The use of induced pluripotent stem cells to model neurodevelopmental disorders: *MECP2* duplication syndrome as an example

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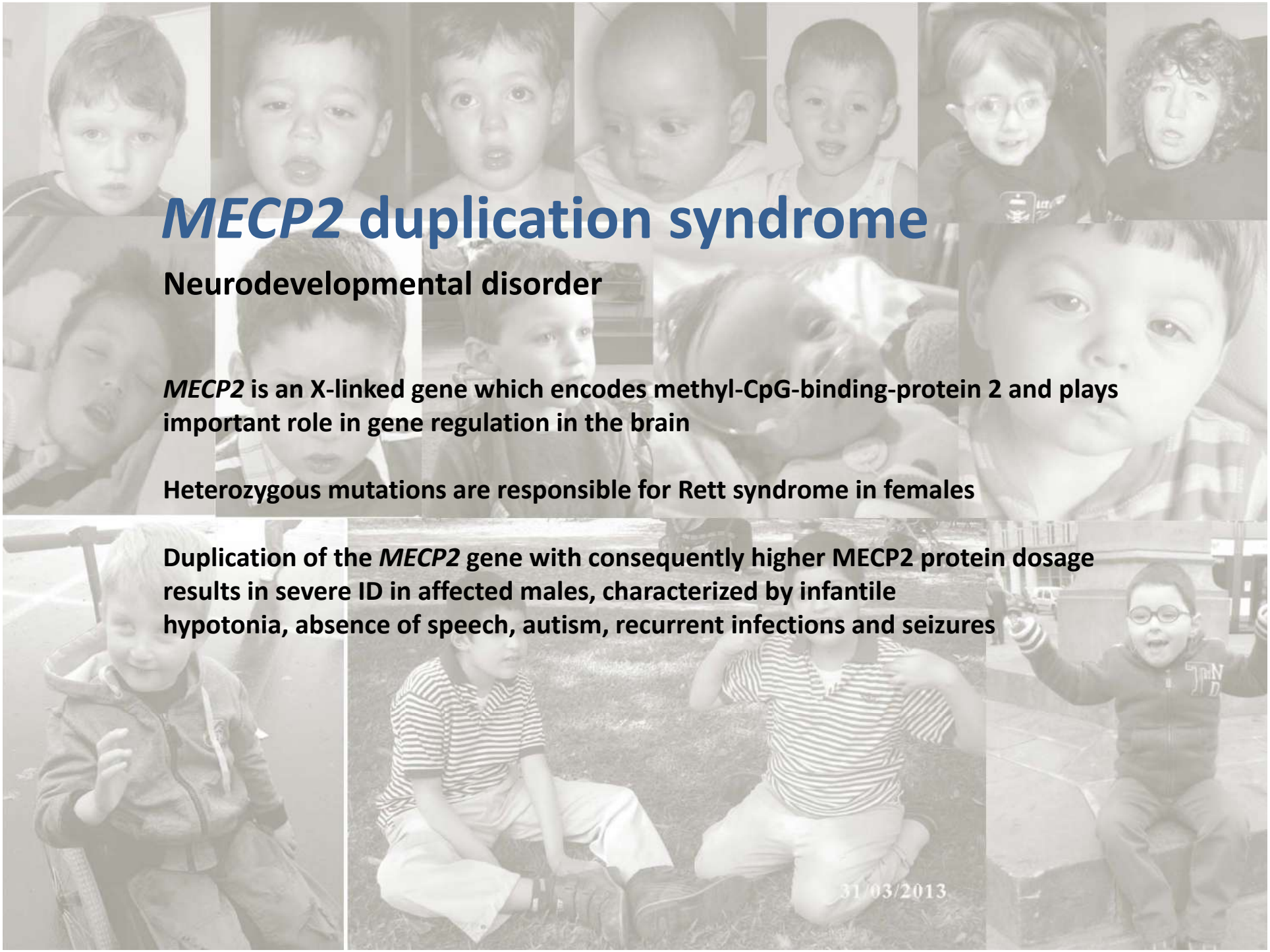


MECP2 duplication syndrome

Neurodevelopmental disorder

MECP2 **is an X-linked gene which encodes methyl-CpG-binding-protein 2 and plays important role in gene regulation in the brain**

Heterozygous mutations are responsible for Rett syndrome in females

Duplication of the *MECP2* gene with consequently higher MECP2 protein dosage results in severe ID in affected males, characterized by infantile hypotonia, absence of speech, autism, recurrent infections and seizures

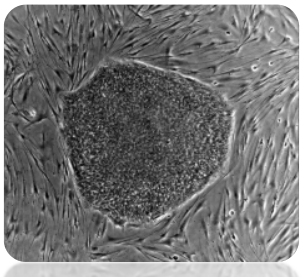
Cellular reprogramming in disease modeling

