28 FEBRER 2019

DIA MUNDIAL DE LES MALALTIES MINORITÀRIES

# Epigenetics and Rett Syndrome


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**Genetics Professor, University of Barcelona**
**ICREA Research Professor**
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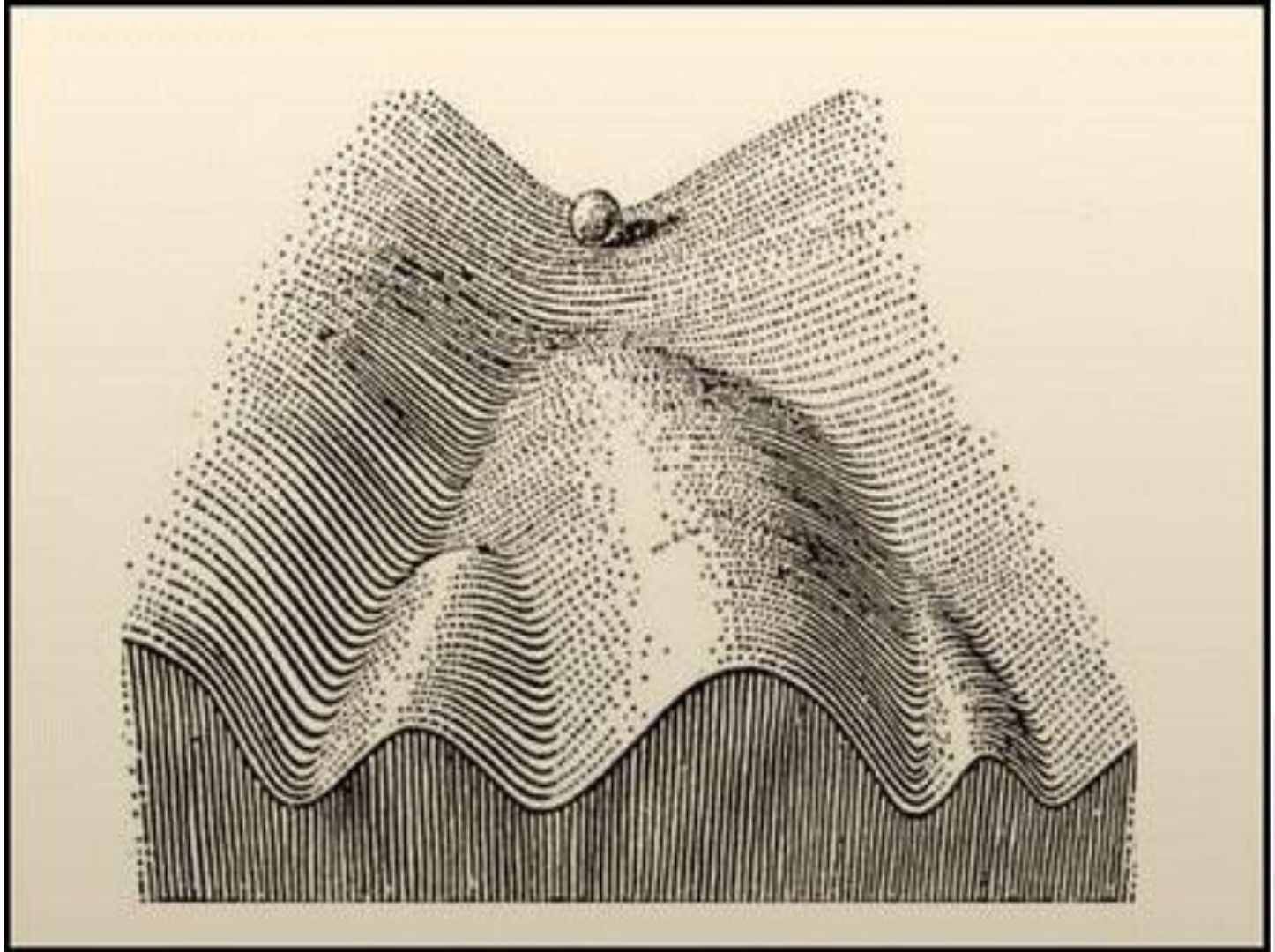
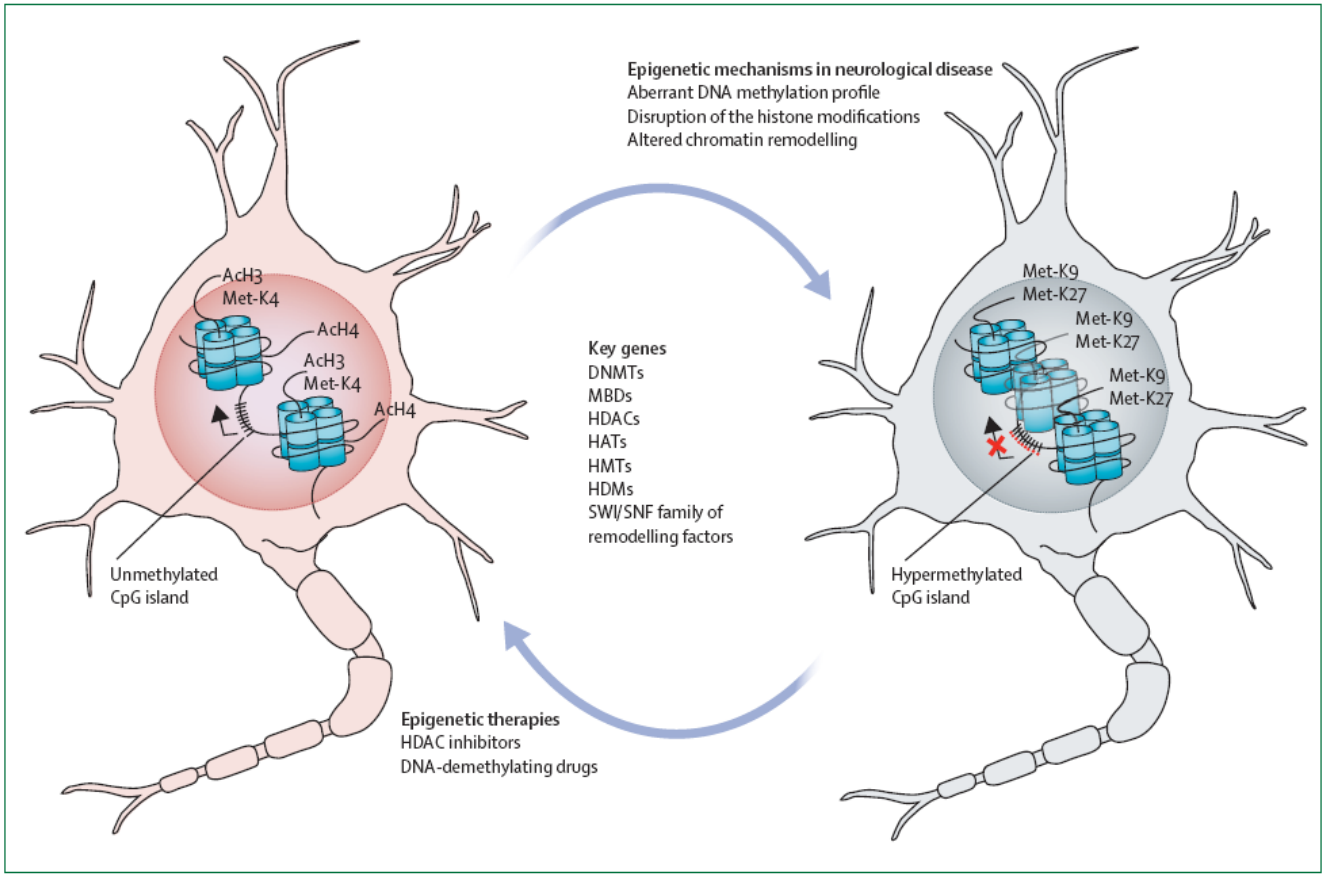
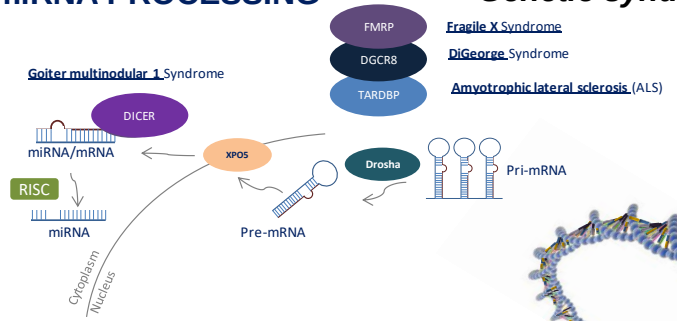




