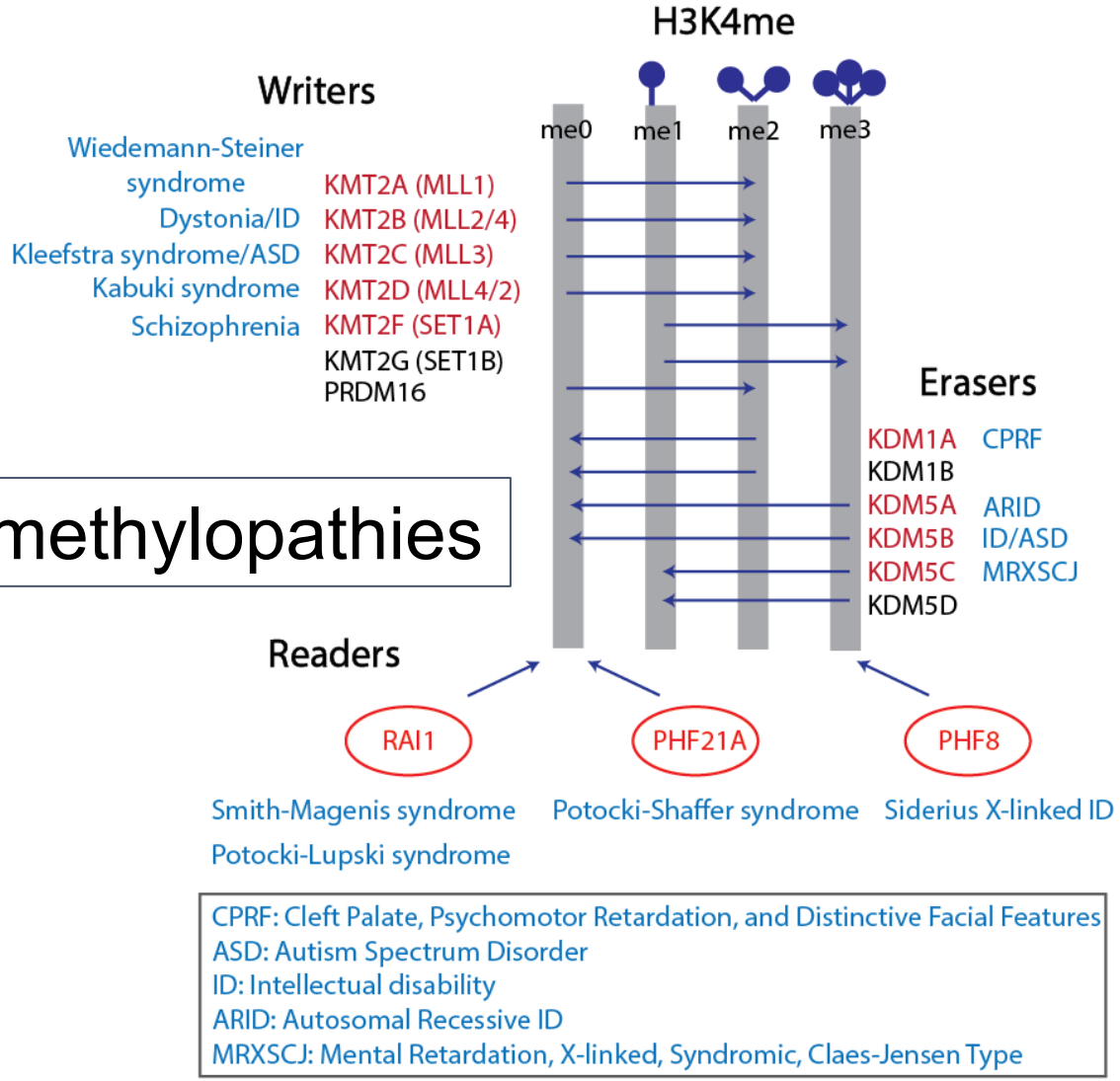
— HistoneH3K4 methylopathies

Shigeki Iwase Ph.D.
Department of Human Genetics

Disrupted intricacy of H3K4me regulation in the neurodevelopmental disorders

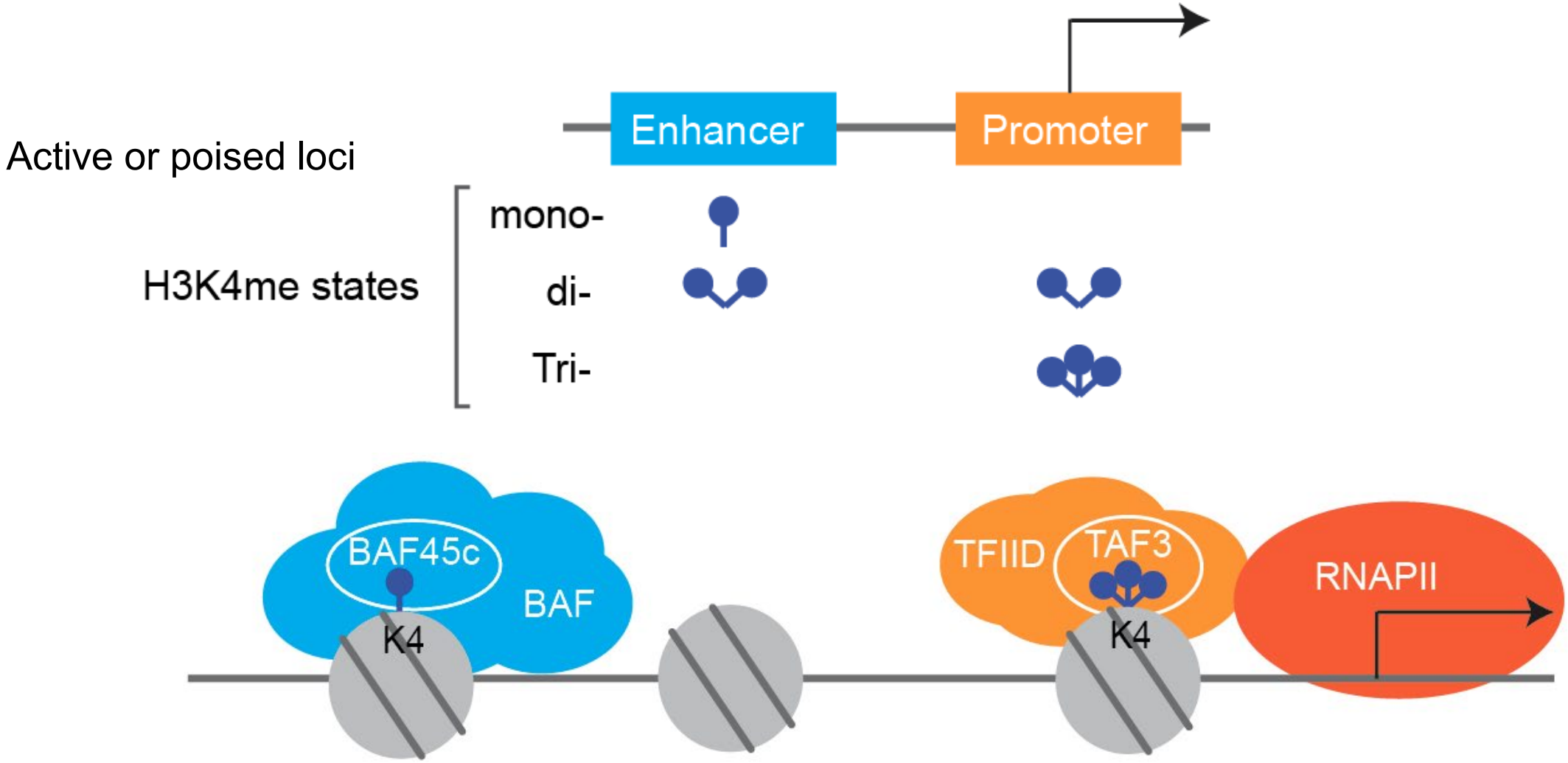


Histone H3K4 methylopathies

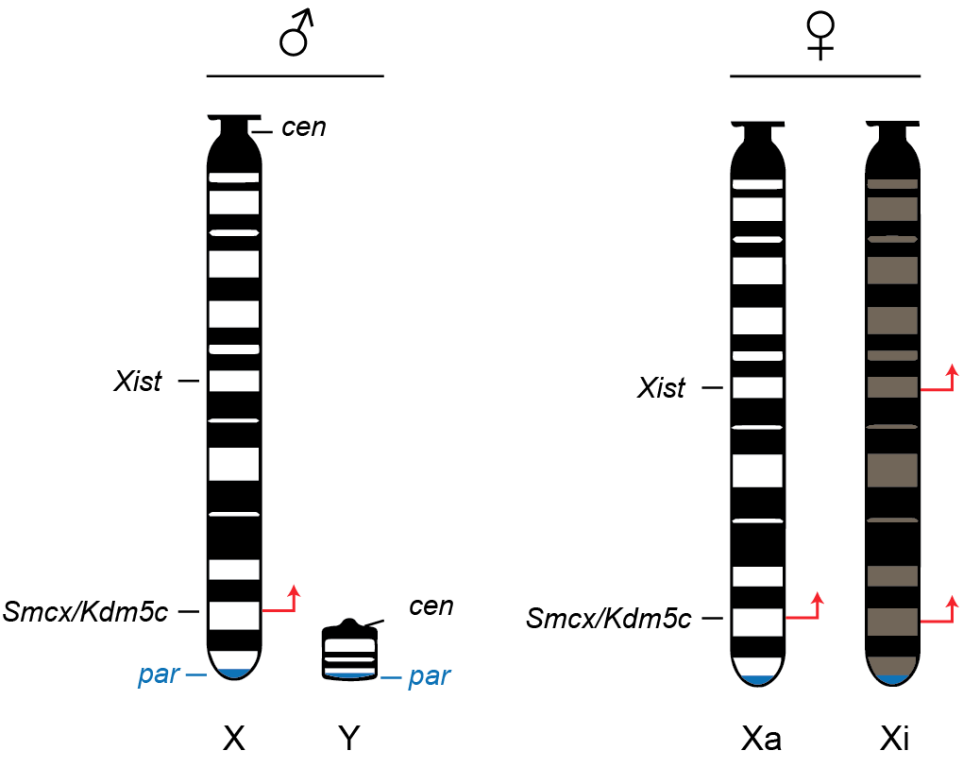


CPRF: Cleft Palate, Psychomotor Retardation, and Distinctive Facial Features
ASD: Autism Spectrum Disorder
ID: Intellectual disability
ARID: Autosomal Recessive ID
MRXSCJ: Mental Retardation, X-linked, Syndromic, Claes-Jensen Type