- Lack of human brain tissue is a major hurdle to study the pathophysiology
- Mouse models, however, often fail to recapitulate all features observed in humans which greatly affects further insights into the disease pathology
- Alternative approach is to use the **induced pluripotent stem cell (iPSC) technology** to develop a valid disease model
- iPSCs are similar to embryonic stem cells in gene expression, ability to form all the three germ layers and *in vivo* chimera formation

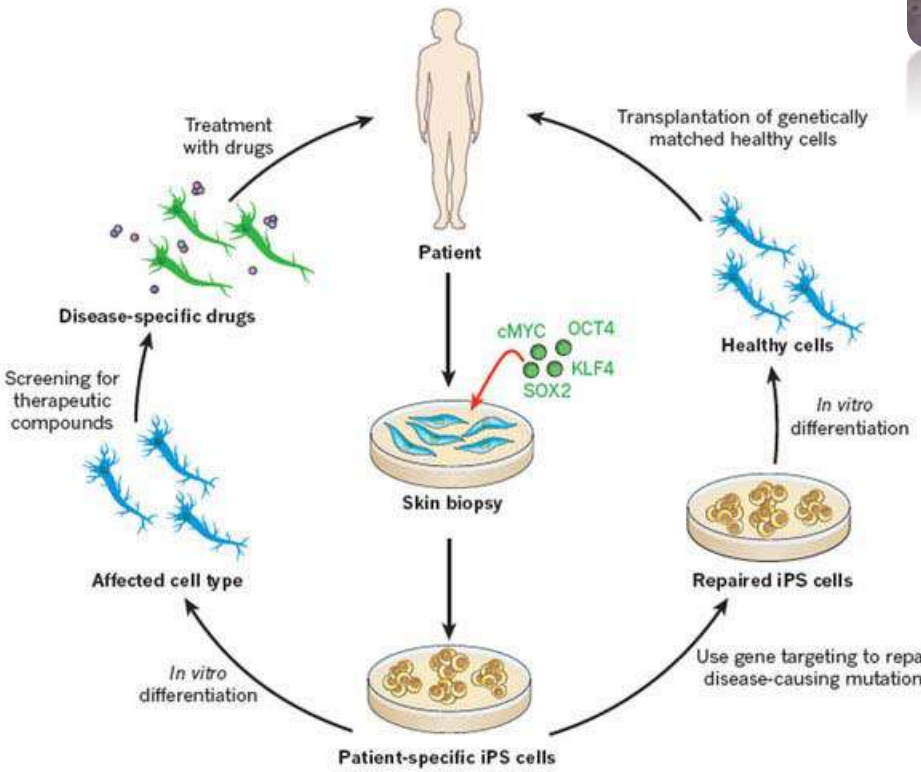
Applications of induced pluripotent stem cells

Fibroblast

Oct4
Sox2
Klf4
Myc



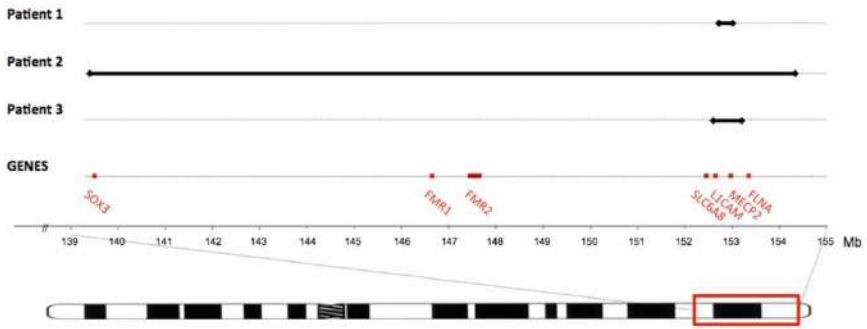
iPSC

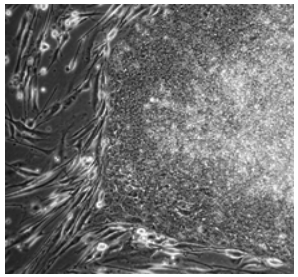


- Humanized model: mechanistic insight
- Pre-clinical model: compound screening
- Cell therapy and tissue engineering

The potential of human iPSCs to differentiate into cells of almost any tissue type of the human body provides exciting new opportunities for *in vitro* research and therapeutic intervention