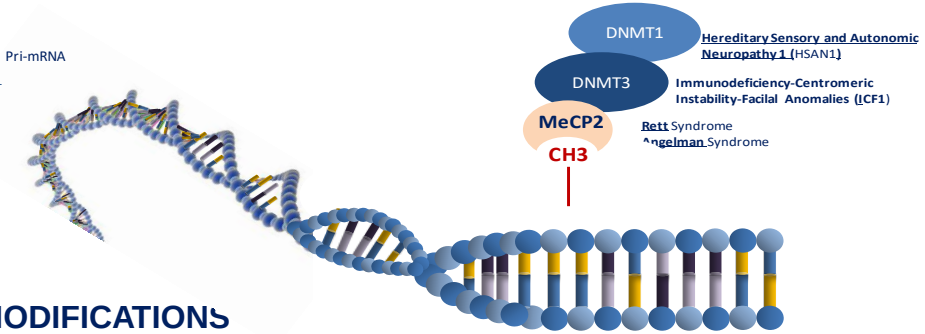

*Urdinguio, Sanchez-Mut and Esteller, Lancet Neurology, 2009*

# Genetic syndromes caused by mutations in Epigenetic genes

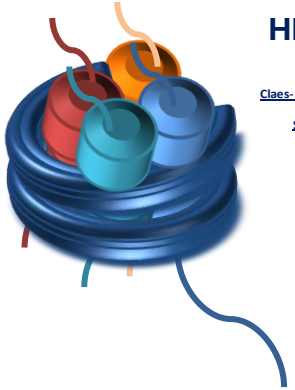
## miRNA PROCESSING

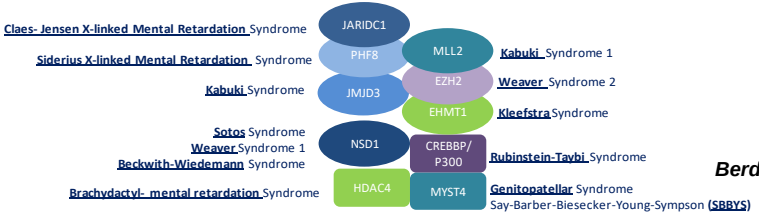



## DNA METHYLATION

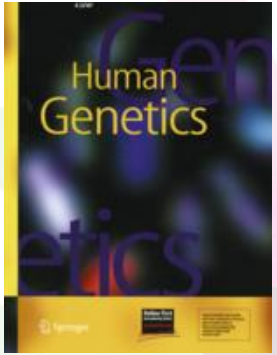



## HISTONE MODIFICATIONS

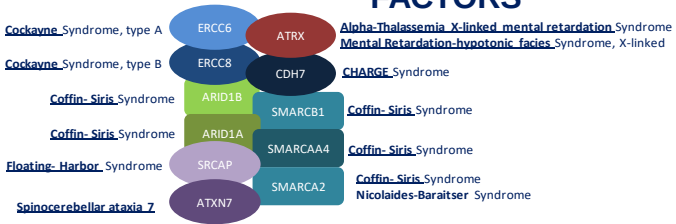





*Berdasco and Esteller, Hum Genet. 2013*




## CHROMATIN- REMODELING FACTORS



# Rett Syndrome

Rett Syndrome (RS) is a neurodevelopmental disorder believed to affect 1 in 10,000 females..

Germline mutations in MECP2 (Methyl-CpG Binding Protein-2) found in 65% of Rett patients. MECP2 is located in Xq28.

Girls with Rett Syndrome appear to develop normally until 6 to 18 months of age. They then enter a period of regression, losing speech and hand skills they had acquired. Most girls develop seizures, repetitive hand movements, irregular breathing and motor-control problems. A slowing of the rate of head growth also becomes apparent. Most researchers agree that RS is a developmental disorder rather than a progressive, degenerative disorder. The girls can live to adulthood, but most never regain the ability to use their hands or to speak.

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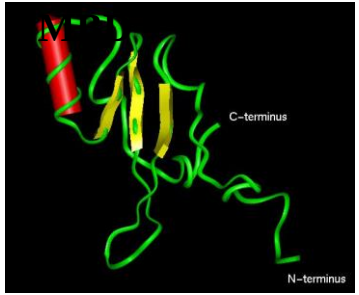
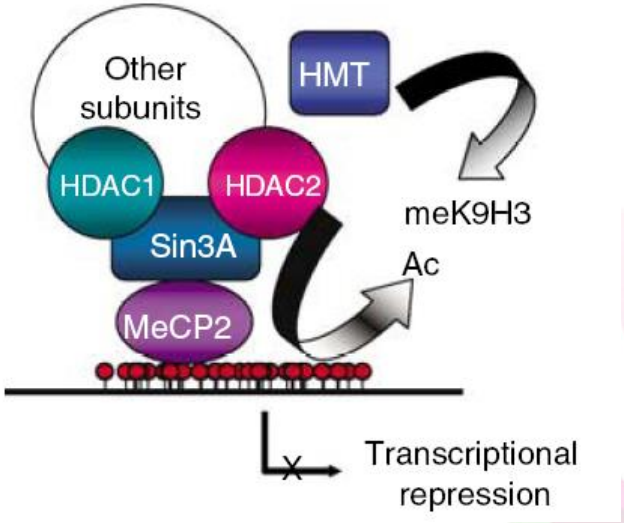
# MeCP2

- **Protein with affinity for methylated DNA (CpG pairs).**
  - **i.e. Fraga et al. NAR 2003; Ballestar et al. EMBO J 2003**
- **Transcriptional repressor.**
  - **i.e. Fournier et al. EMBO J 2002; Ballestar et al. EMBO J 2003; Lopez-Serra et al. Cancer Res 2006; Lopez-Serra et al., Oncogene 2008**