H3K4me states contribute to distinct processes of transcriptions



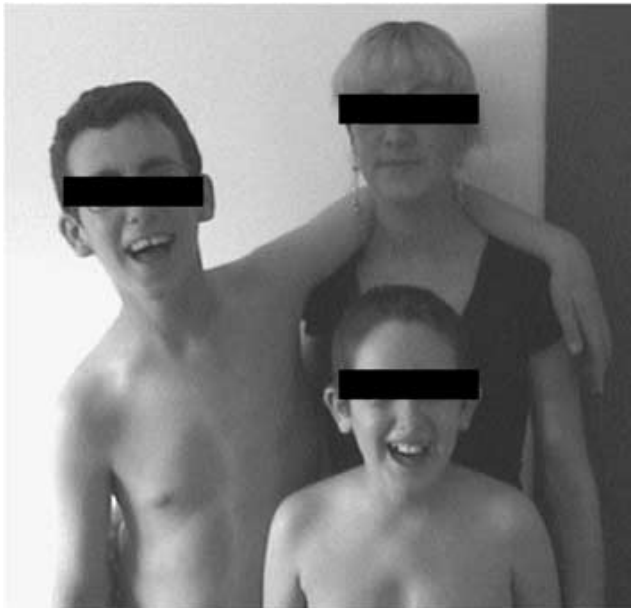
SMCX is mutated in mental retardation, X-linked, syndromic, Claes-Jensen type; (MRXSCJ, OMIM# 300534)



Wu and Shapiro et al., 1994, Human Mol Genet

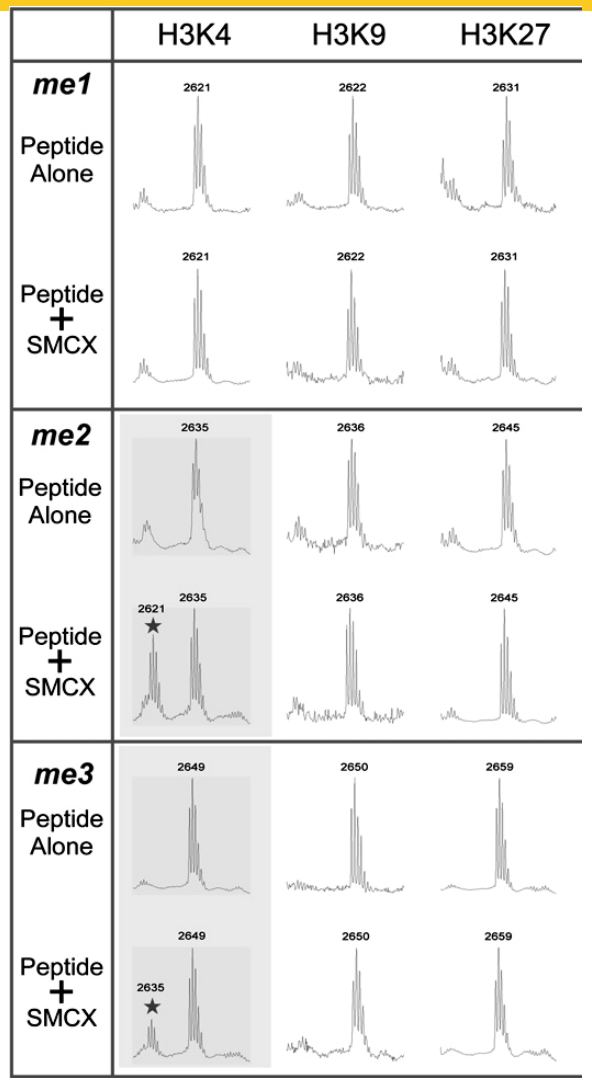
- Predominantly males. Mild to severe ID (IQ: 20~70)
- Account for 2 % of X-linked Intellectual Disability

- Aggressive behavior
- Spontaneous smiling
- Autistic behavior
- Epilepsy
- Short stature