Generation and pluripotent gene analysis of Mecp2dup-iPSCs

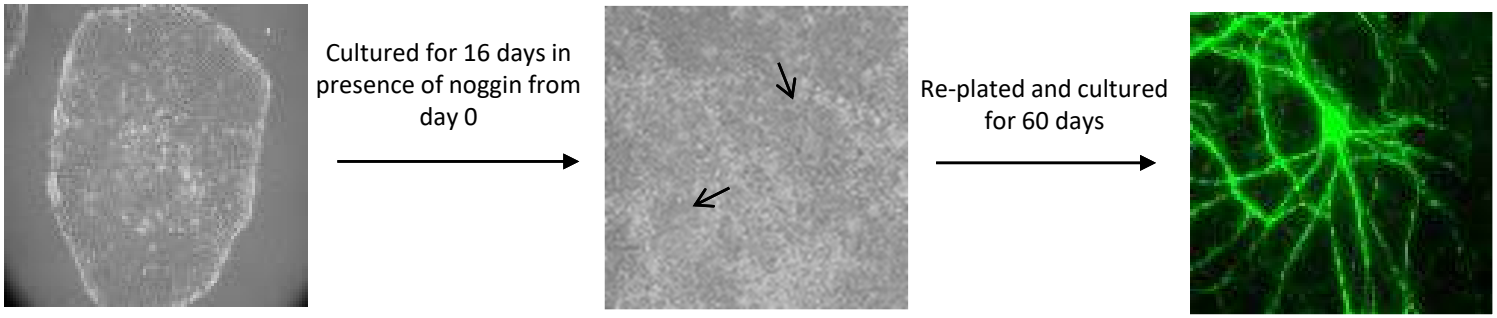




Readout of an authentic iPS cell:

- Pluripotent gene expression analysis
- Silencing of transgenes
- Immunofluorescence analysis
- Teratoma assay

Differentiation of pluripotent cells to neurons of cortical identity

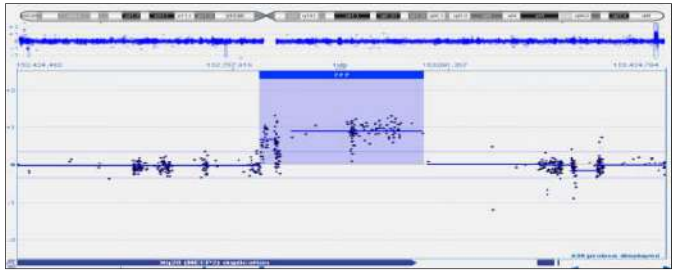


- ESCs upon differentiation, efficiently generated cortical-like neurons which expressed cortical neuronal genes like *RELN*, *CTIP2* and *TBR1*

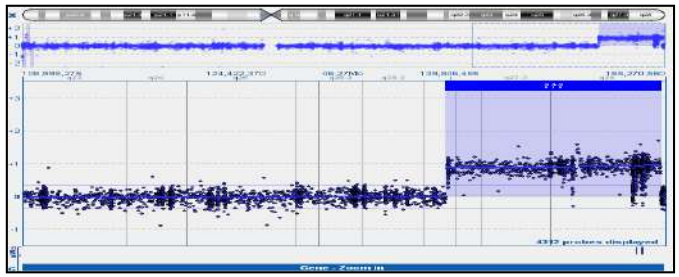
Espuny-Camacho I *et al*. Neuron 2013

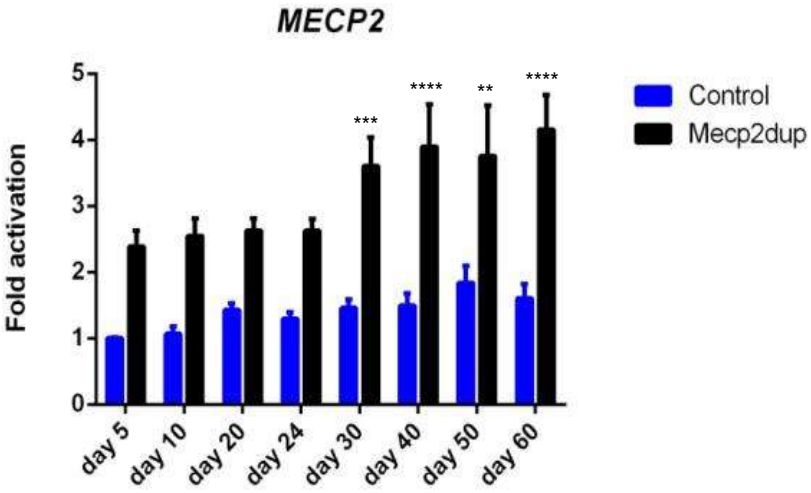
Analysis of MECP2 expression in differentiated neurons