**Two main functional domains:**



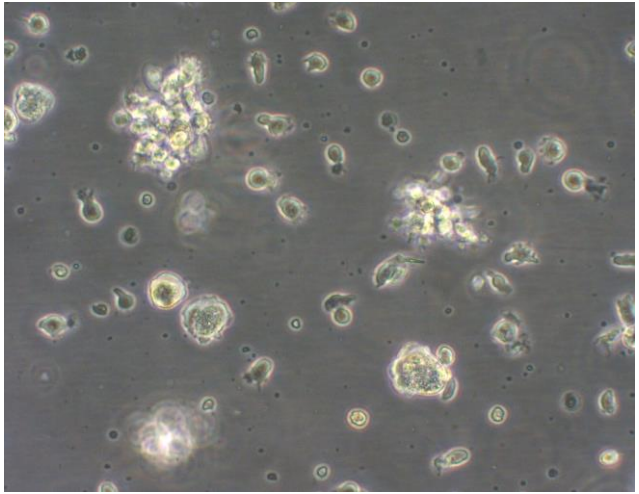

⇨**TRD**



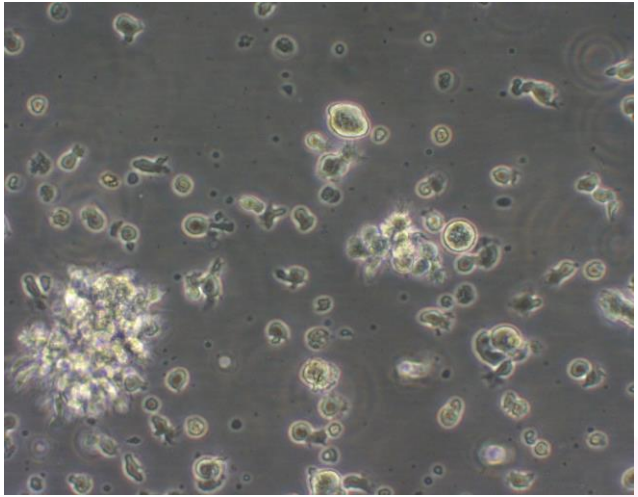

*Lopez-Serra et al., British Journal of Cancer 2008*

# Establishment of Immortalized Lymphocyte Cell Lines

## Healthy Donor



## MeCP2 Rett Patient



## Lymphoblastoid Cell Lines Available

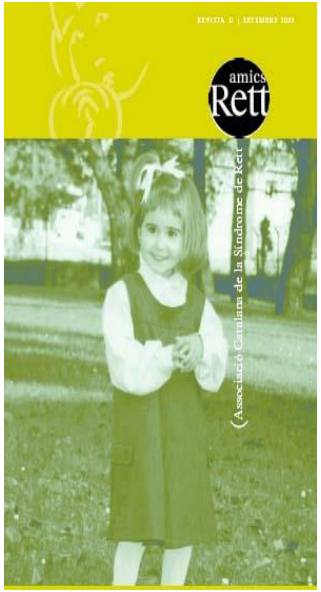
**MeCP2**
n=23

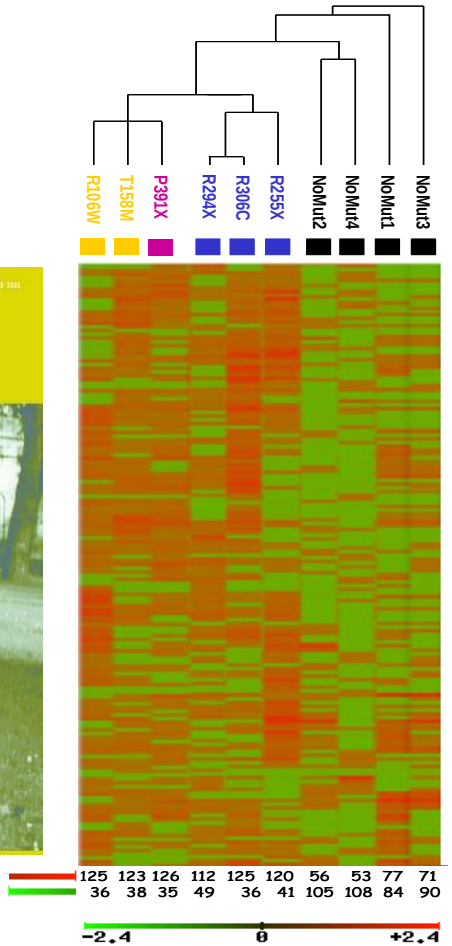
**CDKL5**
n=7

**NTG1**
n=3

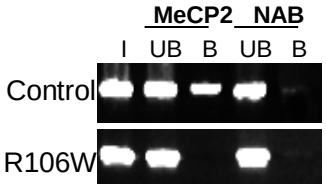
**FOXG1**
n=2

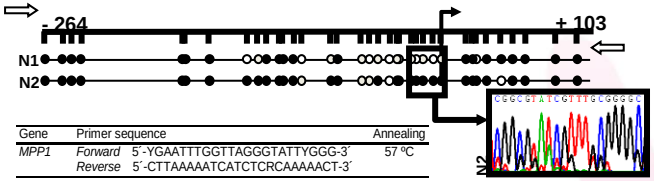
# Clustering expression analysis and release of MeCP2- Silencing in Rett Syndrome Patients




## MPP1

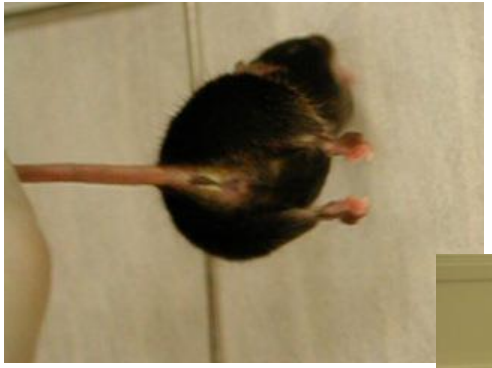


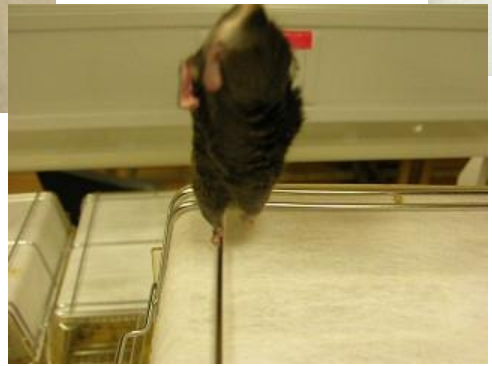



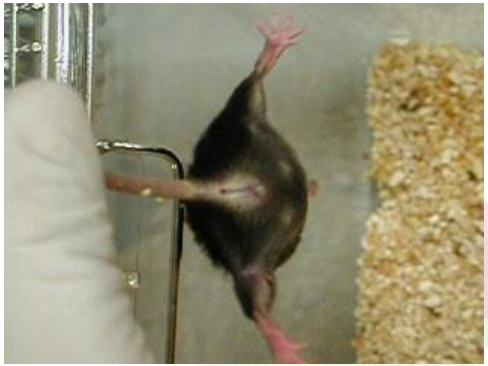

| Gene | Primer sequence | | Annealing |
|---|---|---|---|
| *MPP1* | *Forward* | 5´-YGAATTTGGTTAGGGTATTYGGG-3´ | 57 ºC |
| | *Reverse* | 5´-CTTAAAAATCATCTCRCAAAAACT-3´ | |

MPP1

*Ballestar et al., Human Mutation, 2005*



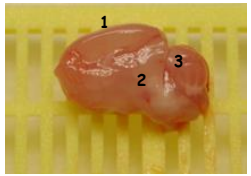




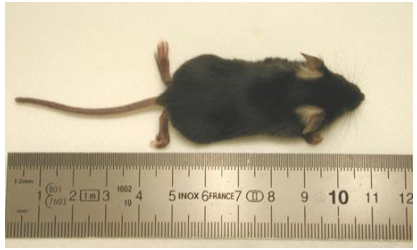
# Mouse Model of Rett

7.5gr/10.25cms

299mg

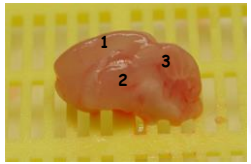



1.-Encéfalo
2.-Mesencéfalo
3.-Cerebelo



8.1gr/11.05cms

310mg



# Looking for transcriptional changes

**Wild type mice**

Cortex
Midbrain
Cerebellum

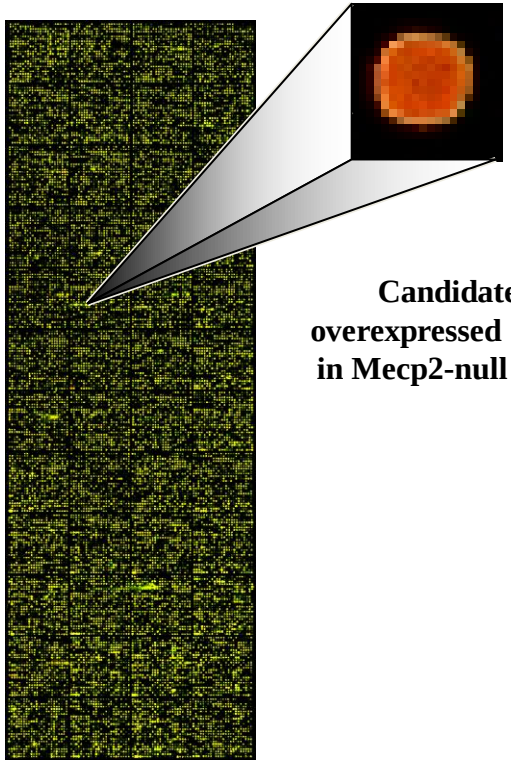
**Mecp2-null mice**

Cortex
Midbrain
Cerebellum

RNA Extraction
↓
Amplification
↓
Reverse transcription and labeling

RNA Extraction
↓
Amplification
↓
Reverse transcription and labeling

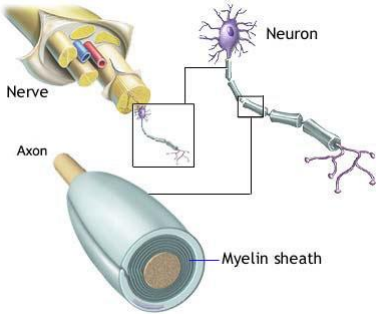
Hybridization
↓
Image scanning
↓
Data analysis