Santos *et al*. European Journal of Human Genetics (2006) 14, 583–586

SMCX/KDM5C is an H3K4me2/3 demethylase



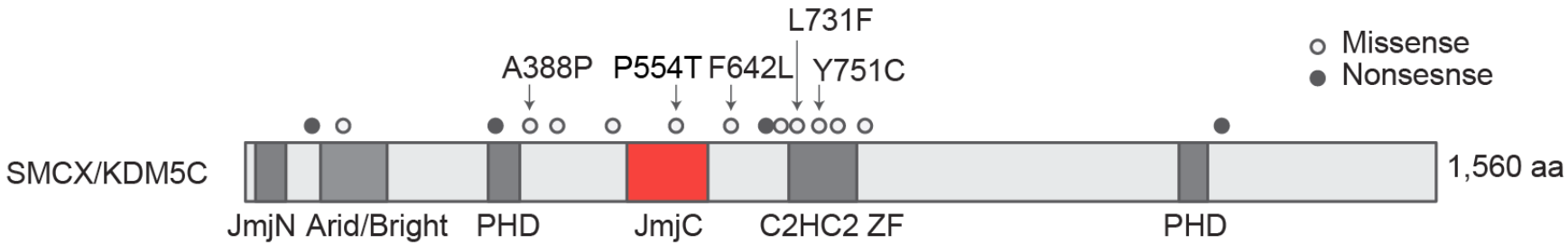
Iwase *et al*. Cell. 2007. 389: 251-260

SMCX

↓

KDM5C

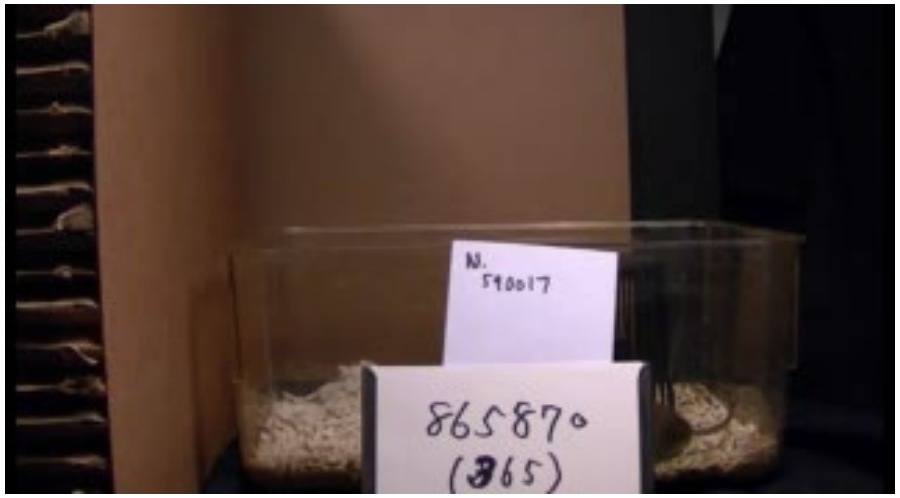
(Lysine DeMethylase 5C)



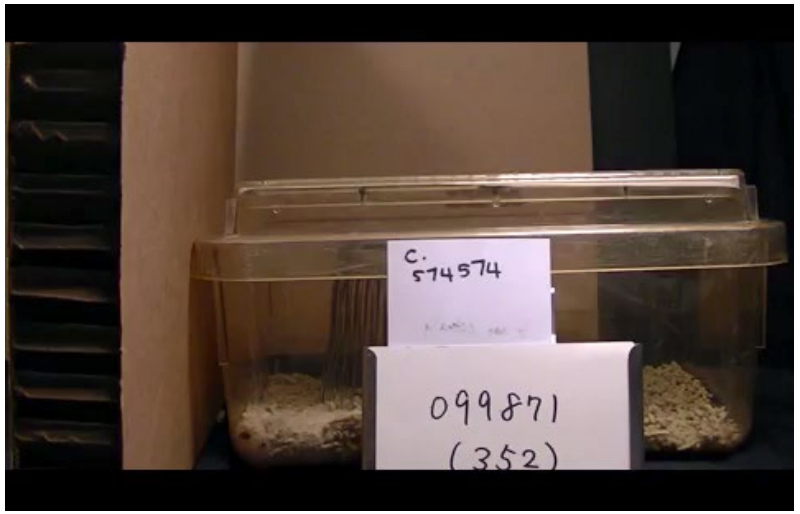
Kdm5c-KO male mice are a good model of MRXSCJ

Resident-intruder test

WT

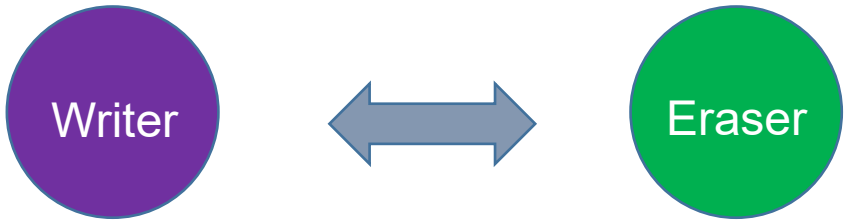


Kdm5c KO



Iwase *et al*. Cell Reports. 2016. 9;14(5):1000-9

Small molecules were able to correct phenotypes in models of chromatin-associated neurodevelopmental disorders



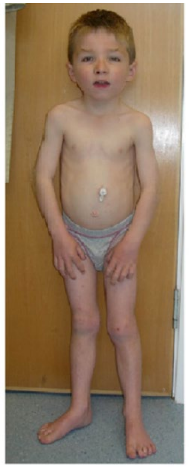
Genes	Encoded protein	Disorder	Small molecule inhibitors	Inhibitor's targets	References
Cbp (+/–)	Histone acetyltransferase	Rubinstein–Taybi syndrome	SAHA	HDAC1, 2, 3, 4, 5, 6, 7, 8, 9, 10	Alarcon et al. 2004. Neuron
Kmt2d (+/–)	H3K4 methyltransferase	Kabuki syndrome	AR-42	HDAC1, 2, 3, 4, 5, 6, 7, 8, 9, 10	Bjornsson et al.2014. Sci Transl Med.
Fmr1 (–/y)	FMR1: regulator of protein translation	Fragile X	JQ1	BRD2, 3, 4, BRDT	Korb et al. Cell. 2017

Can we modulate single enzyme to ameliorate brain histone methylopathies?

Phenotypic similarities between WSS and MRXSCJ

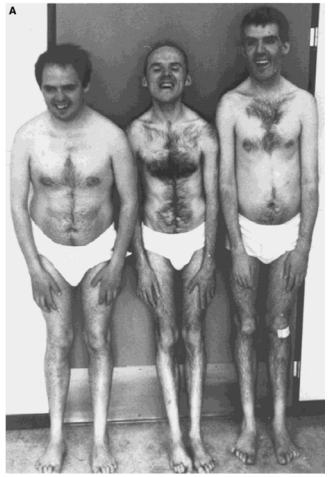
KMT2A: Weidemann-Steiner Syndrome

- Autosomal dominant
  - *De novo*
  - Males & females
- Hairy elbows, specific facial features