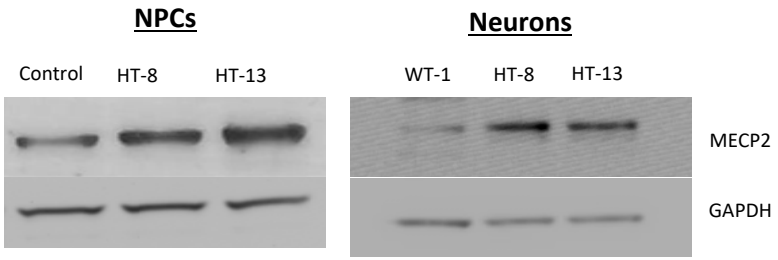
HT-13

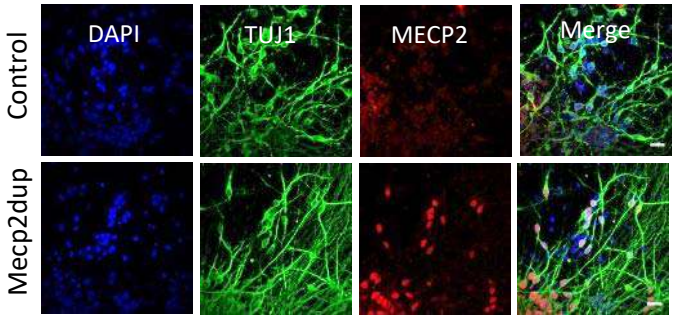


2MC3



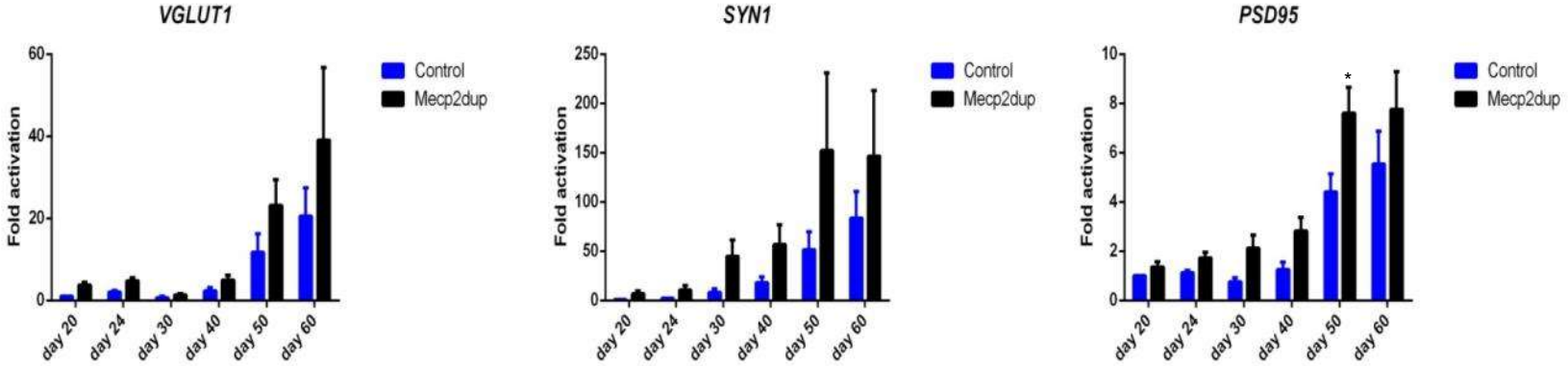


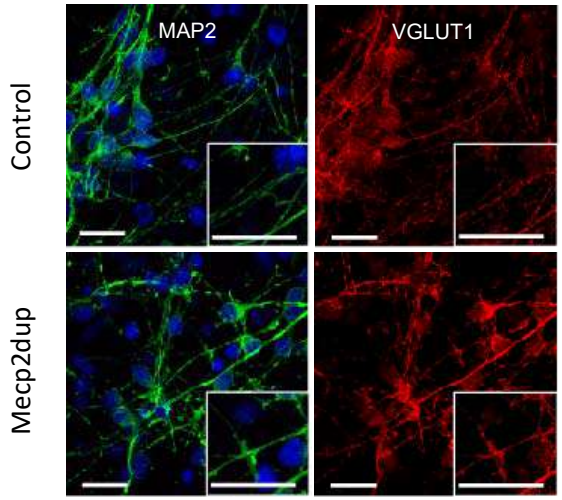


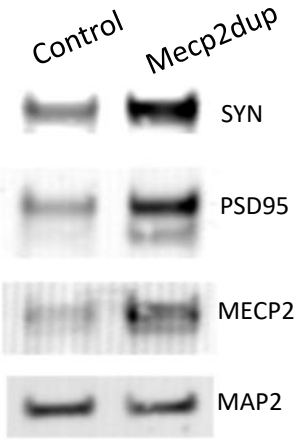


- Duplication of *MECP2* gene is maintained in disease neurons

Expression analysis of synaptic gene transcripts and proteins



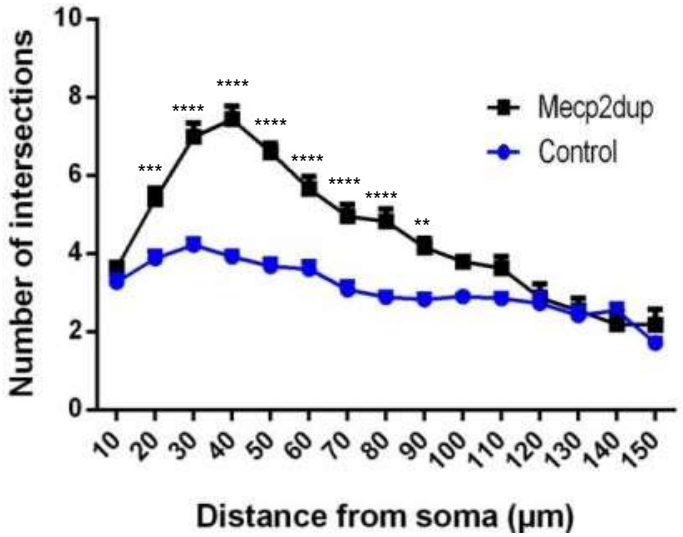


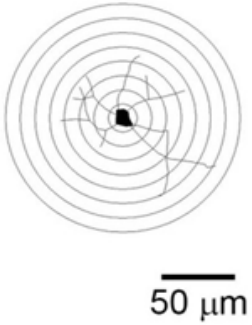


- Mecp2dup neurons show enhanced expression of *VGLUT1*, *SYN1*, and *PSD95* when compared to control neurons

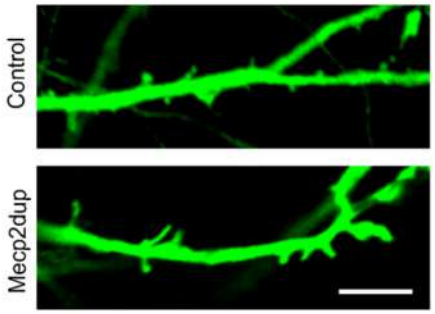
