

***Modeling Rett syndrome with
human neurons – insights into
regression***

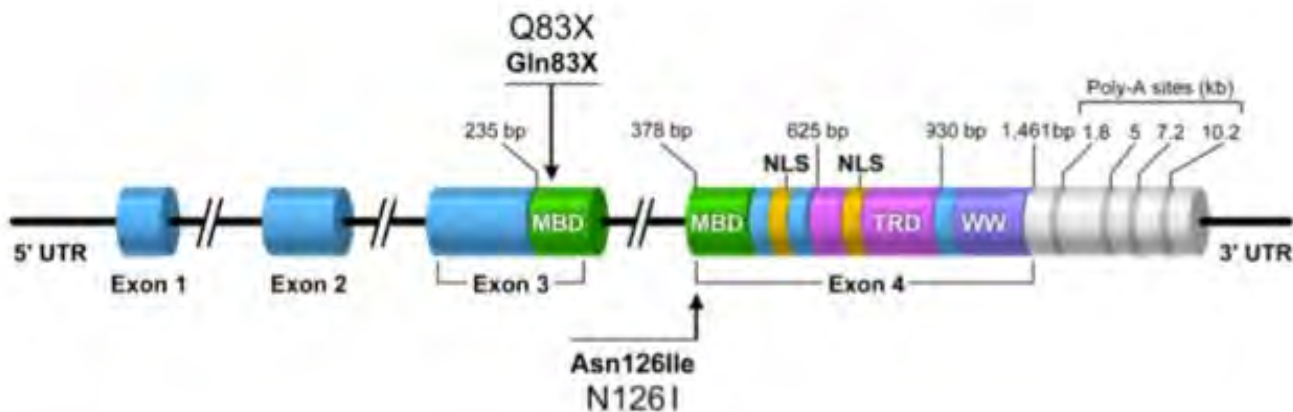
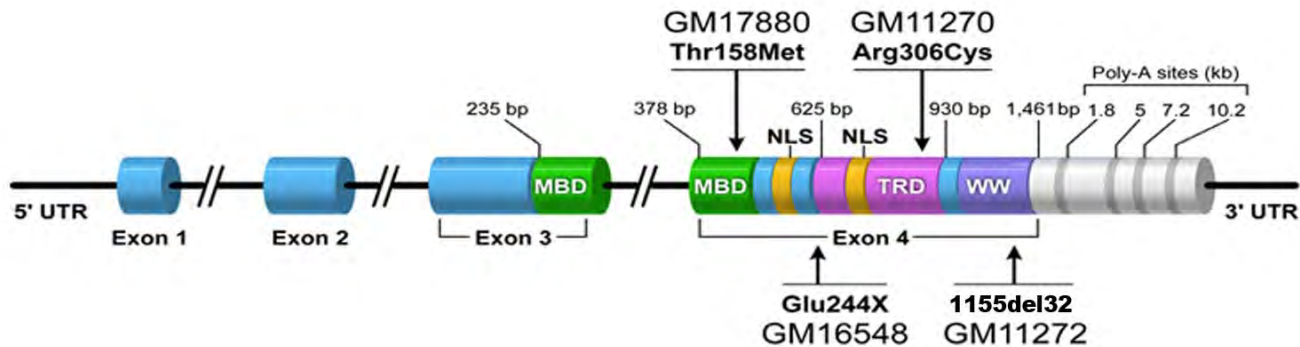
Alysson Renato Muotri

University of California San Diego

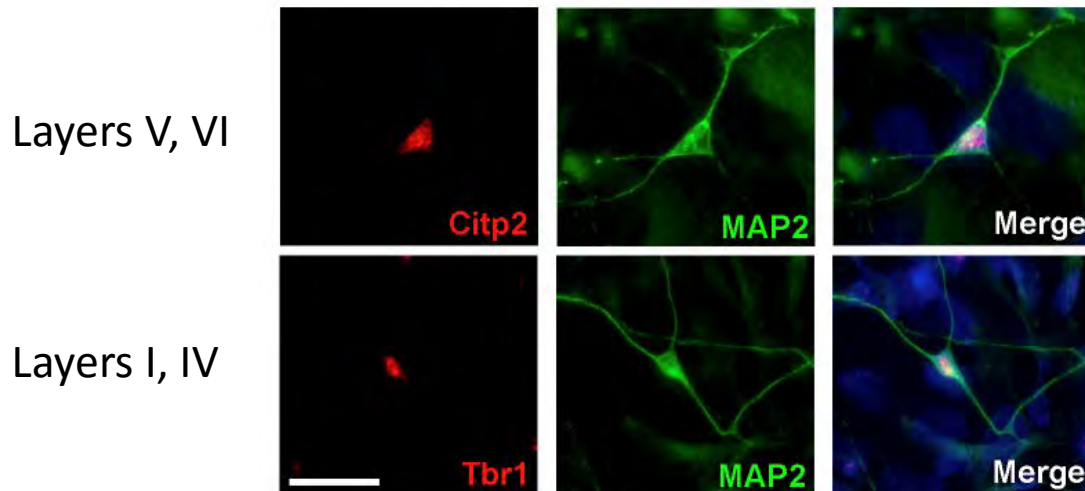
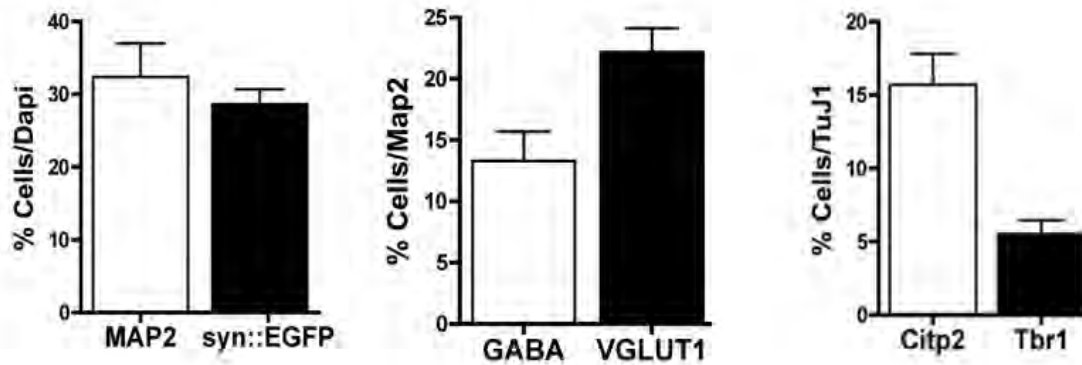
Dept. Pediatrics/Cellular Molecular Medicine

MeCP2 mutants (X-LINKED GENE)