**Candidate overexpressed genes in Mecp2-null mice**

# Mobp

Myelin-associated oligodendrocytic basic protein




Family of abundant CNS myelin proteins

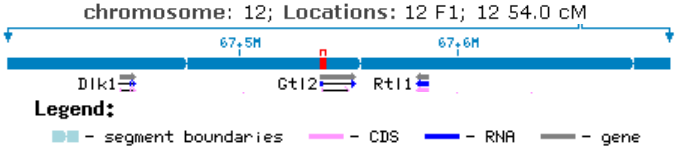
Localizes to major dense line of compact myelin

# Dlk1 – Gtl2

*Human Molecular Genetics, 2006, Vol. 15, No. 6* **821–830**
*doi:10.1093/hmg/ddl001*
*Advance Access published on January 26, 2006*

**Imprinted *DLK1* is a putative tumor suppressor gene and inactivated by epimutation at the region upstream of *GTL2* in human renal cell carcinoma**

Takahiro Kawakami[1,2,*], Tokuhiro Chano[2,3], Kahori Minami[2], Hidetoshi Okabe[2], Yusaku Okada[1] and Keisei Okamoto[1]




# Plagl1

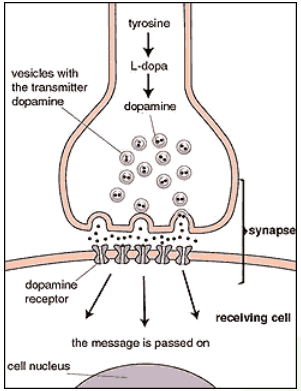
zinc finger protein regulator of apoptosis and cell cycle arrest (Zac1)

Developmental Cell *11*, 711–722, November, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.devcel.2006.09.003

**Zac1 Regulates an Imprinted Gene Network Critically Involved in the Control of Embryonic Growth**

Annie Varrault,[1] Charlotte Gueydan,[1] Annie Delalbre,[1] Anja Bellmann,[1] Souheir Houssami,[1] Cindy Aknin,[1,2] Dany Severac,[1,2] Laetitia Chotard,[1] Malik Kahli,[1] Anne Le Digarcher,[1] Paul Pavlidis,[3,4] and Laurent Journot[1,2,*]

# Ddc



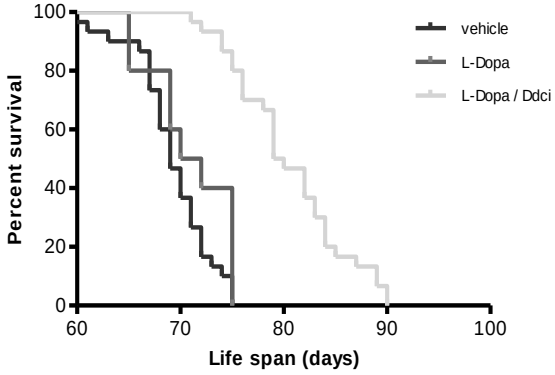

DOPA decarboxylase

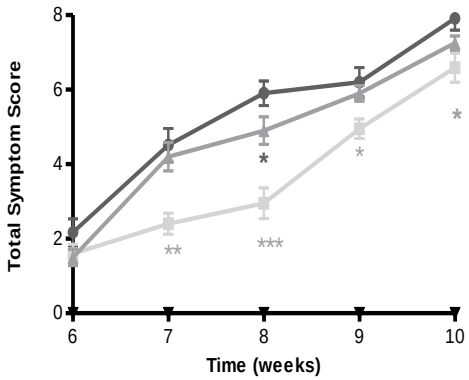
L-Dopa
⇩
Dopamine

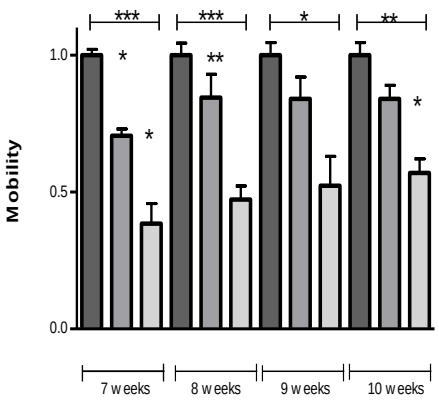
L-5-HTP
⇩
Serotonine

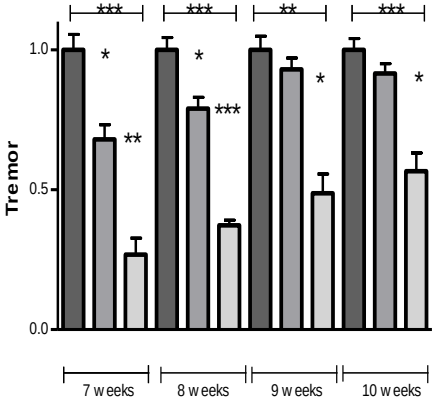
***Urdinguio et al., PLoS ONE, 2008***

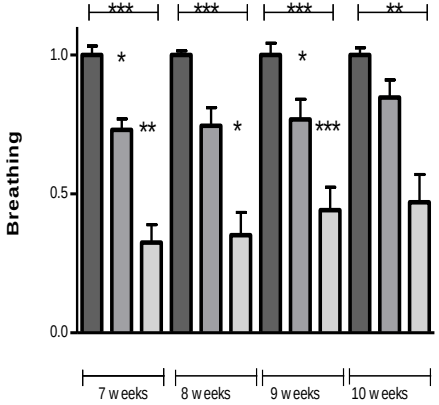
# Effective drugs for Parkinson's reduce symptoms of Rett syndrome in mice




*Szczesna et al., Neuropsychopharmacology 2014*

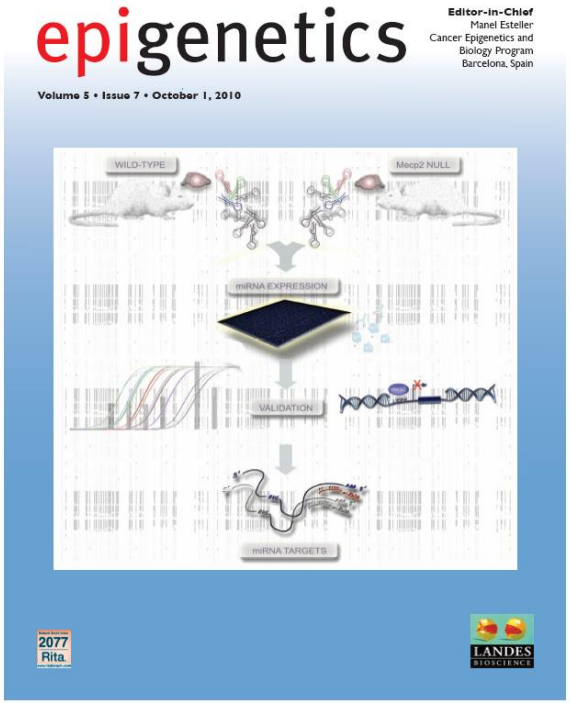
RESEARCH PAPER

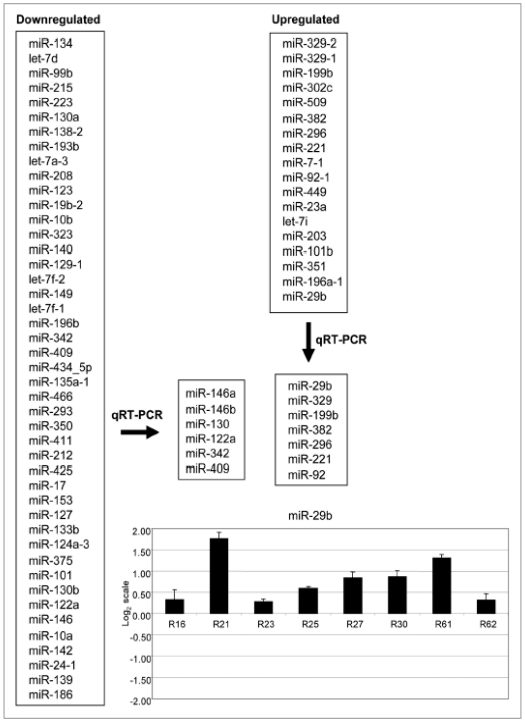
Epigenetics 5:7, 1-8; October 1, 2010; © 2010 Landes Bioscience