KDM5C: MRXSCJ

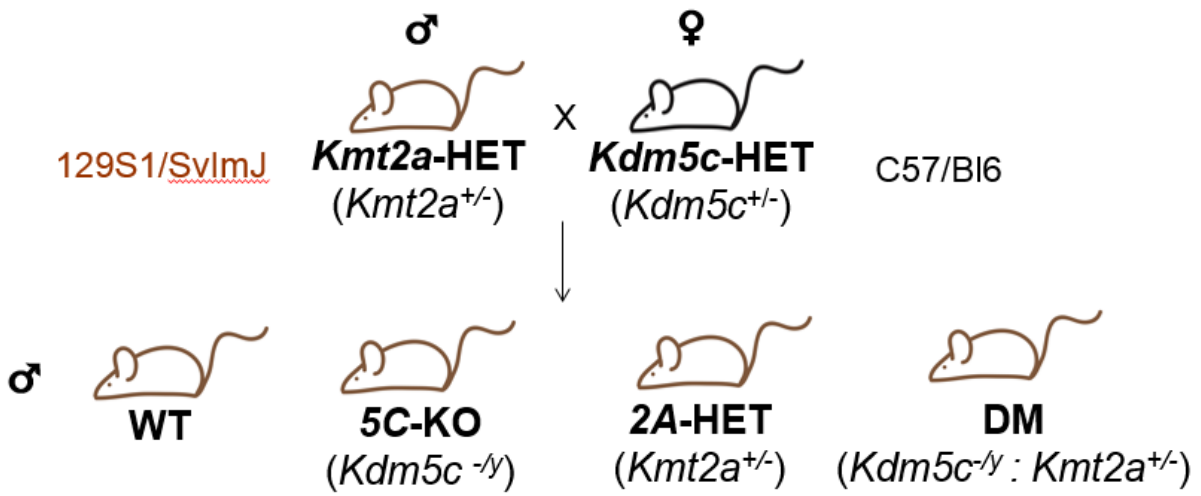
- X-linked
  - Inherited
  - Predominantly males
- Excessive smiling, over-friendly



Common clinical features:
Intellectual disability, thin build, short stature, developmental delay, autism, aggressive behaviors

Weidemann et al., 1989; Jones et al., 2012; Claes et al., 2000; Jensen et al., 2005

Generation of *Kdm5c-Kmt2a*-double mutant (DM) mice

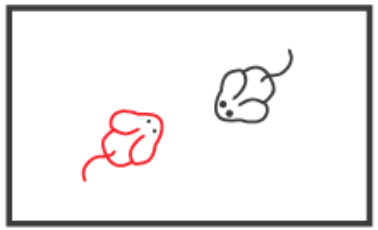


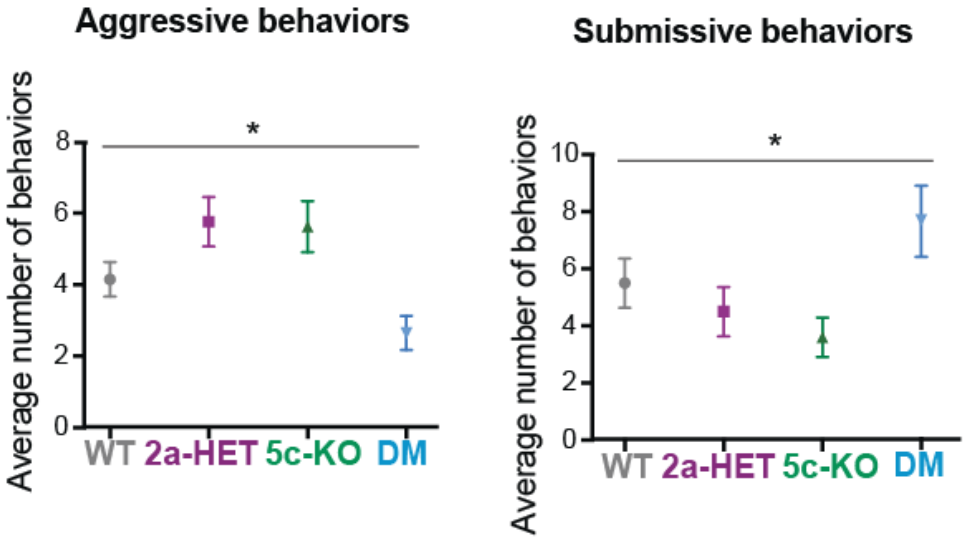
Number of offspring			
17 (25%)	21 (31%)	16 (24%)	14 (21%)
	MRXSCJ model	**WSS model**	

Hypothesis: KDM5C and KMT2A counteract to set the optimal H3K4me levels; therefore, deleting one enzyme corrects deficiency of the opposing enzyme.

Aggression and social dominance

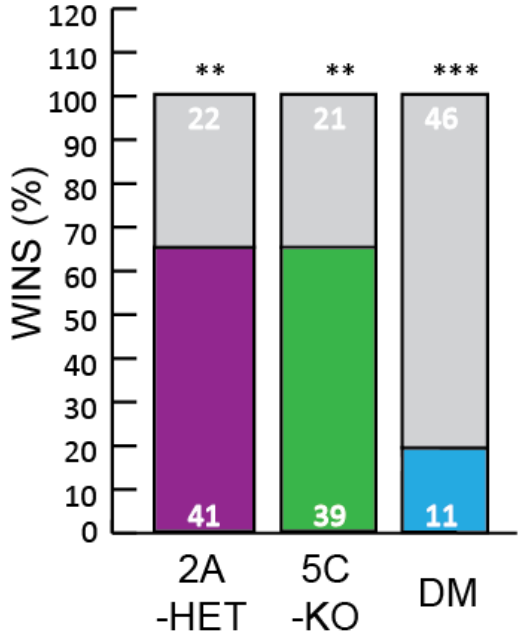
Resident intruder test





One-way ANOVA → Lsd multiple comparison
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

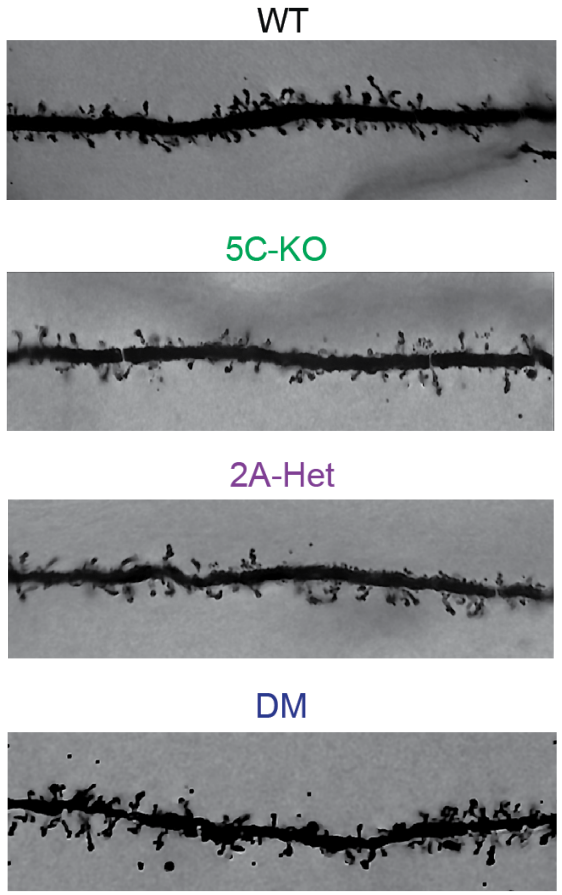
Tube dominance/aggression test

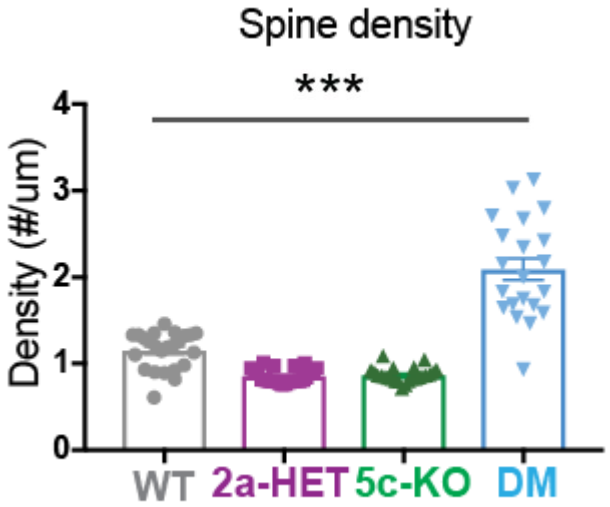


**p<0.01, ***p<0.001, Exact binomial test.

Dendritic spine density was lower in single mutants and reversed in the double mutant