Morphological analysis of Mecp2dup-cortical neurons

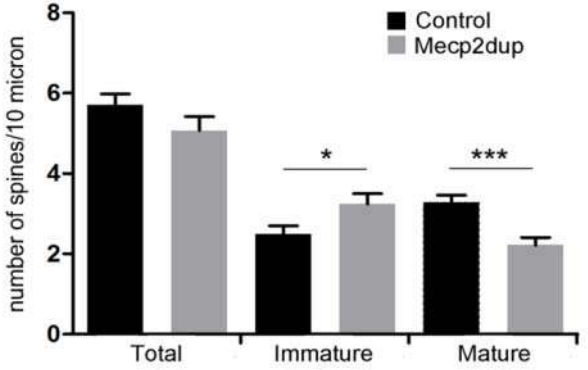
Sholl analysis





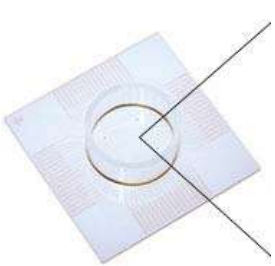
Spine analysis

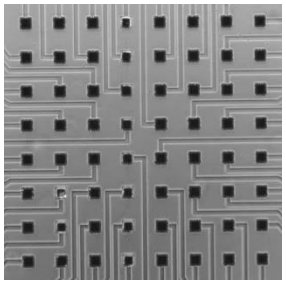


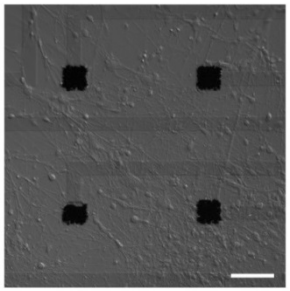


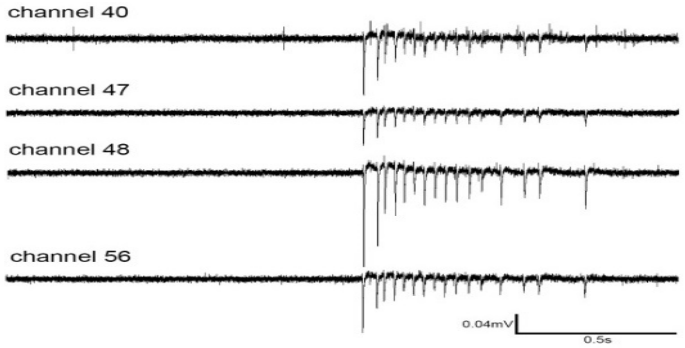
- Patient neurons show significant increase in dendritic complexity
- Significant increase in immature dendritic spine density is observed in Mecp2dup cortical-like neurons

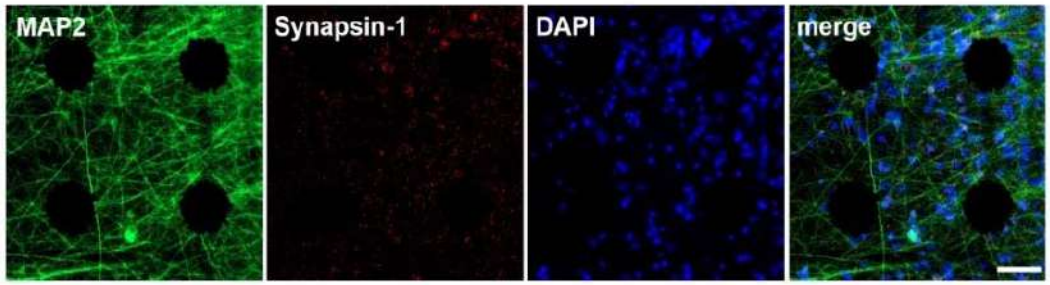
Electrophysiological analysis of Mecp2dup cortical-like neurons