# Disrupted microRNA expression caused by Mecp2 loss in a mouse model of Rett syndrome

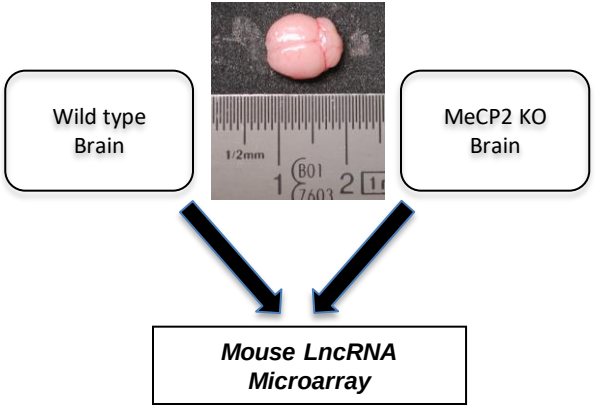
Rocio G. Urdinguio,[1] Agustin F. Fernandez,[1] Pilar Lopez-Nieva,[1] Simona Rossi,[2,†] Dori Huertas,[1] Marta Kulis,[1] Chang-Gong Liu,[2] Carlo Croce,[3] George A. Calin[2] and Manel Esteller[1,4,*]

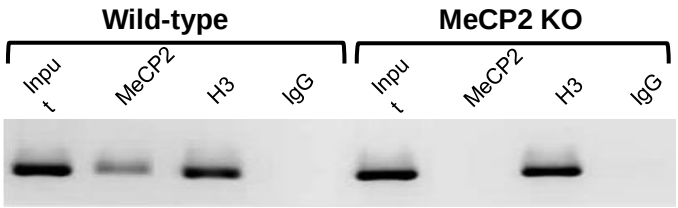
[1]Cancer Epigenetics and Biology Program (PEBC); Bellvitge Biomedical Research Institute (IDIBELL); L'Hospitalet; [4]Institucio Catalana de Recerca i Estudis Avançats (ICREA); Barcelona, Catalonia Spain; [2]Experimental Therapeutics and The Center for RNA interference and non-coding RNAs; MD Anderson Cancer Center; Houston, TX USA; [3]Department of Molecular Virology; Immunology and Medical Genetics; Comprehensive Cancer Center; Ohio State University; Columbus, OH USA

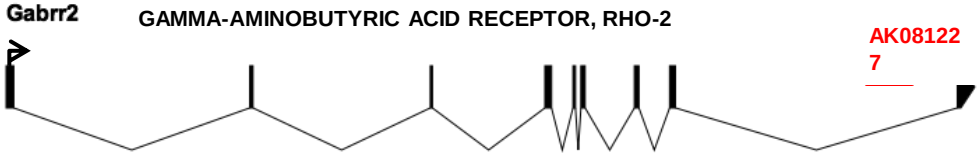


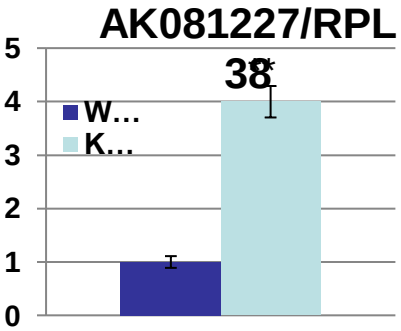



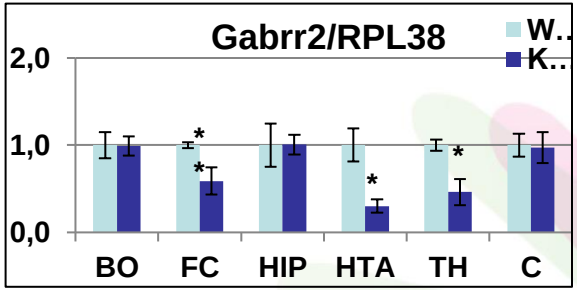
# An Impact of MeCP2 Disruption on lncRNA Transcription: The Mouse Model of Rett Syndrome



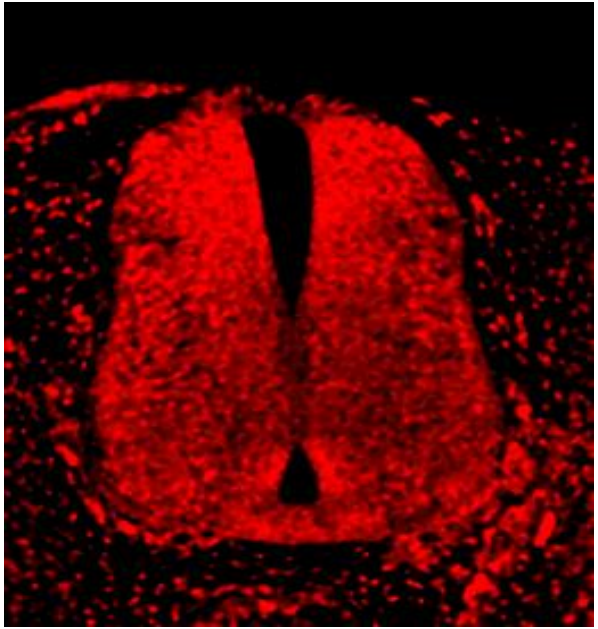




*Petazzi et al., RNA Biology, 2013*

# MECP2 duplication syndrome

**Intellectual disability, weak muscle tone in infancy, feeding difficulties, poor or absent speech, seizures, delayed development of motor skills, such as sitting, loss of previously acquired skills (developmental regression), one third cannot walk without assistance.**

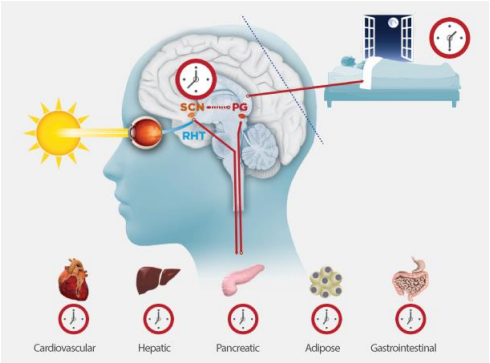
# An Increase in MECP2 Dosage Impairs Neural Tube Formation

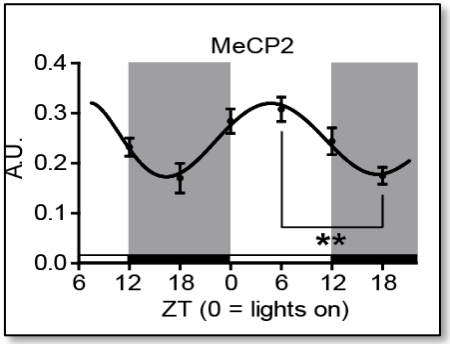


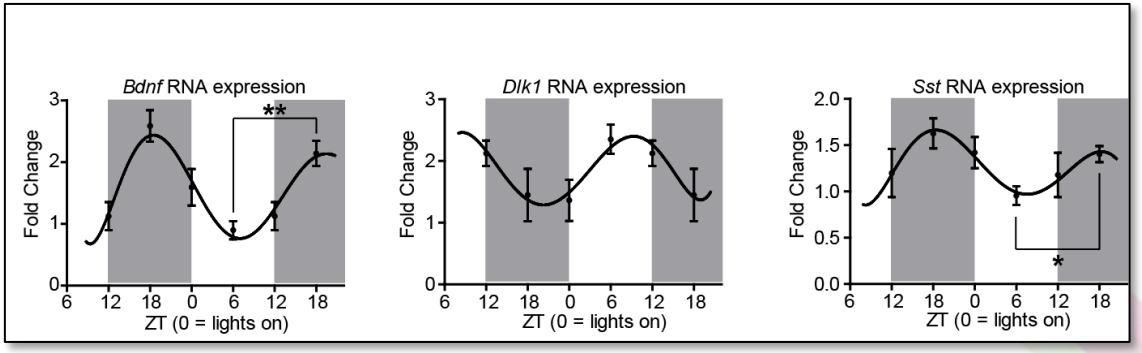
- **We report a new *in vivo* model of MeCP2 overexpression.**
- **The increase in MeCP2 dosage prevents the correct formation of the neural tube.**
- **MeCP2-electroporated cells show an ectopic neuronal localization.**
- **Neuroblasts overexpressing MeCP2 undergo decreased proliferation and increased death.**