Girls

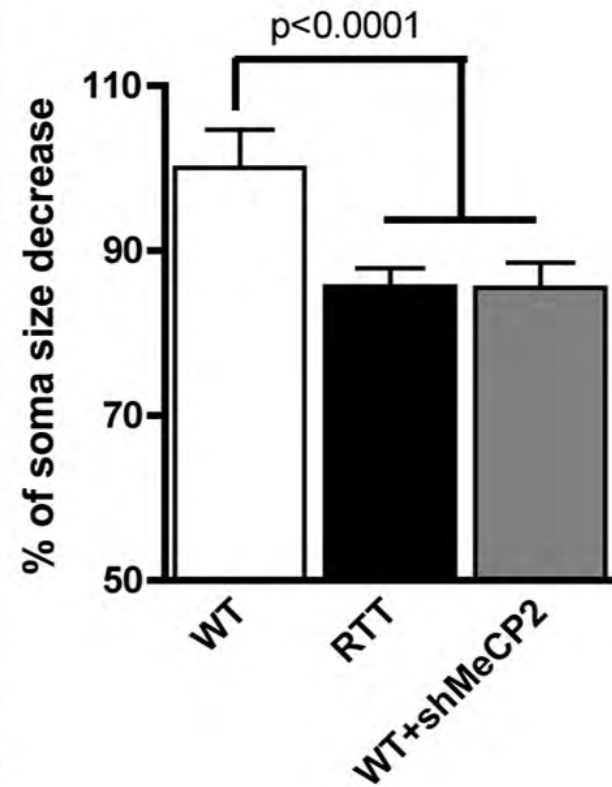
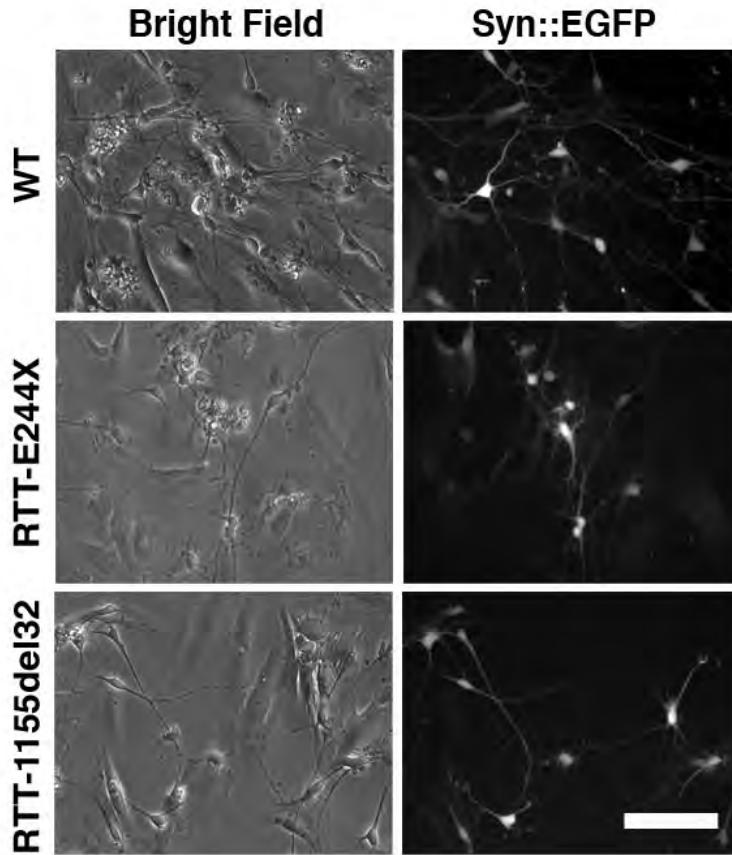


iPSC-derived cortical neurons

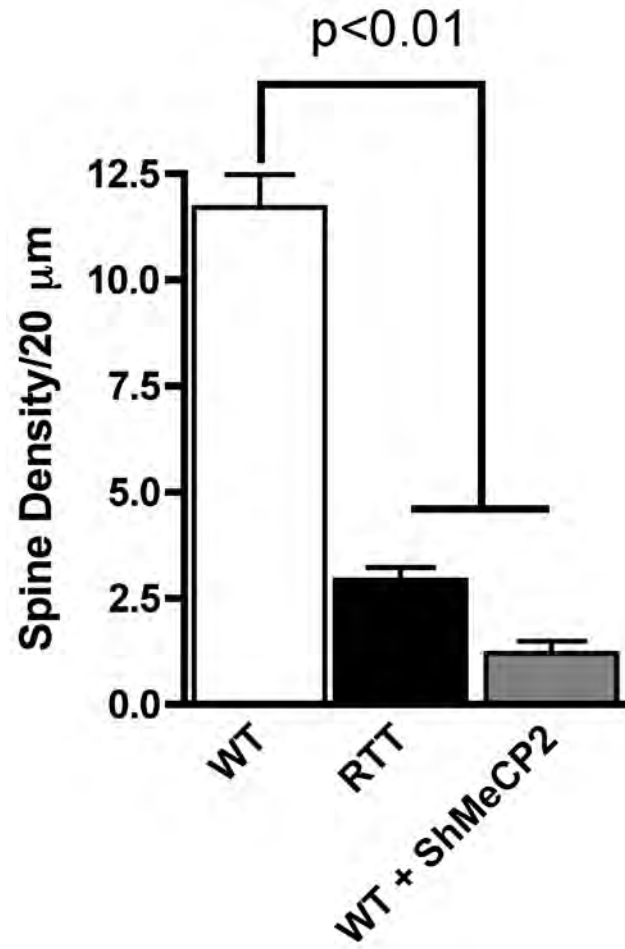
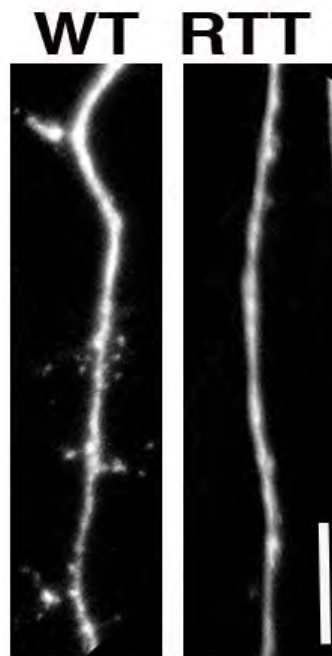


*Expression of Peripherin and En1 (midbrain) were not detected.

RTT neurons have smaller cell neuronal soma

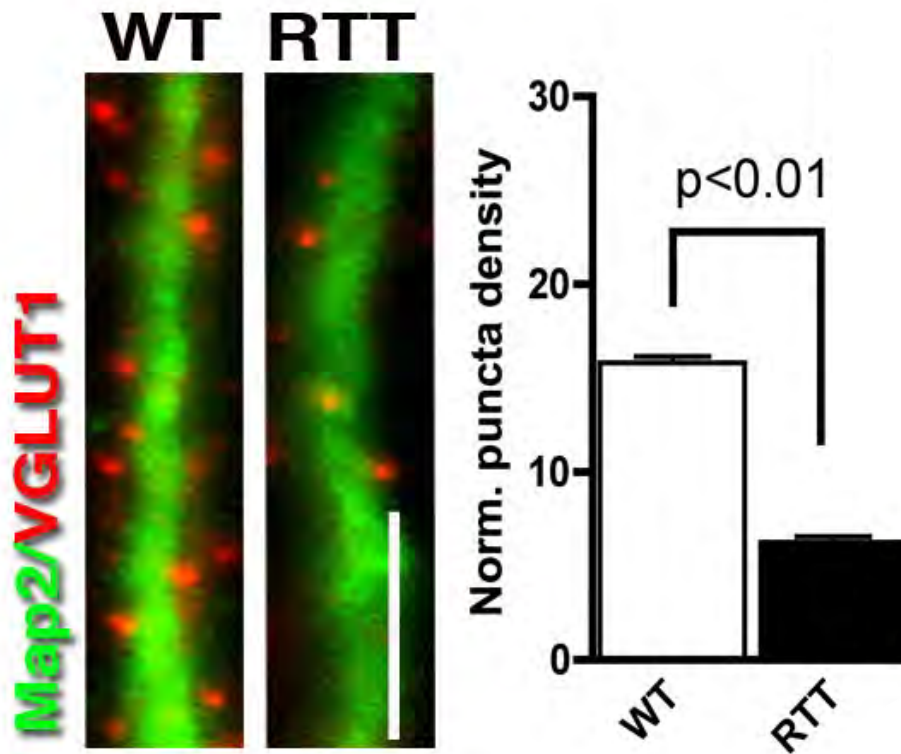
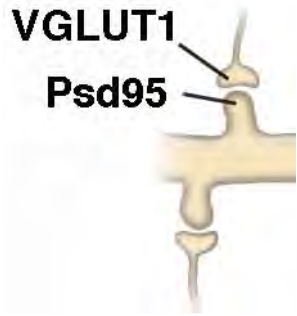