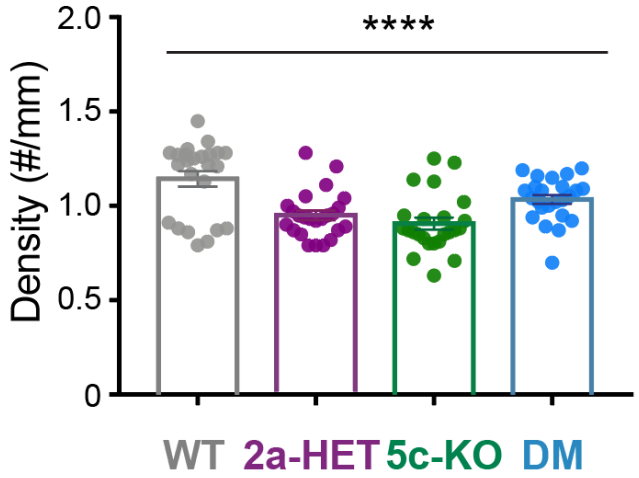
A Basolateral amygdala, adult





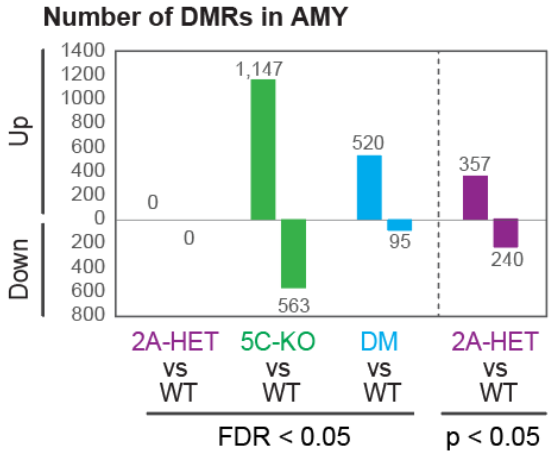
One-way ANOVA → Tukey multiple comparison
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

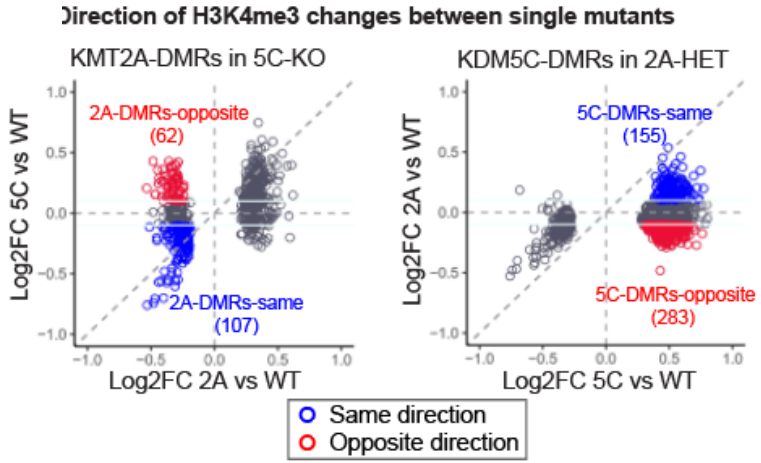
B Dorsal hippocampal CA1



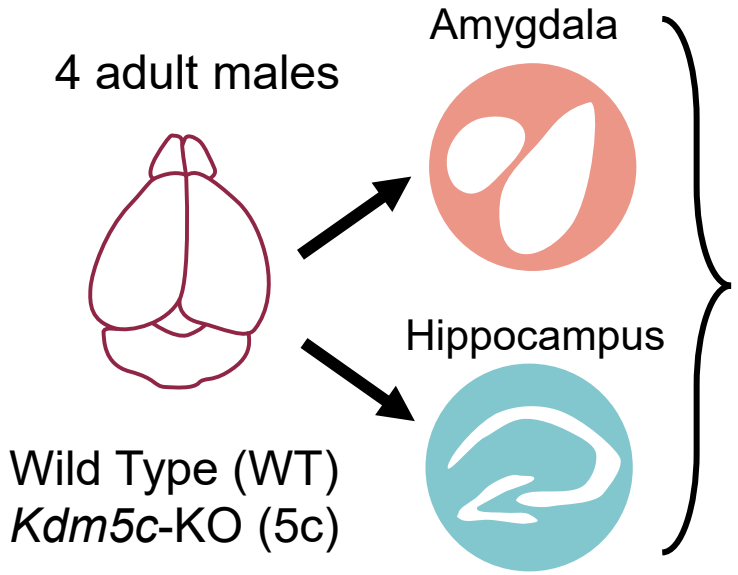
*Golgi staining: Pyramidal neurons

KMT2A-KDM5C antagonism over H3K4me3 (ChIP-seq)





Many genes overexpressed in the *Kdm5c*-KO brain are testis genes with no known brain function



Bulk RNA-seq
DESeq2

Katherine Bonefas

Top 10 *Kdm5c*-KO differentially expressed genes (DEGs) compared to WT

Gene	log2FoldCh	padj
D1Pas1	3.66108	3.38E-06
Pnmt	3.10739	1.47E-09
Tex14	2.89637	3.79E-26
Cyct	2.66877	0.00563
Tspo2	2.51655	0.00698
Fbxw23	2.43673	0.00257
F9	2.35591	0.00046
4930524B	2.35321	3.12E-08
Zar1	2.30852	8.32E-09
Hsf2bp	2.22283	3.67E-05