***Petazzi et al., Neurobiology of Disease, 2014***

# Circadian Cycle-Dependent MeCP2 and Brain Chromatin Changes



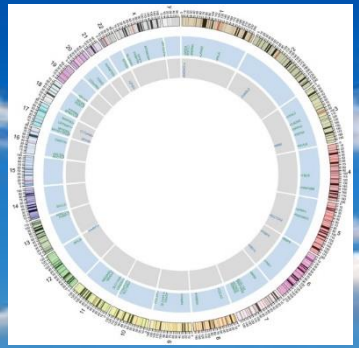

**Martínez de Paz *et al*, PLoS One (2015)**

# Genomics

## Girls with Clinical Features of Rett Syndrome but without any mutation in the “usual suspect” genes

Whole-Exome Sequencing

22 Patients
+
42 Parents



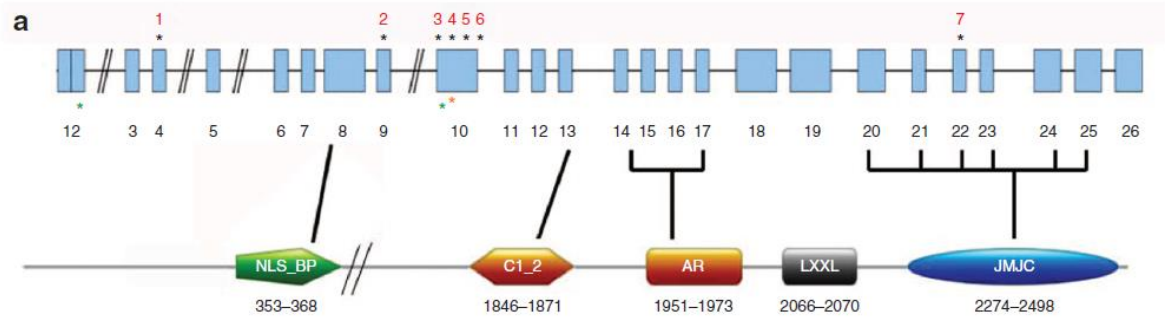
32 % Absence of Mutations

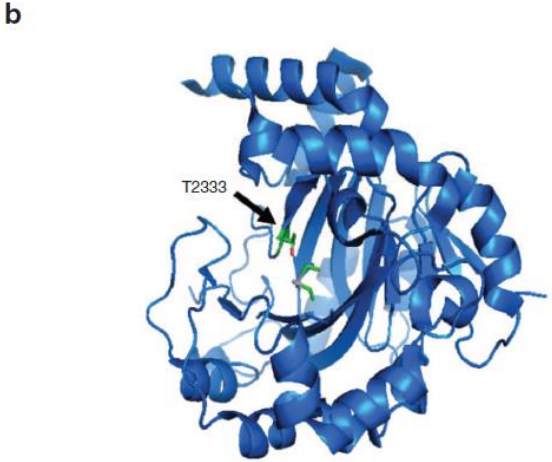
67 % Presence of Mutations

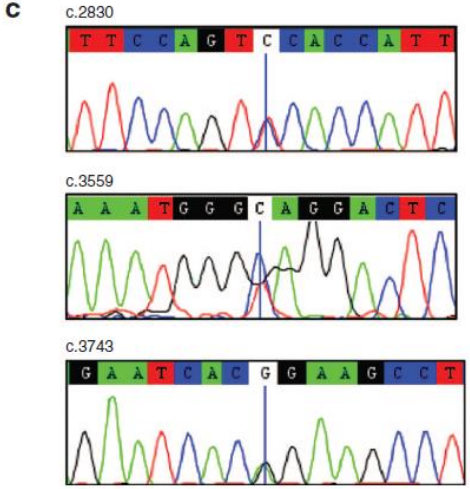
23 % Other Neurodevelopmental Disorders

67 % New Mutations

# Mutations in JMJD1C (jumonji domain containing 1C) are involved in Rett syndrome and intellectual disability



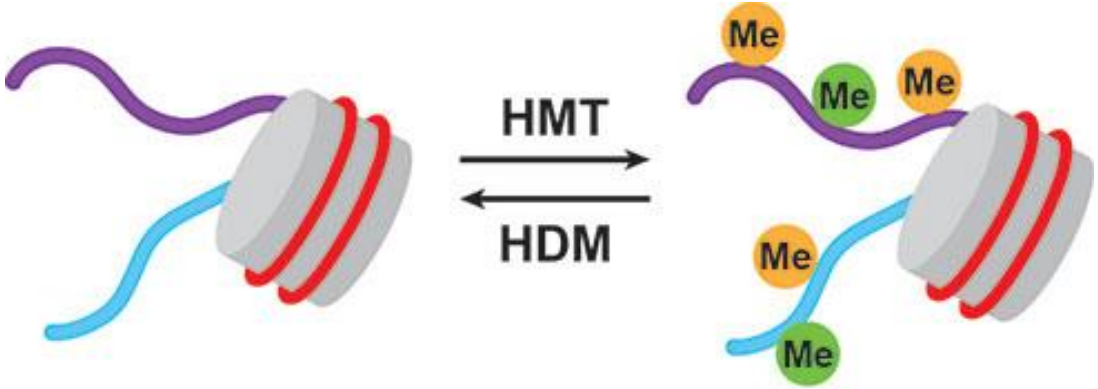




*Saez et al., Genetics in Medicine , 2015*

## Other Mutations in Histone Modifiers

## Are Also Involved in Intellectual Disability




**Dynamic methylation of histones**

HMT: histone methyltransferases; HDM: histone demethylases

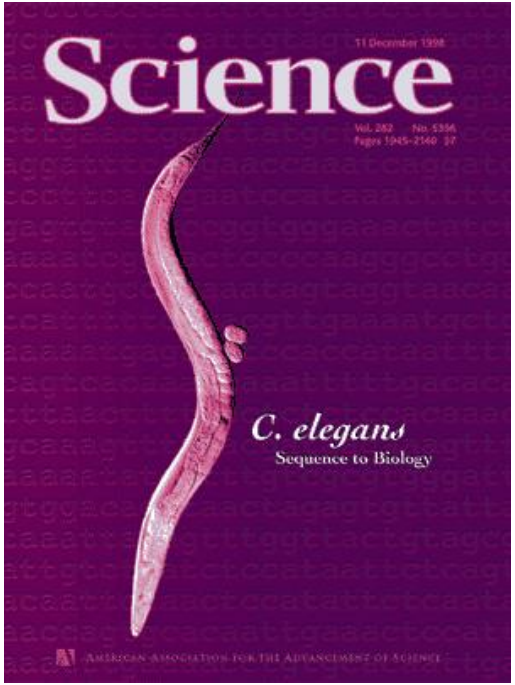
**Table 2** List of patients with variants found in genes previously associated with neurodevelopmental phenotypes

| Proband | Gene | Protein | NM number | Variant: genomic coordinates | cDNA change | Protein change | Gene-disease association |
|---|---|---|---|---|---|---|---|
| 4 | *HCN1* | Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 1 | NM_021072.3 | 5:45396665 | c.1159G > T | p.Ala387Ser | Early infantile epileptic encephalopathy 24 |
| 8 | *SCN1A* | Sodium Channel Protein Type I Subunit Alpha | NM_001165963.1 | 2:166866266 | c.3965C > G | p.Arg1322Thr | Dravet syndrome |
| 10 | *TCF4* | Transcription Factor 4 | NM_001243236.1 | 18:52901827 | c.958delC | p.Gln320Ser_fs8X | Pitt–Hopkins syndrome |
| 11 | *GRIN2B* | Glutamate receptor ionotropic, NMDA 2B | NM_000834.3 | 12:13764782 | c.1657C > A | p.Pro553Thr | Autosomal Dominant Mental Retardation 6; Early infantile epileptic encephalopathy 27 |
| 17 | *SLC6A1* | Solute Carrier Family 6 Member 1 | NM_003042.3 | 3:11067528 | c.919G > A | p.Gly307Arg | Myoclonic-atonic epilepsy and schizophrenia |