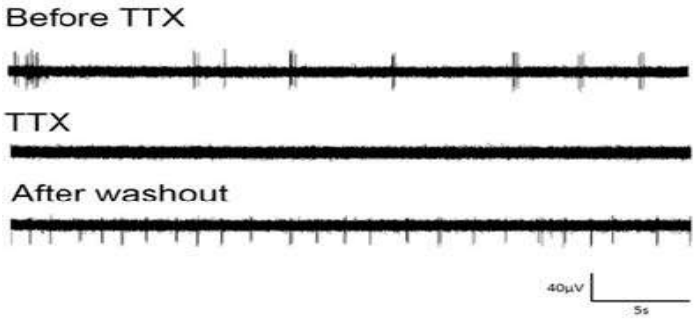








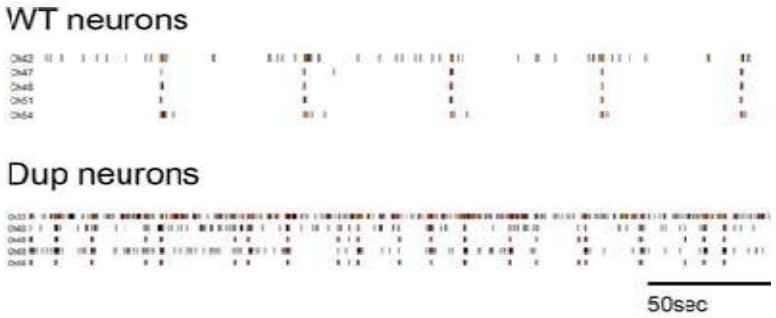


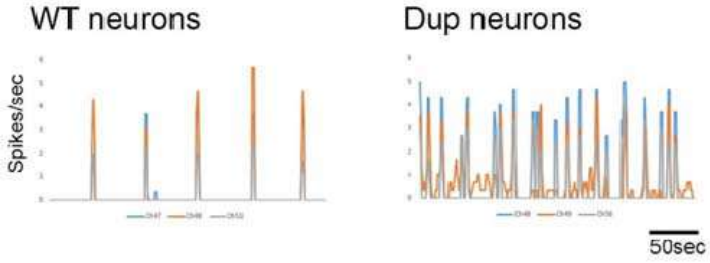


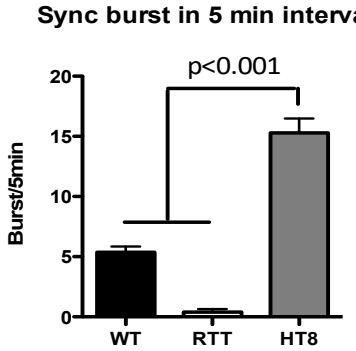
In collaboration with Prof. Muotri, UCSD

- Spontaneous neuronal activity of 4 independent channels show synchronized bursts
- Immunostaining of MAP2 and SYN1 positive Mecp2dup-neurons growing on MEA Chip.

Electrophysiological analysis of Mecp2dup cortical-like neurons







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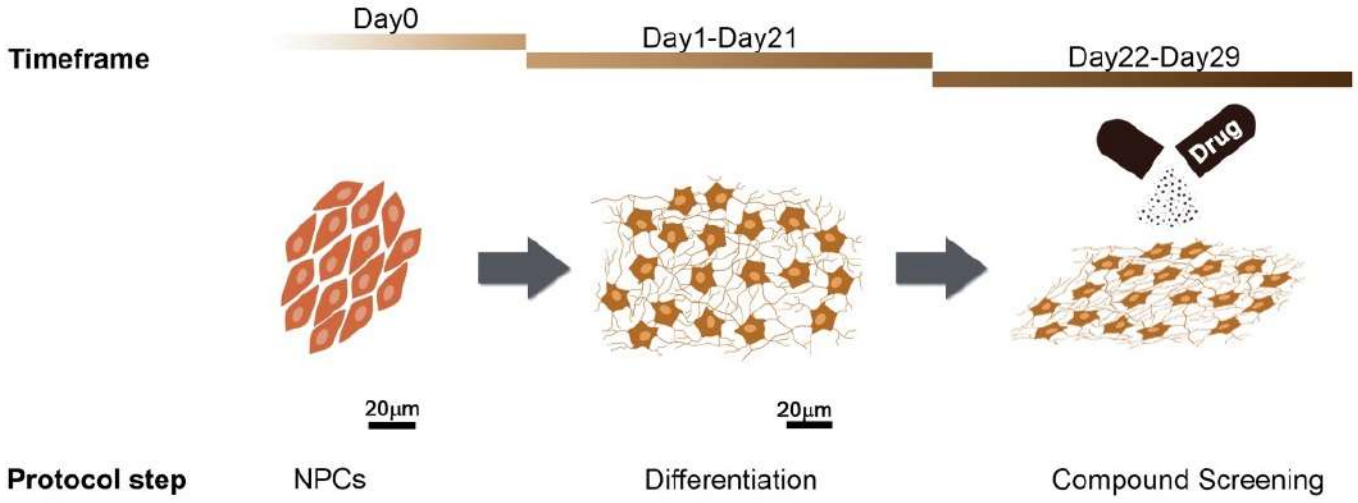
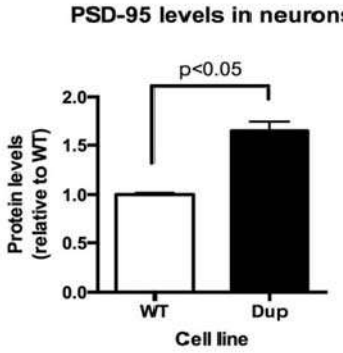
- Mecp2dup-neurons display altered electrophysiology network properties
- iPSC derived neurons are functional and Mecp2dup-neurons exhibit enhanced sync burst activity compared to WT neurons which is opposite to what is seen in case of RTT neurons