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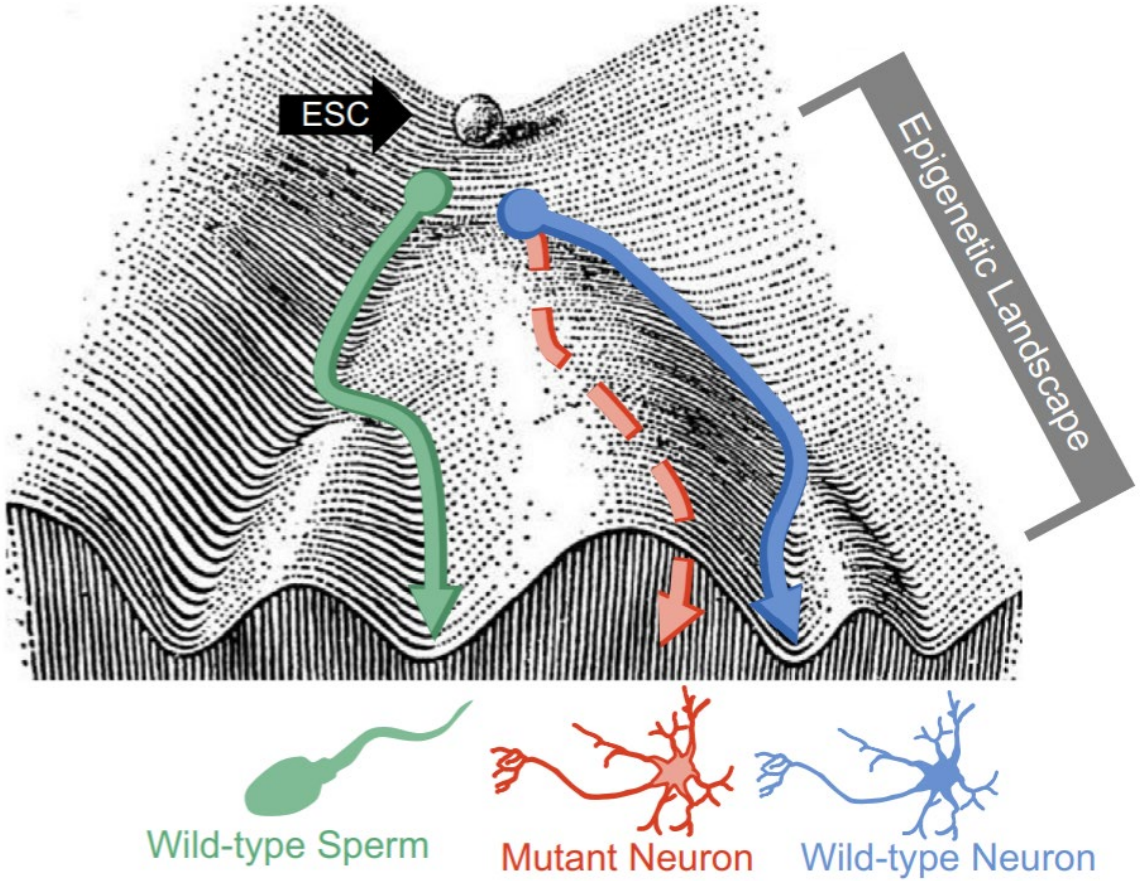
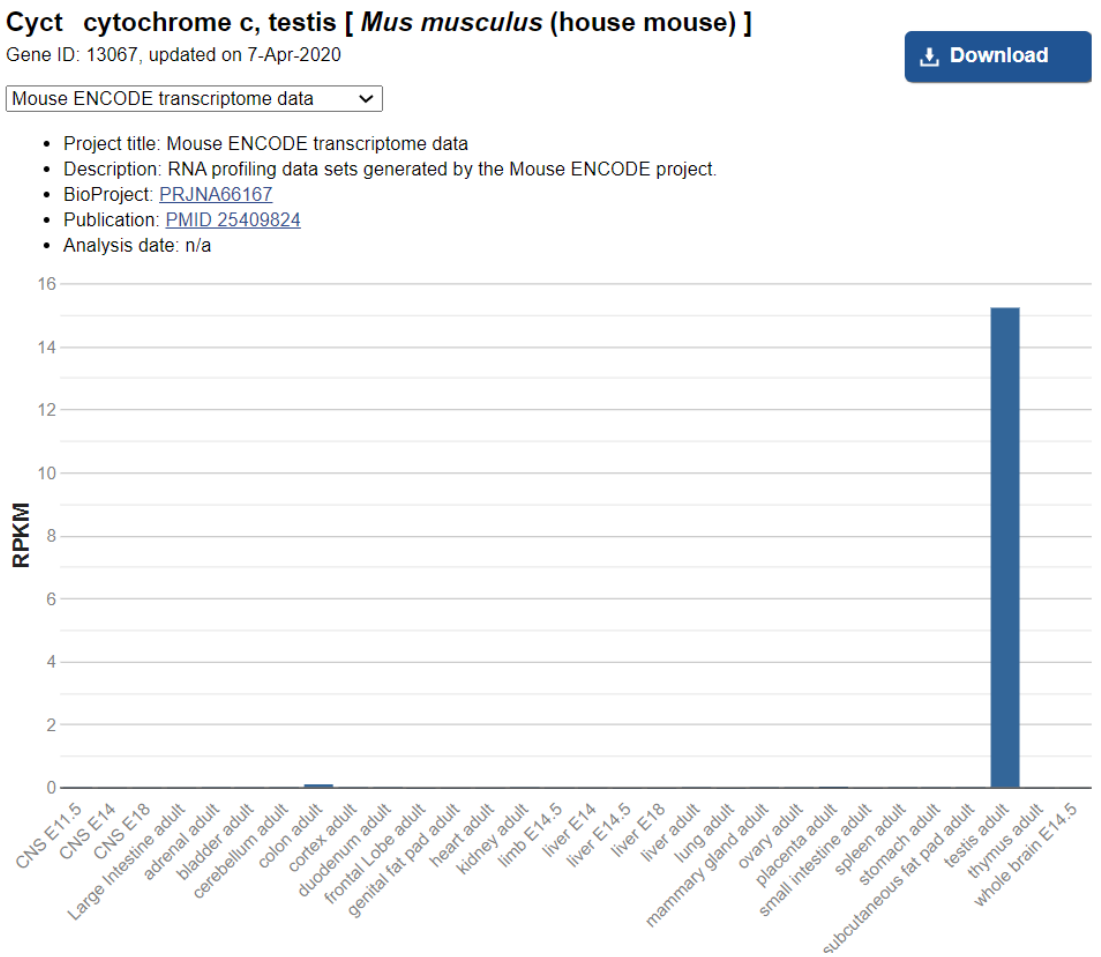
Sorted by Log2FC and q-value

Soma-to-germline transformation in KDM5C disorder?