Dravet syndrome
SCN1A mutation

Pitt-Hopkins syndrome
TCF4 mutation

*Lucariello et al., Human Genetics 135, 1343-1354, 2016*



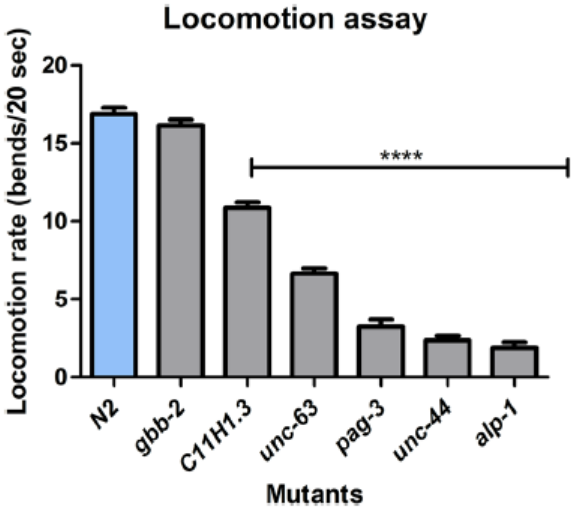




**Fig. 2** Locomotion assay in *Caenorhabditis elegans*. Functional validation of mutations was performed by measuring the locomotion rate, expressed in average of measuring, *in C. elegans*. Each mutant strain was compared to a wild-type N2 control strain by measuring worm body bends during 20 s in three independent sets of experiments. Locomotion rates of mutants, represented by *C11H1.3* (*MGRN1*), *unc-63* (*CHRNA5*), *pag-3* (*ZNF620*), *unc-44* (*ANKRD31*) and *alp-1* (*PDLIM7*) are significantly lower compared to that of the N2 control strain ($p < 0.0001$), on the contrary *gbb-2* (*GABBR2*) mutant move similarly. Standard error of the mean (SEM) values is shown. $p$ values obtained according to Student's $t$ test. ***$p < 0.0001$

# Final Update

# Mllt2h

Suggested transcriptional factor

Specifically expressed in Purkinje cell (Af4)

The Journal of Neuroscience, March 1, 2003 • 23(5):1631–1637 • 1631

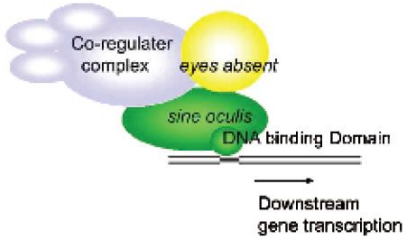
A Mutation in *Af4* Is Predicted to Cause Cerebellar Ataxia and Cataracts in the Robotic Mouse

Adrian M. Isaacs,[1*] Peter L. Oliver,[1*] Emma L. Jones,[1] Alexander Jeans,[1] Allyson Potter,[1] Berit H. Hovik,[1] Patrick M. Nolan,[2] Lucie Vizor,[2] Peter Glenister,[2] A. Katharina Simon,[3] Ian C. Gray,[4] Nigel K. Spurr,[6] Steve D. M. Brown,[2] A. Jackie Hunter,[5] and Kay E. Davies[1]

# Eya2

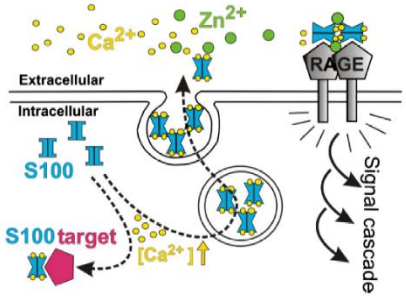
Transcriptional coactivator

Expressed during development of several organs




# S100a9

S100 calcium binding protein A9 (calgranulin B)




Marenholz I, Heizmann CW and Fritz G. (2004) Biochem Biophys Res Commun. 322:1111-22

Human diseases associated with S100 proteins

| Protein | Disease association |
|---|---|
| S100A1 | Cardiomyopathies |
| S100A2<br>S100A3<br>S100A4<br>S100A5<br>S100A6<br>S100A10<br>S100P<br>S100B | Cancer |
| S100A7<br>S100A7L1/A15 | Psoriasis |
| S100A8<br>S100A9<br>S100A12 | Inflammatory disorders |
| S100B | Neurodegeneration |

OPEN ACCESS Freely available online PLoS one

*Mecp2*-Null Mice Provide New Neuronal Targets for Rett Syndrome

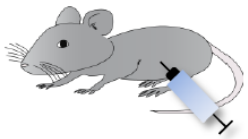
Rocio G. Urdinguio[1], Lidia Lopez-Serra[1], Pilar Lopez-Nieva[1], Miguel Alaminos[1], Ramon Diaz-Uriarte[2], Agustin F. Fernandez[1], Manel Esteller[1,3,4]

1 Cancer Epigenetics Laboratory, Spanish National Cancer Research Centre (CNIO), Madrid, Spain, 2 Statistical Computing Team, Spanish National Cancer Research Centre (CNIO), Madrid, Spain, 3 Cancer Epigenetics and Biology Program (PEBC), Catalan Institute of Oncology (ICO) and Institut d'Investigacio Biomedica de Bellvitge (IDIBELL), Barcelona, Catalonia, Spain, 4 Institucio Catalana de Recerca i Estudis Avancats (ICREA), Barcelona, Catalonia, Spain

**IRAK1 !!!!**

*Urdinguio et al., PLoS ONE, 2008*

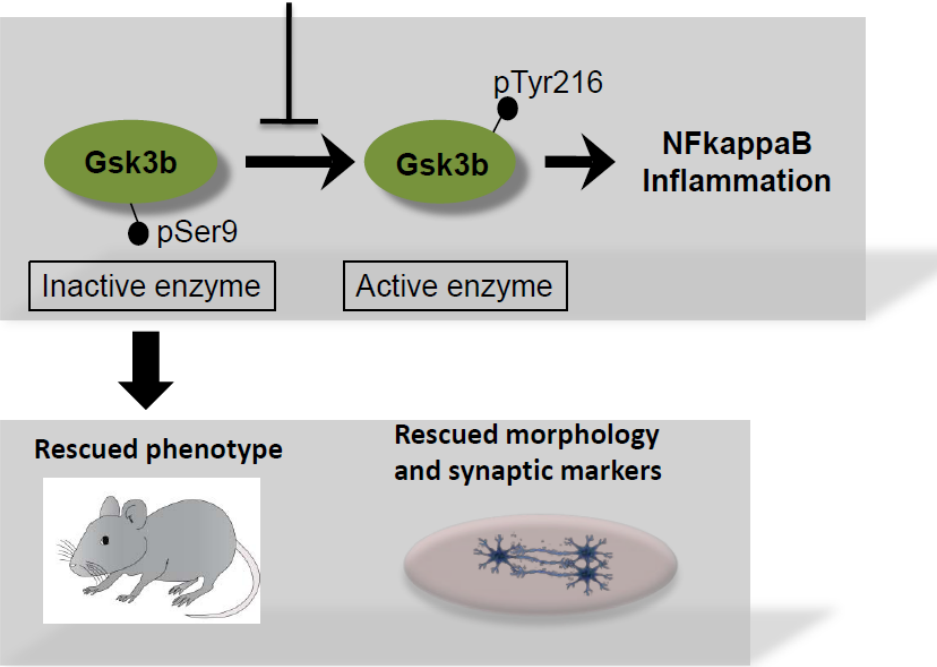
## Inhibition of Gsk3b reduces NFk-B signaling and rescues synaptic activity to improve the Rett syndrome phenotype in Mecp2-knockout mice