Rescue of the disease phenotype ?

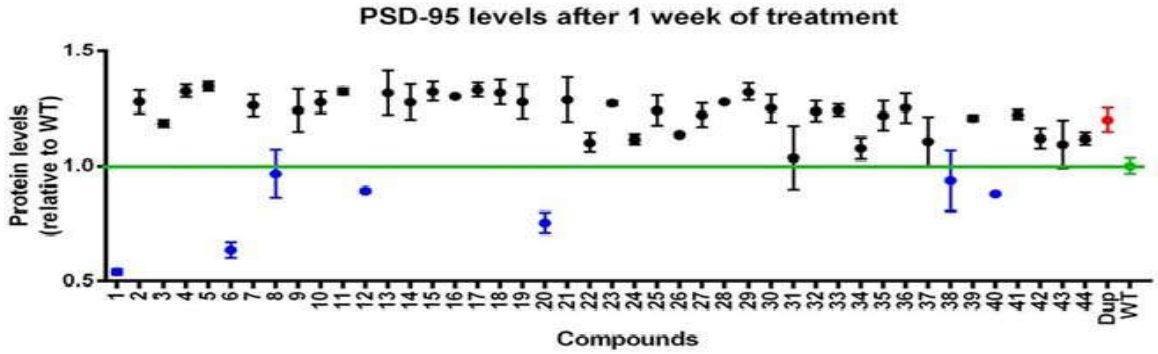
Screening of epigenetic compounds in Mecp2dup-neurons



Screening of epigenetic compounds in Mecp2dup-neurons



No.	Compound	Mechanism of Action	Average Value
8	Scriptaid	HDAC inhibitor	0.966
22	BML-266	SIRT2 inhibitor	1.101
24	Fluoro-SAHA	HDAC inhibitor	1.115
26	AGK2	SIRT2 inhibitor	1.136
34	Oxamflatin	HDAC inhibitor	1.077
38	NCH-51	HDAC inhibitor	1.112
42	BML-281	HDAC-6 inhibitor	1.119



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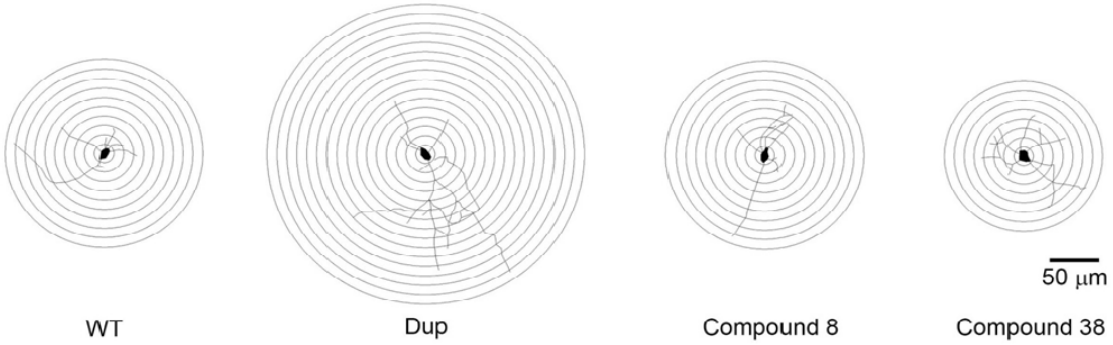
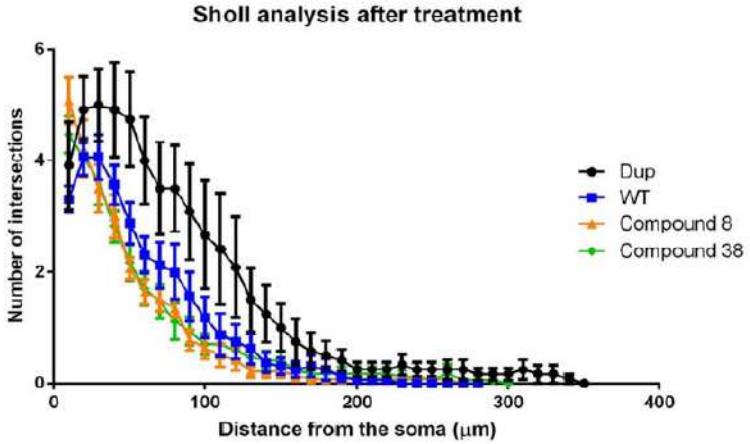
- Mecp2dup-neurons exhibit enhanced post-synaptic protein PSD-95
- Compound screening using library of epigenetic modulators showed rescue of PSD-95 level