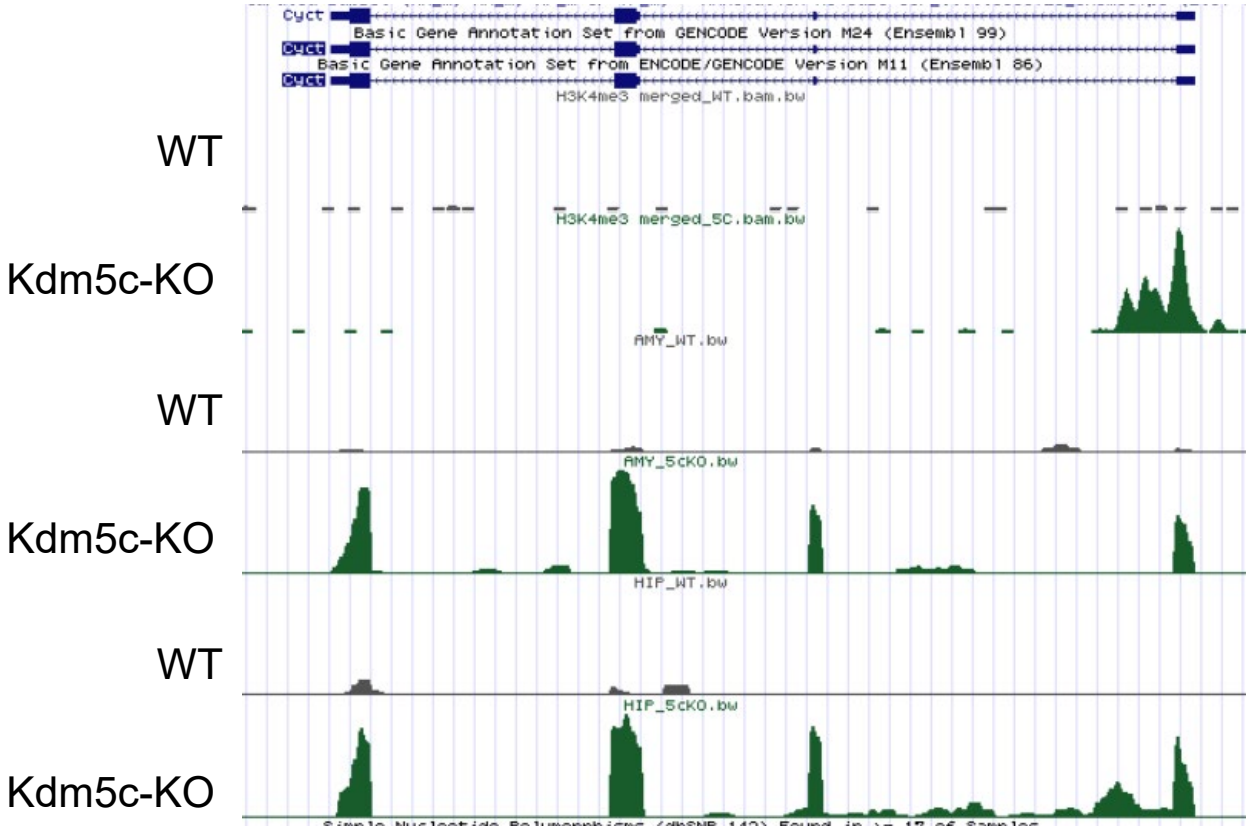


Cytochrome C testis specific (Cyct) is expressed in the Kdm5c-KO brain

Cyct expression in mouse tissues



Cyct expression in KDM5C-KO amygdala and hippocampus

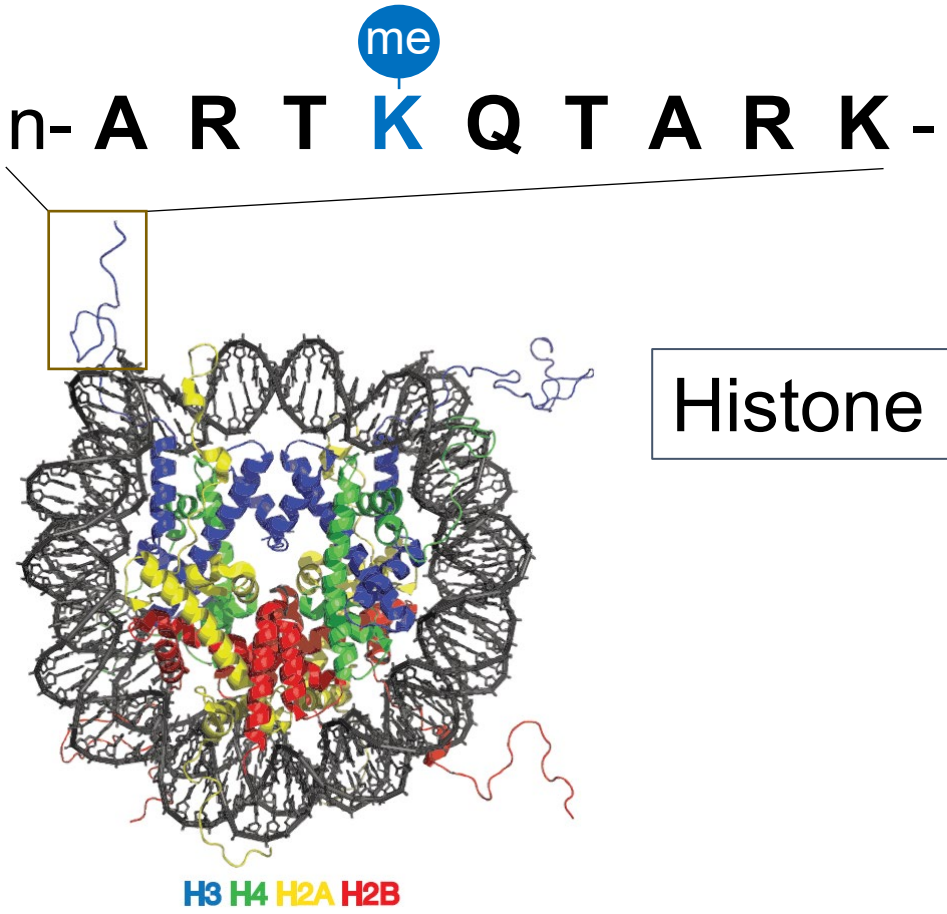


RPKM in KO: ~1.5 (10% of testis expression)

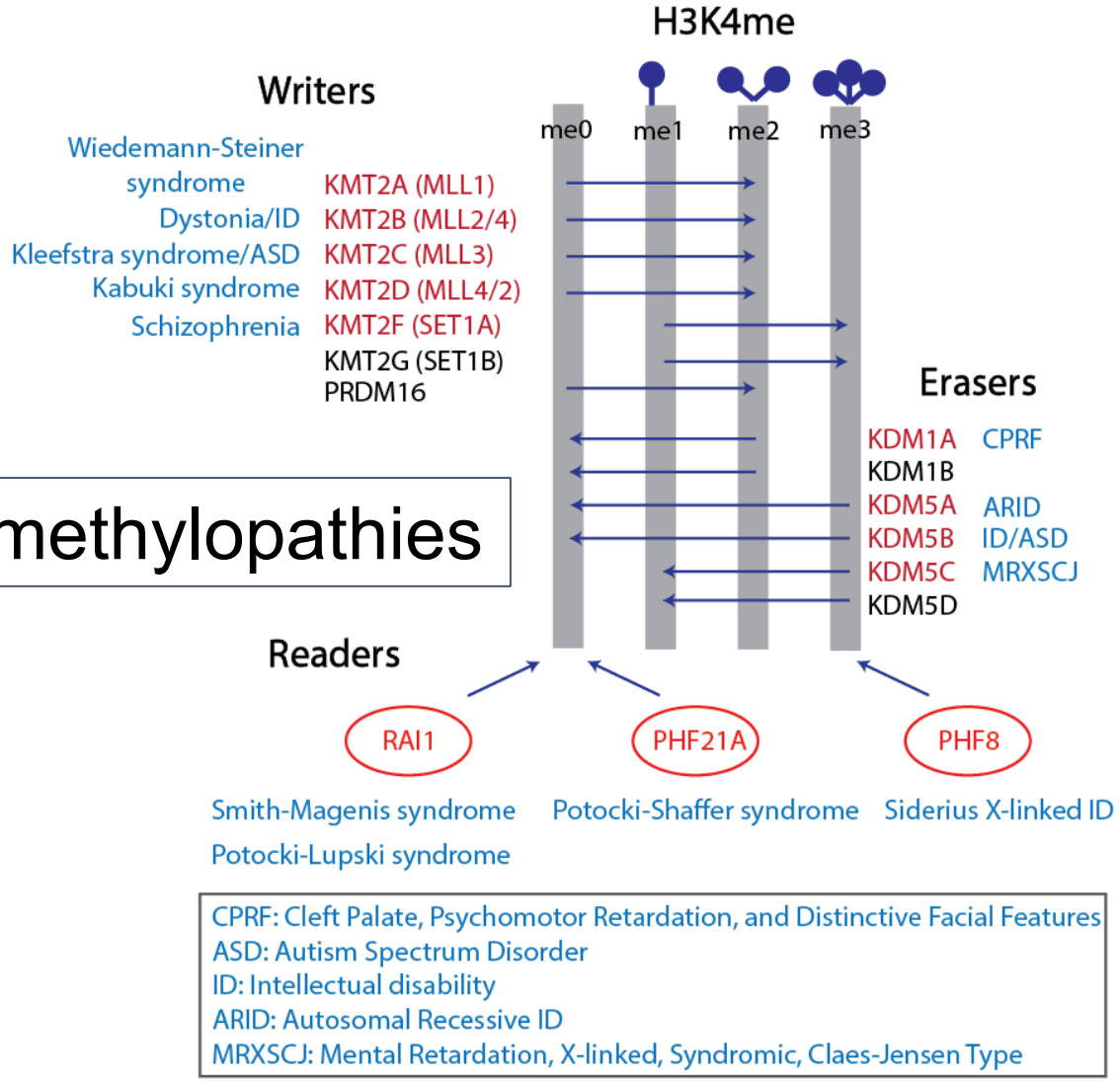
Possible soma-to-germline transformation in other rare chromatin disorders