RTT neurons have lower spine density

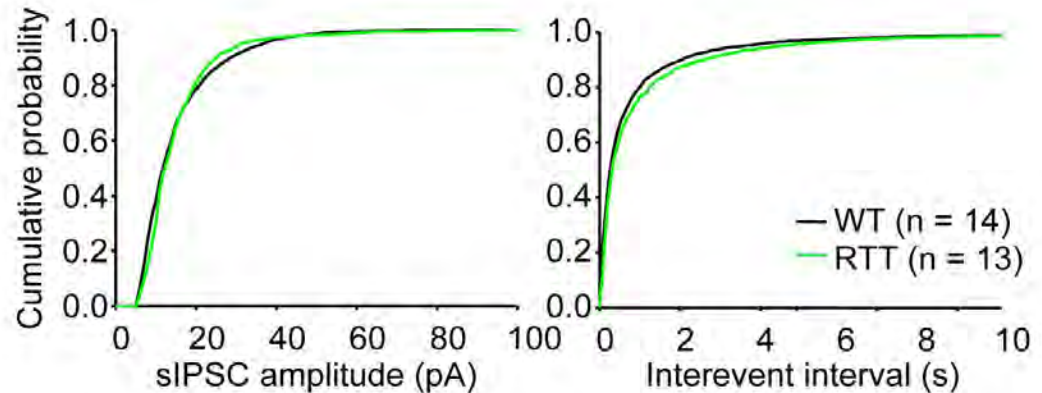
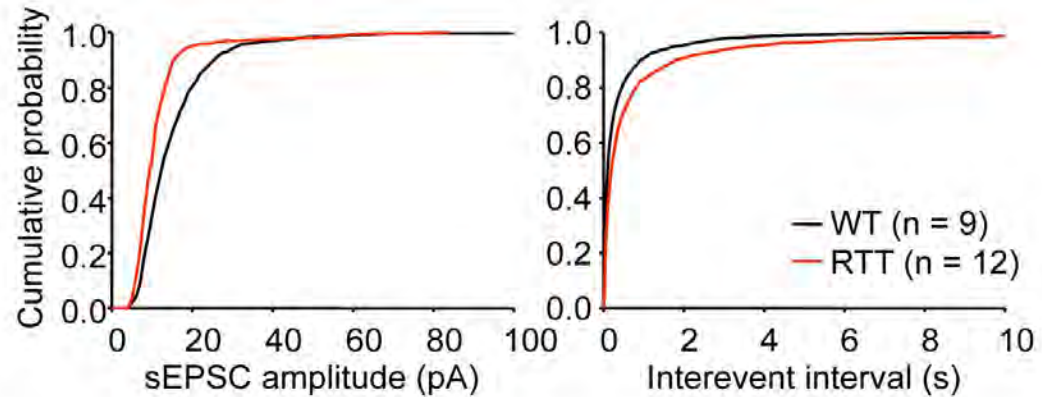
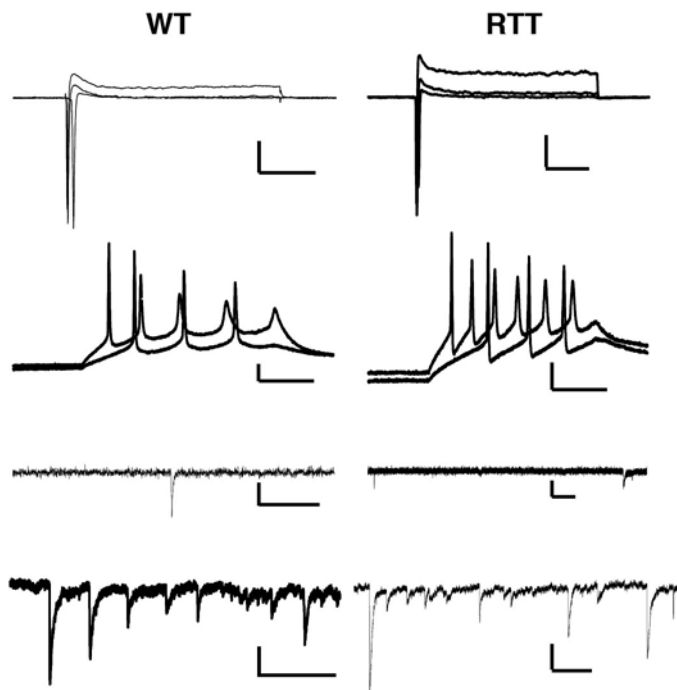
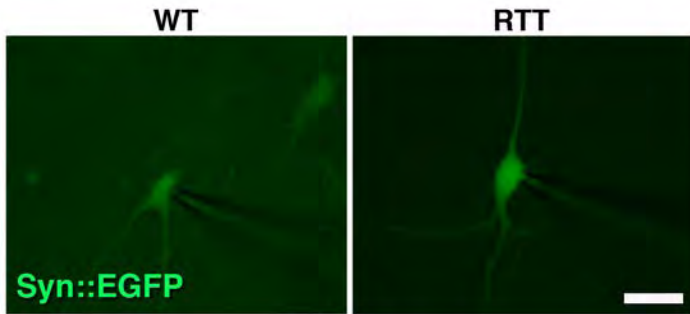




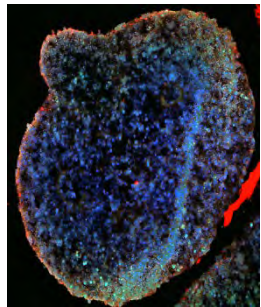
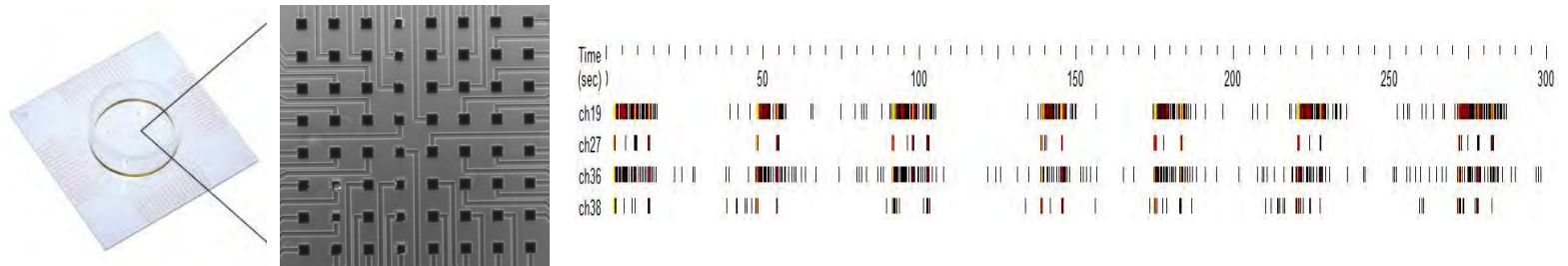
RTT neurons have fewer glutamatergic synapses