***Mecp2*** $^{-/y}$

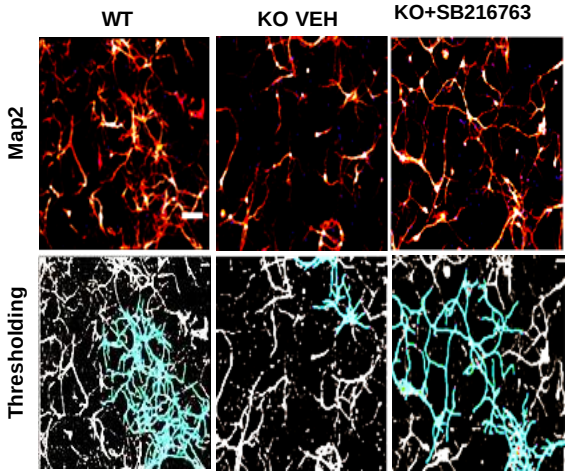
**Rett syndrome mouse model**

**SB216763 inhibitor**

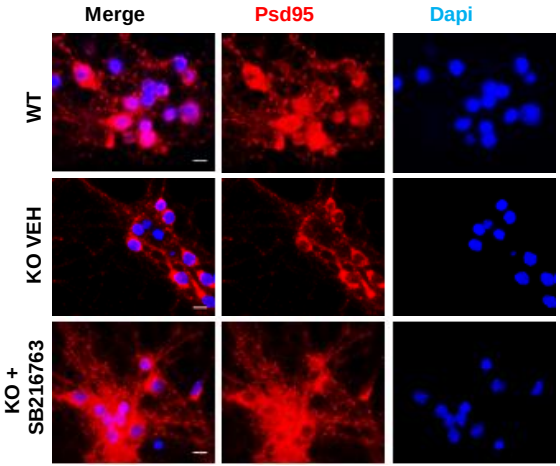



*Jorge-Torres et al., Cell Reports 2018*

# Treatment with a GSK3β inhibitor (SB216763) Improves Neuronal Functionality




**Branching**

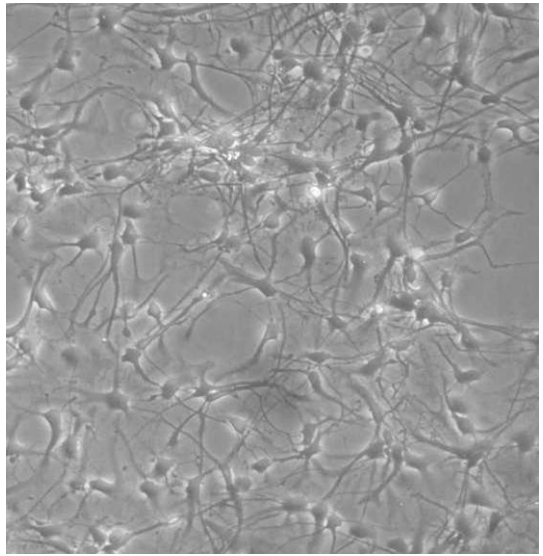



**Postsynaptic**

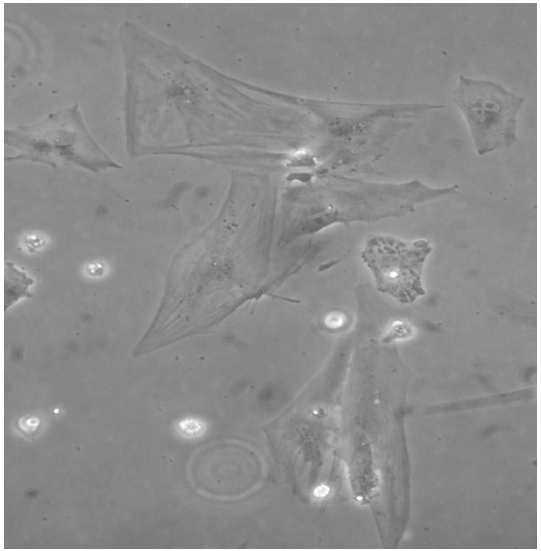
*Jorge-Torres et al., Cell Reports 2018*

# Future

# Genetic Edition of MeCP2 in Neurons

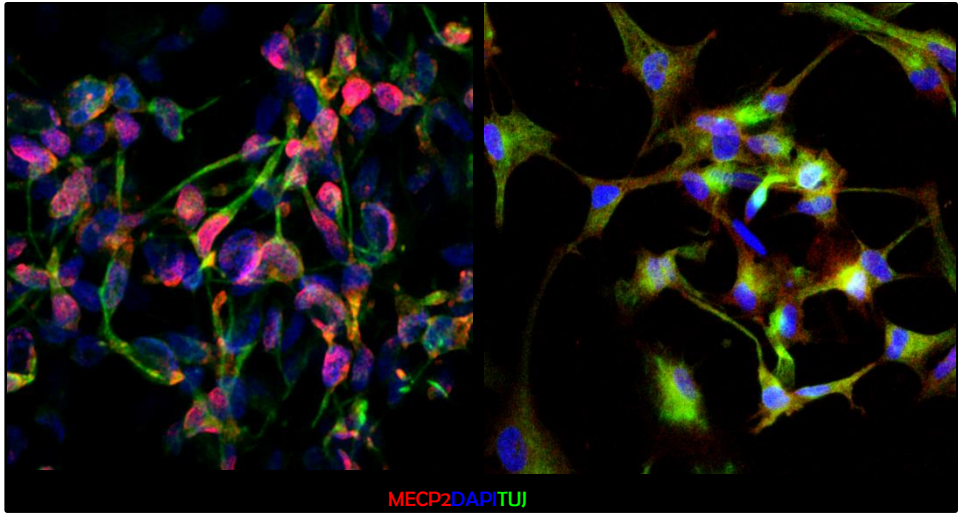


Normal Neurons

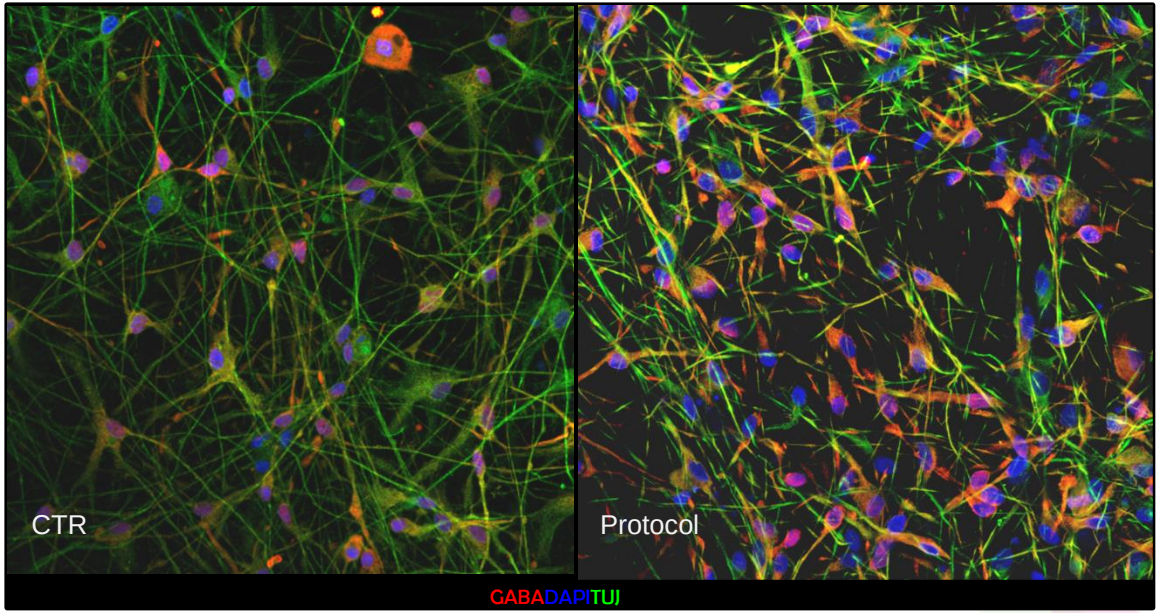


Neurons with MeCP2 Loss

# Genetic Edition of MeCP2 in Neurons



# Obtaining GABAergic Neurons



# Thanks to all the families and research supporters….

Sonia Guil

Raul Delgado-Morales

Catalan Rett Syndrome Association

Valencian Rett Syndrome Association

EuroRETT
European Network on Rett Syndrome

dischrom

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