Gene Name	Major Chromatin Function	Associated NDD(s)	Reports of Ectopic Germline Genes in Brain	Example Germline Genes
Dnmt3b (DNA-methyltransferase 3 beta)	De novo DNA CpG methylation	Immunodeficiency, Centromere instability, Facial anomalies (ICF) syndrome	Velasco et al, PNAS, (2010)	Ddx4, Tex11, Mael, Syce1
G9a/Glp (G9a and G9-a Like Protein)	H3K9me1/2 methyltransferase	Kleefstra syndrome	Schaefer et al, Neuron, (2009)	Dnah1, Dazl, Spag6
Mecp2 (Methyl CpG binding protein 2)	Binds methylated CpG DNA	Rett syndrome (RTT) MeCP2 duplication syndrome (MDS)	Ben-Shachar et al, Human Mol Genet (2009) Samaco et al, Nature Genetics, (2012)	Spag6, Spata1
Kdm5c (Lysine demethylase 5c)	H3K4me2/3 demethylase	Mental Retardation, X-linked Syndromic – Claes Jensen Type (MRXSCJ)	Iwase et al, Cell Reports, (2016) Scandaglia et al, Cell Reports, (2017)	Tex14, Cyct, Ddx4, Dnah1, Rnf17, D1Pas1, Spag16

Disrupted intricacy of H3K4me regulation in the neurodevelopmental disorders



Histone H3K4 methylopathies



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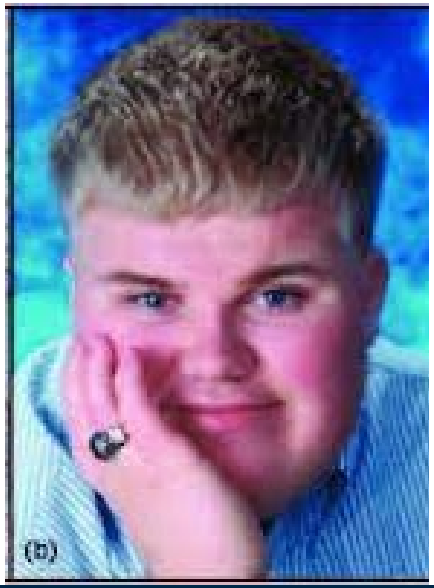
Smith-Magenis syndrome



- Identified as conditions associated with ~3.7M de novo deletion of 17p11.2.

  (Patil and Barley. 1984, Smith et al. 1986)

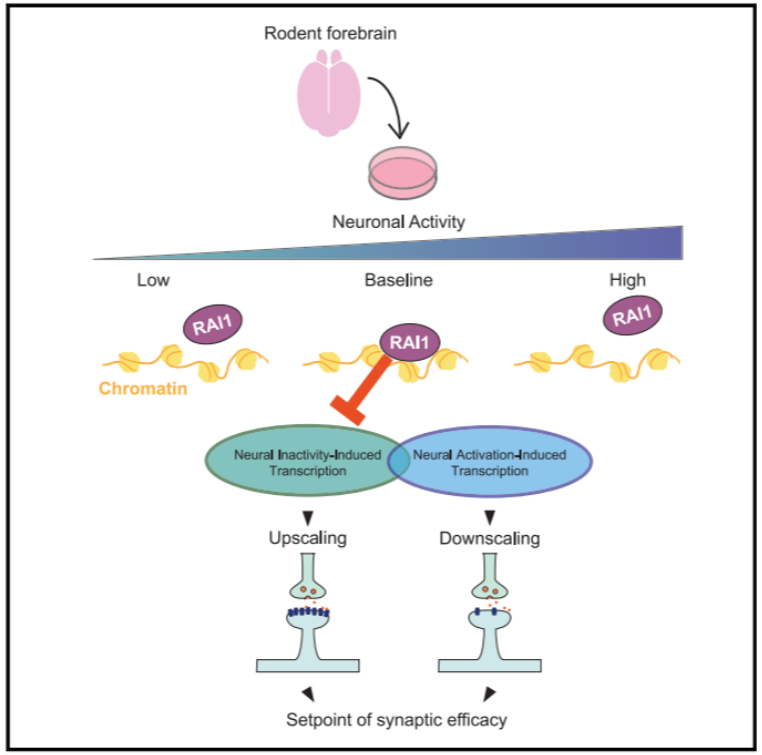
- Obesity
- Mental and behavioral problems.
- **Sleeping disturbance**
- **Inverted Melatonin cycle**
- RAI1 — responsible for most of the symptoms

Cell Reports

Article

RAI1 Regulates Activity-Dependent Nascent Transcription and Synaptic Scaling

Graphical Abstract



Authors

Patricia M. Garay, Alex Chen, Takao Tsukahara, ..., Kevin S. Jones, Michael A. Sutton, Shigeki Iwase

Correspondence

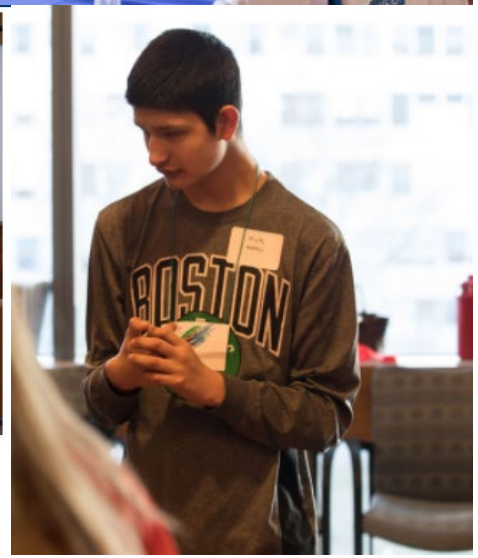
masutton@umich.edu (M.A.S.), siwase@umich.edu (S.I.)

In Brief

Garay et al. adopt BrU-seq to profile bona fide transcriptional dynamics triggered by neuronal activity shifts. An integrated genomics approach including BrU-seq, combined with electrophysiology, reveals that RAI1, the Smith-Magenis syndrome protein, controls baseline synaptic strength and homeostatic synaptic upscaling by regulating the transcriptome associated with network inactivity.

Working with RDD families

The Foglio family shared their experience establishing a foundation to fund research of La Salla disease with the Robl family, who plan to start a foundation to address disorders related to KDM5C mutations



Rare Disease Day event at Einstein College of Medicine, 2020, March, NY

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