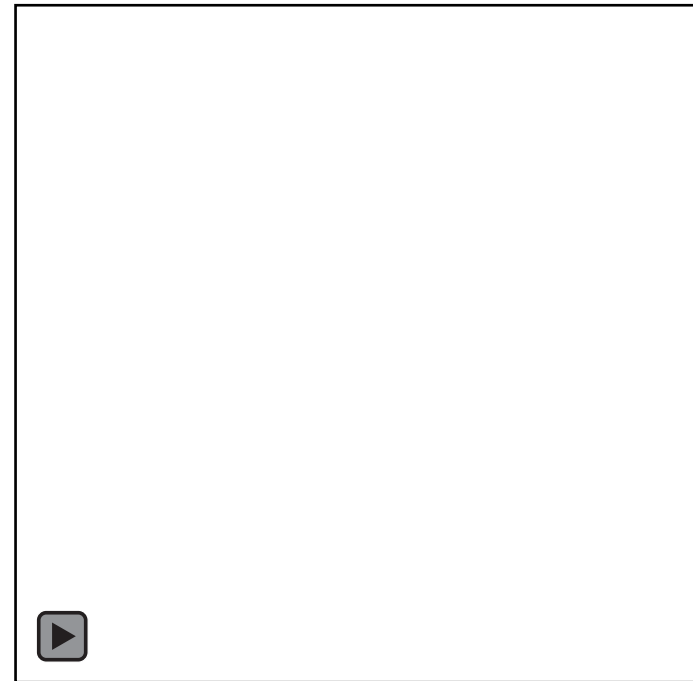
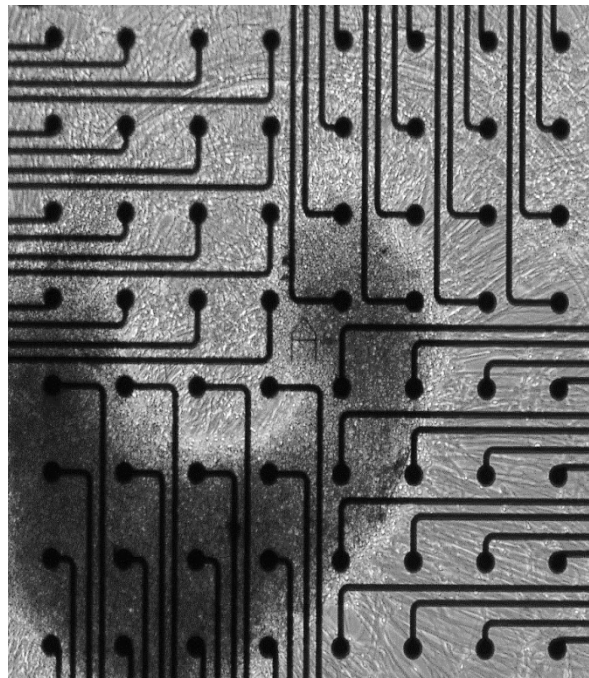
Decrease frequency of spontaneous postsynaptic currents in RTT neurons



RTT neuronal networks are not synchronized

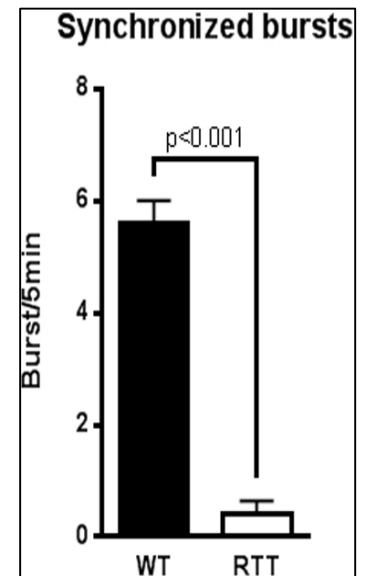
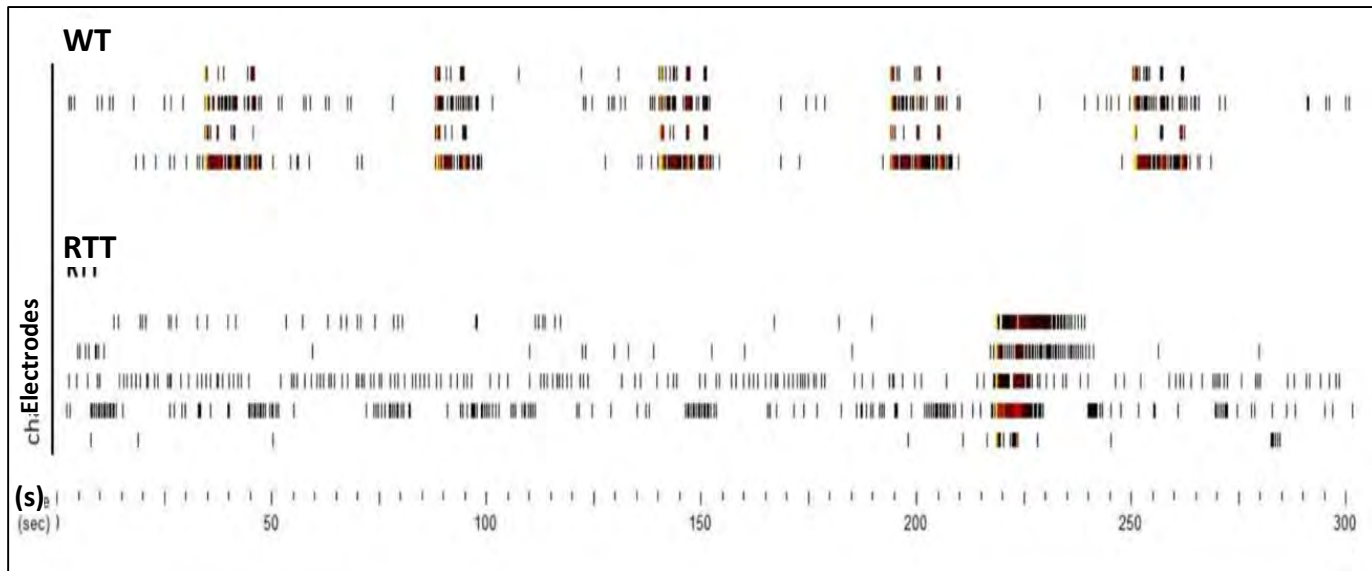