Morphological rescue

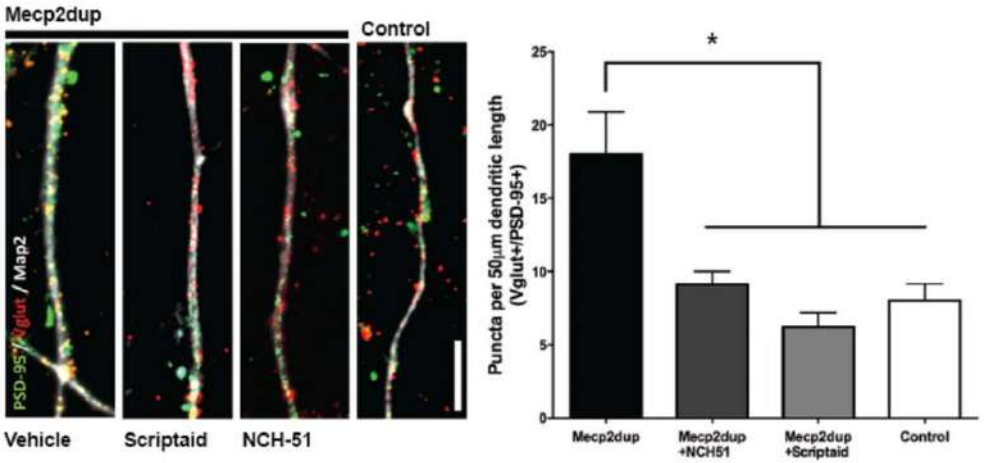




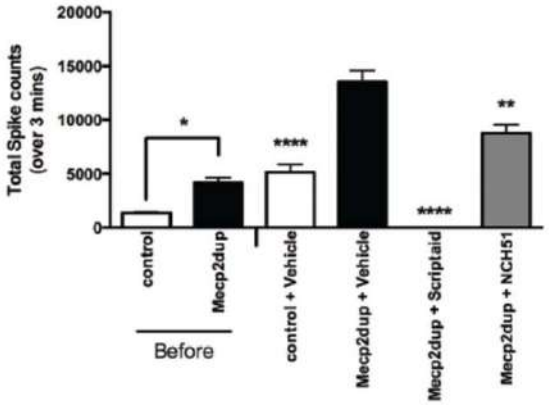
- Among the screened compounds, compound 8 and 38 which corresponds to HDAC inhibitors showed effective rescue in PSD-95 and morphological phenotype

Functional rescue

Glutamatergic puncta after treatment



MEA rescue after treatment



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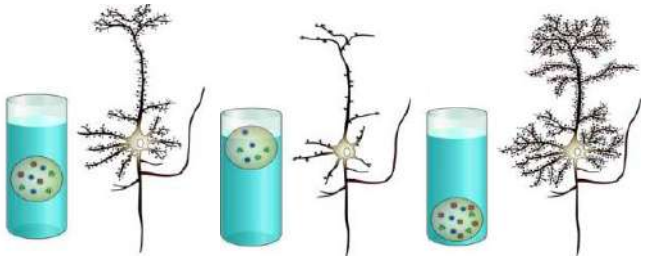
- Among the screened compounds, only one compound could also rescue the altered neuronal network activity

Conclusions of this study

- Epigenetic modifiers were able to restore PSD-95 protein level and also rescue the morphology of the affected neurons similar to that of control neurons
- One modifier was also able to rescue the electrophysiological phenotype
- The data obtained from our study and from studies using *MECP2*-Tg mice and human Rett iPSC derived neurons convey that **balanced dosage of MECP2 is critical for normal brain function**



➡ **iPSCs can be considered as an ideal tool for designing a human model to understand the mechanism of neurodevelopmental disorders**

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Limitations & Challenges

- Human induced pluripotent stem cells are not always genetically stable and the role of epigenetic factors and processes during reprogramming and differentiation is not well understood
- Dish ≠ living organism
- Reproducibility can be a problem, many biological replicates are necessary/more patients
- High throughput screening is very costly
- Human brain consists of many different cell types that “live and work” together
- Ideal tool for screening of compounds, however, still difficult to predict effect of same compound in living organism/ side effects

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Patients & families