3D corticaloids

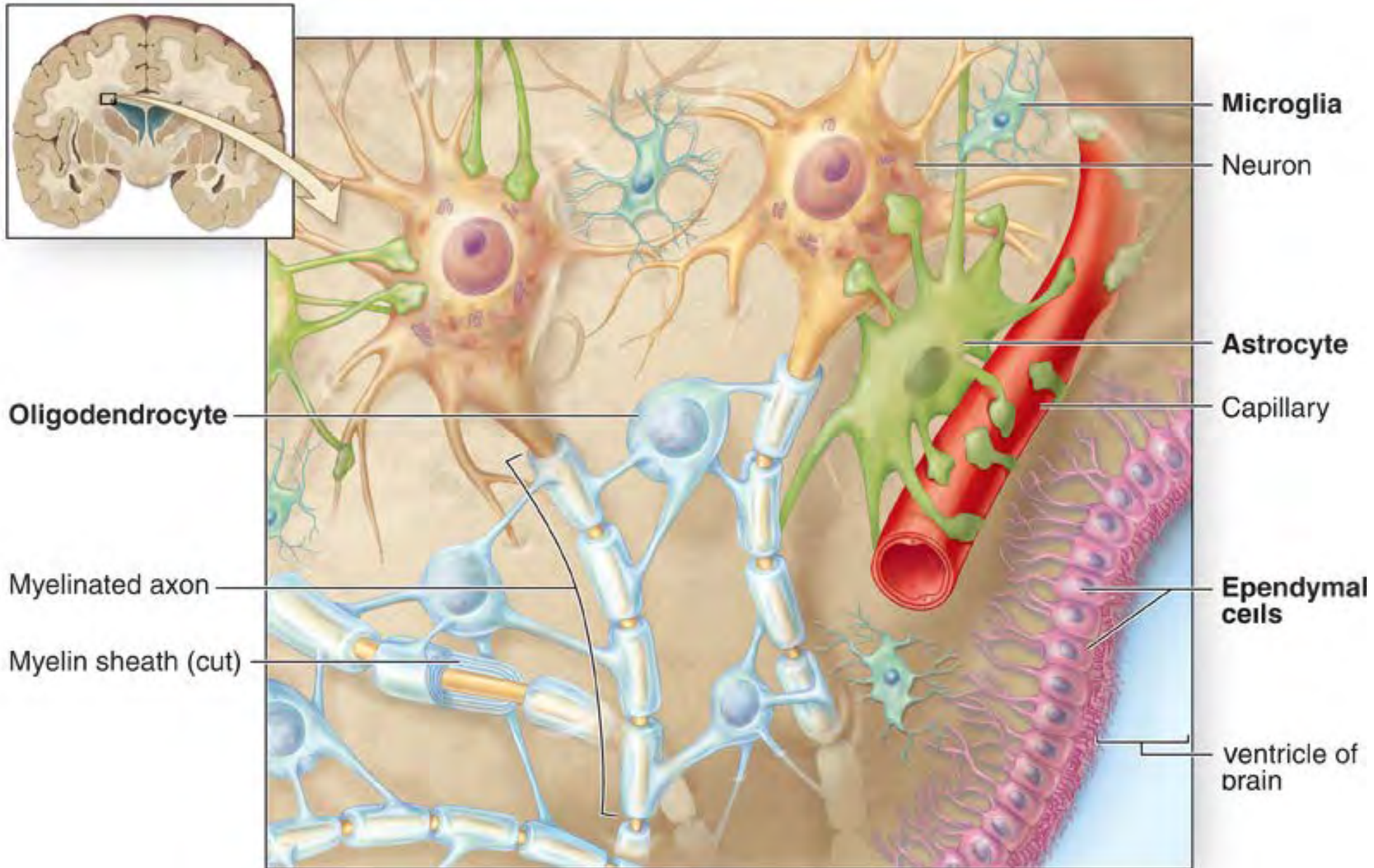


Activity Map

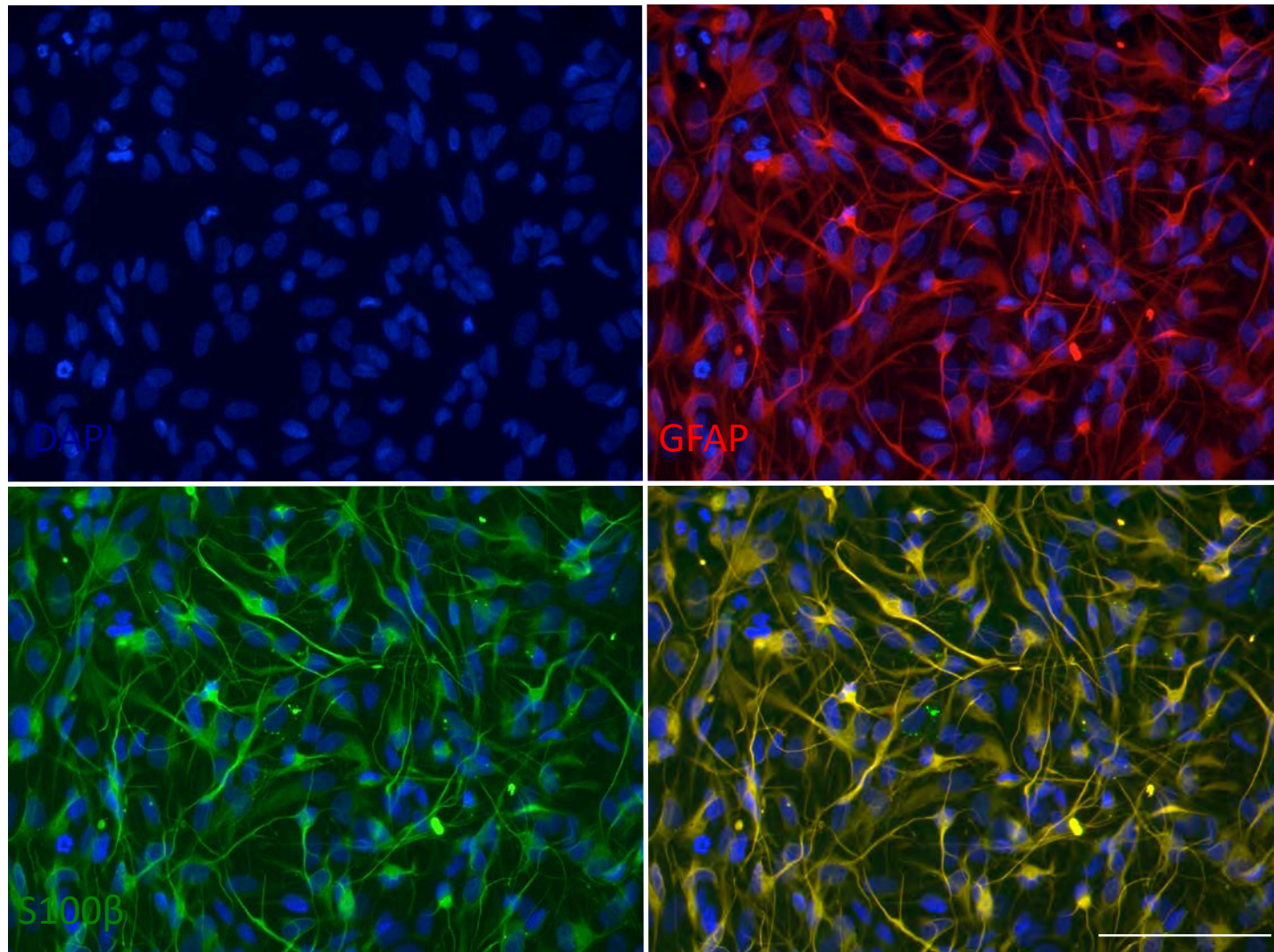
RTT neuronal networks are not synchronized



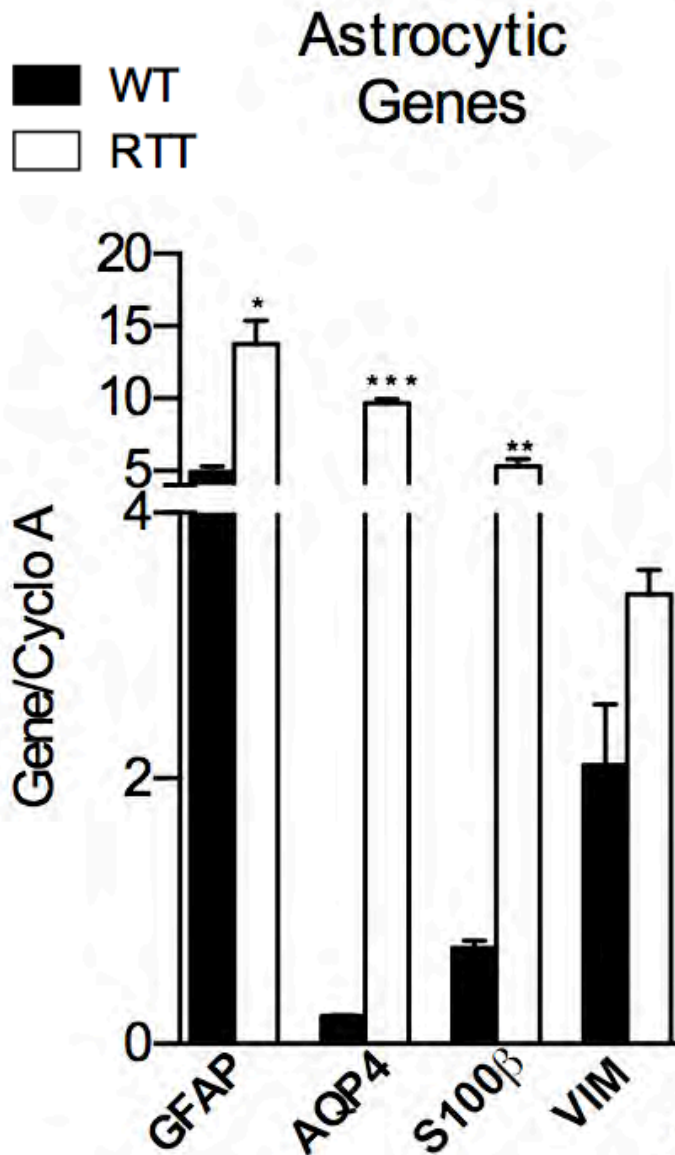
GLIA



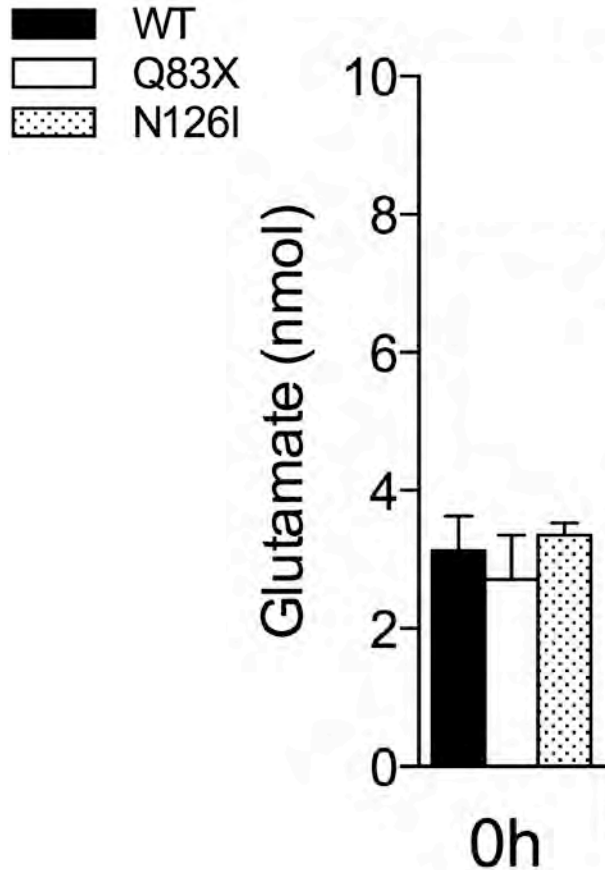
Muotri lab iPSC astrocyte protocol (30 days, no growth factors)



RTT astrocyte altered gene expression



RTT astrocytes have slower glutamate clearance



RTT astrocytes have impaired calcium waves

WT

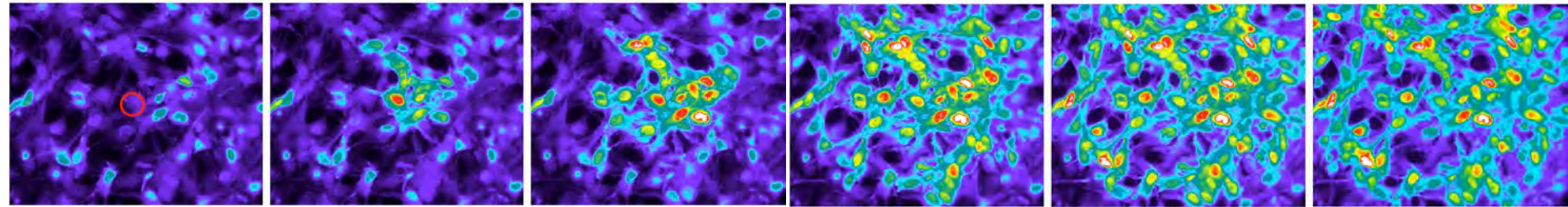
1.5s

1.5s

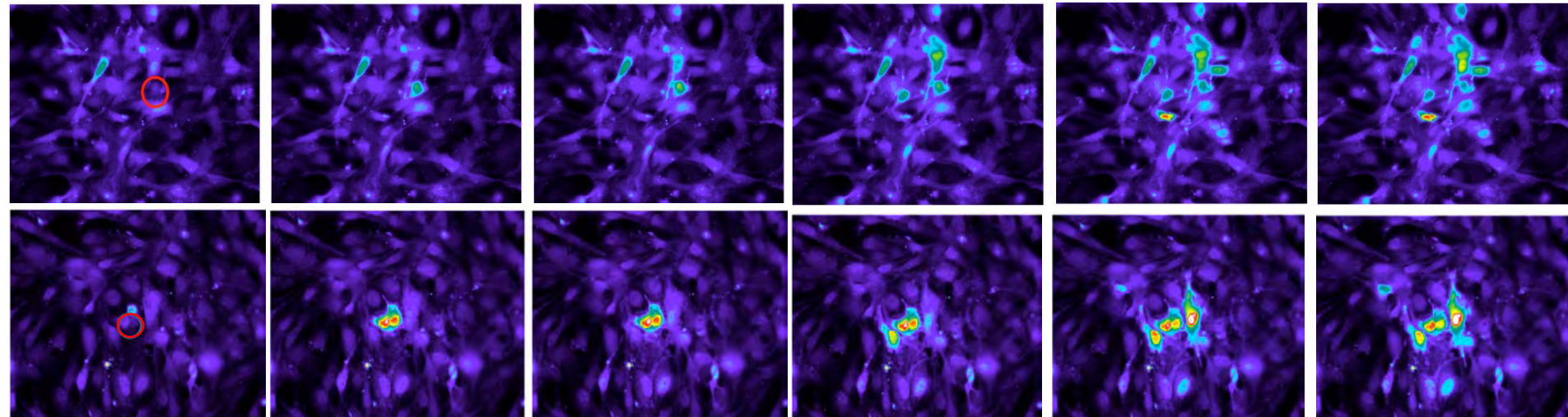
3s

3s

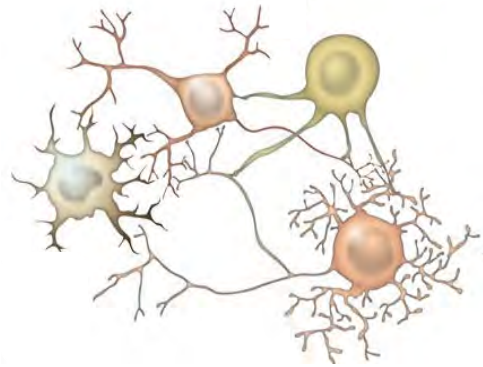
3s



RTT



Effect of RTT astrocytes on human neurons



Mixed cell population
4 weeks of neuronal induction

Magnetic sorting



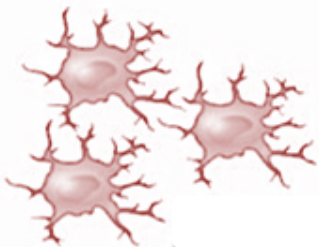
CD44 -
CD184 -



WT
RTT

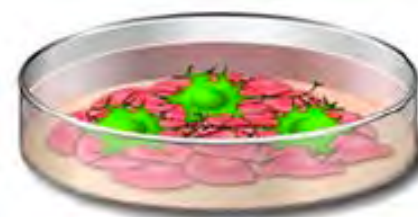
Neuron enriched population

WT
RTT



Astrocytes

Plated on coverslips



2w

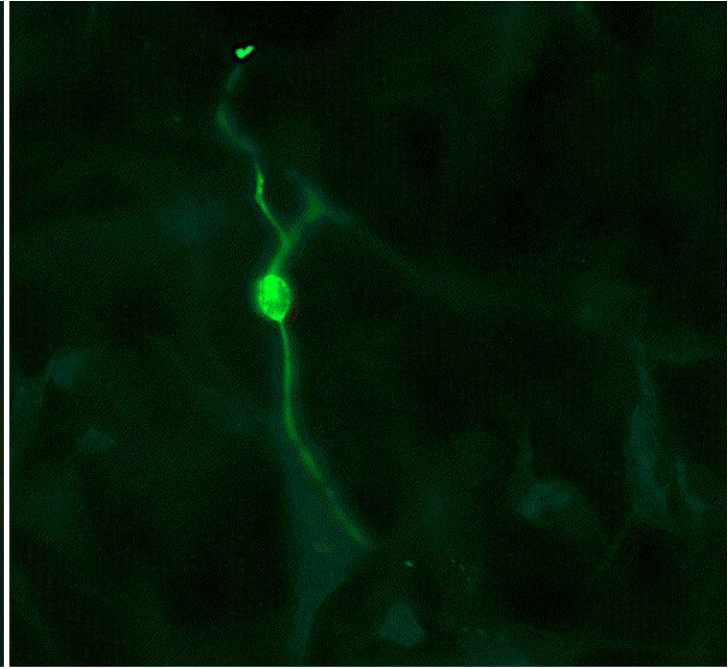
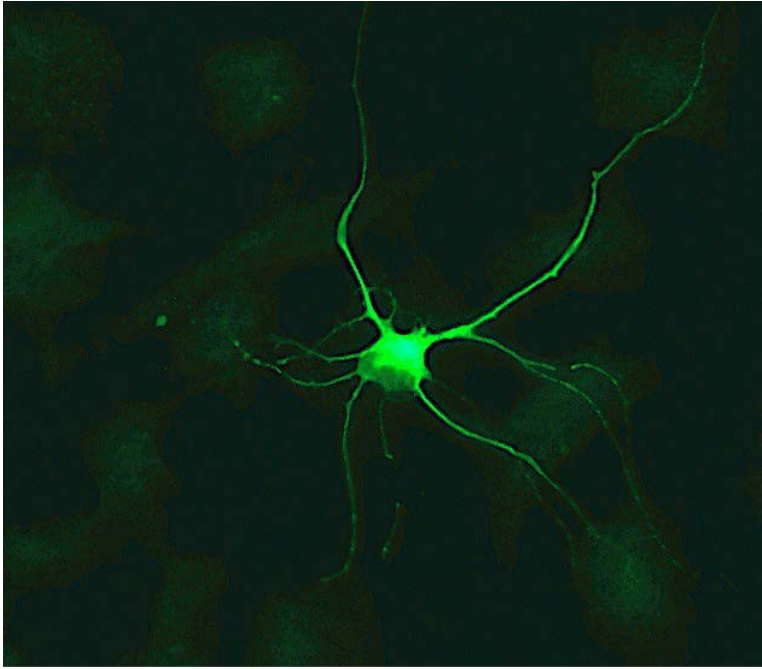


Fix
&
Stain

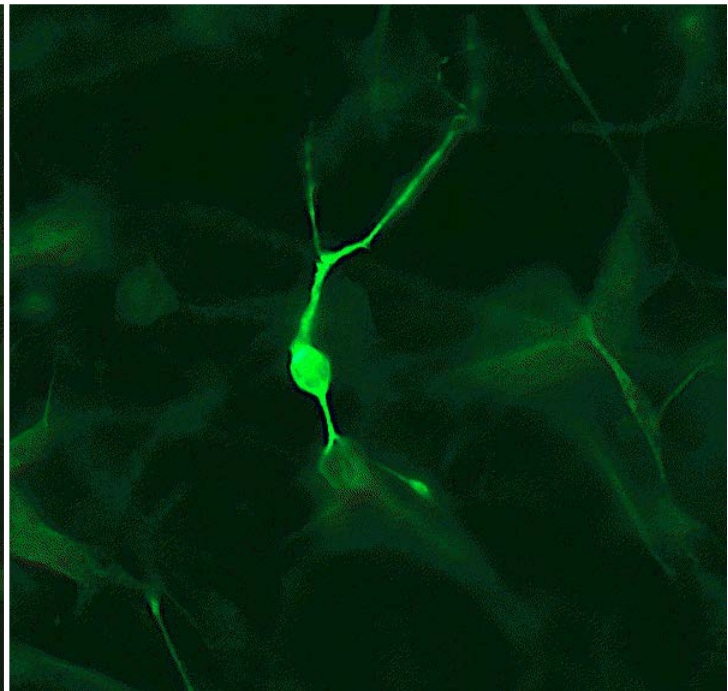
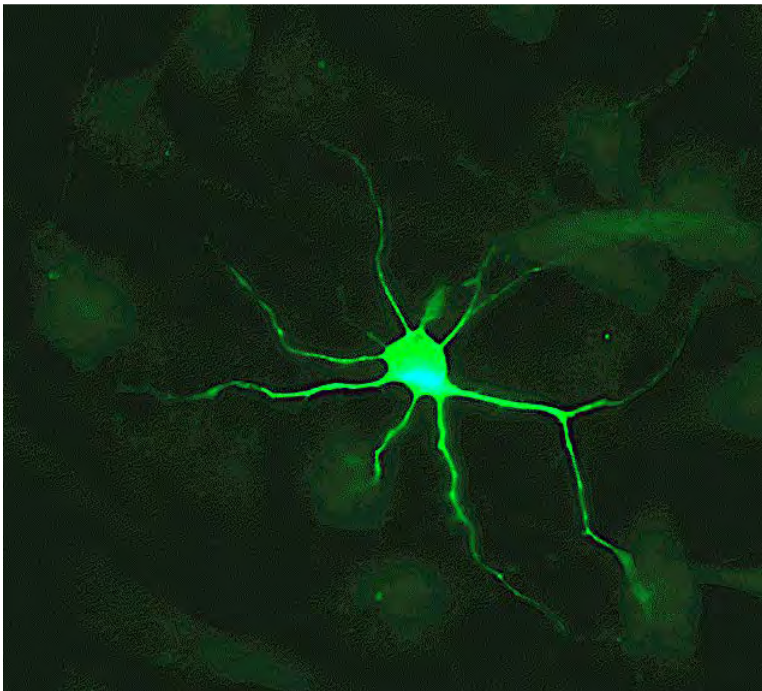
WT Astrocytes

RTT Astrocytes

WT Neurons



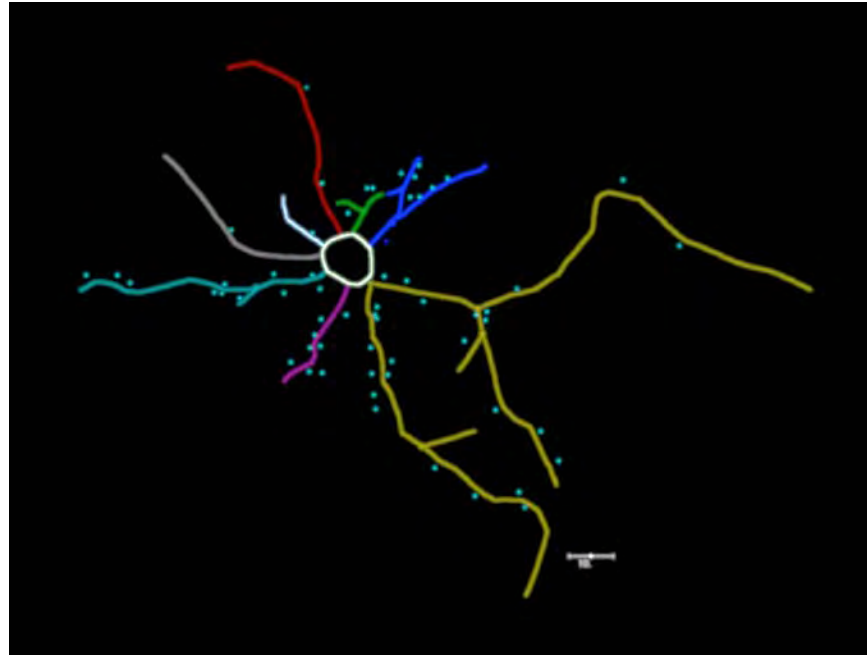
RTT Neurons



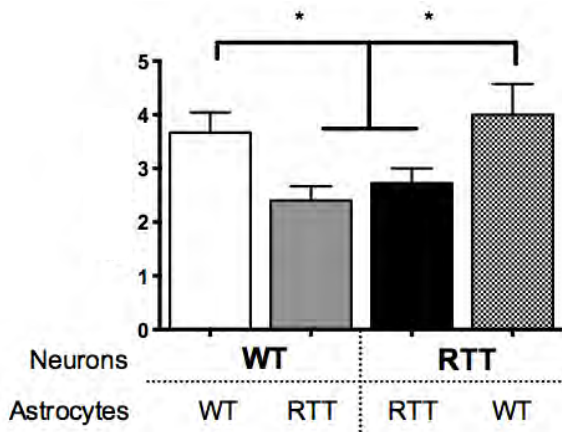
RTT neuronal rescue by astrocytes



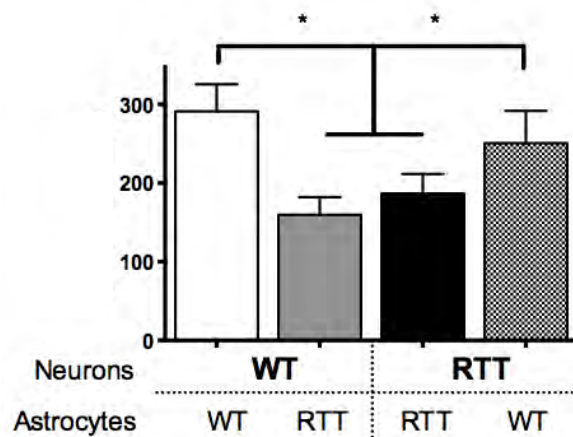
Branka



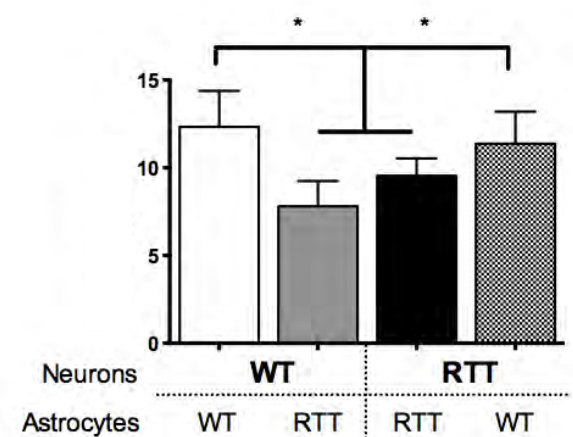
Dendrites



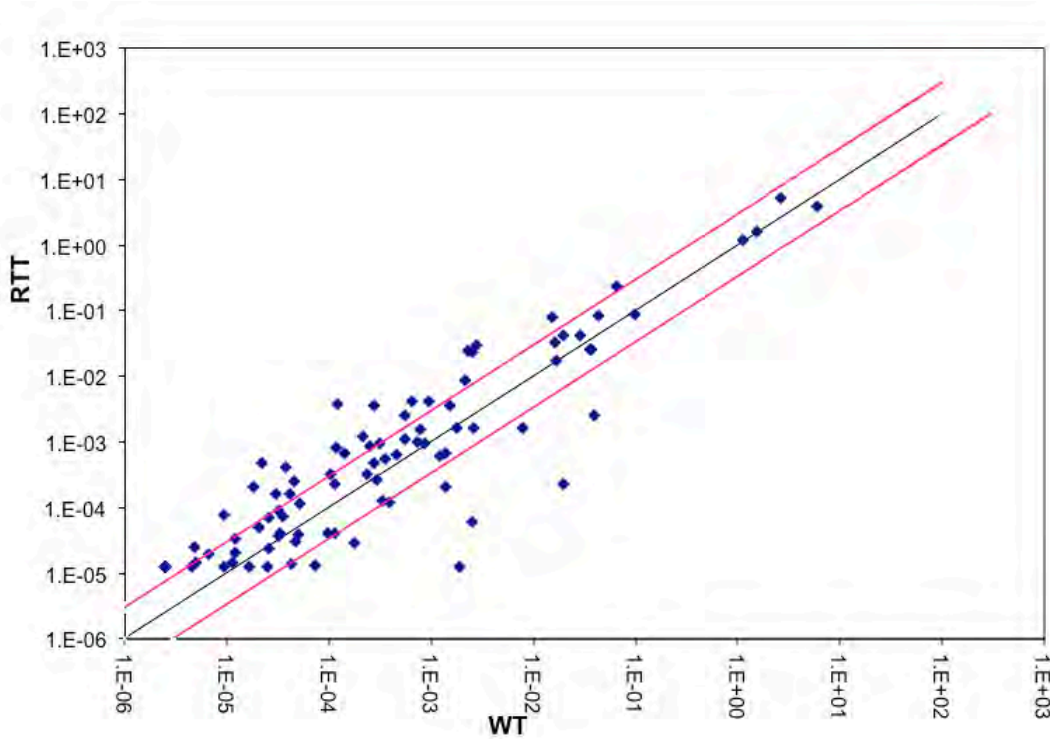
Neuronal Length



Segments



RTT astrocytes aberrant cytokines

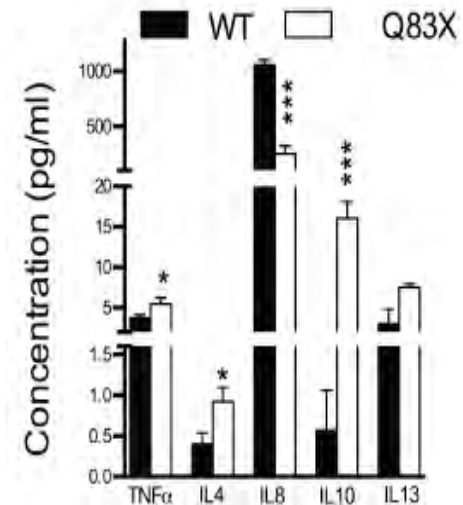


Up-regulated

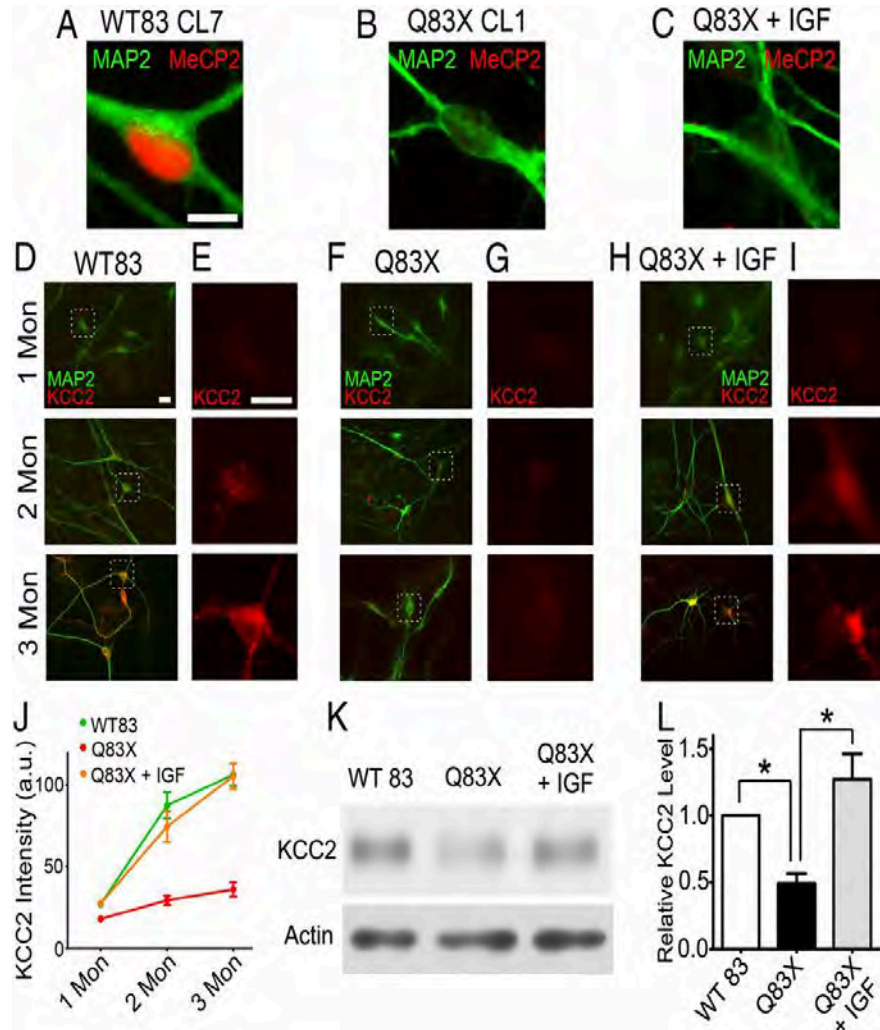
Symbol	Fold dif.
BMP5	4.68
CD40LG	5.36
CSF2	2.29
CSF3	3.89
IFNA4	2.97
IL13	2.69
IL15	6.58
IL23A	2.98
IL3	2.97
IL4	5.42
IL5	5.62
INHBA	5.34
LIF	9.24
TGFB1	1.89
TGFB2	3.55
TGFB3	10.64
TNFSF12	3.98
TNFSF13B	3.39
TNFSF8	5.18
TXLNA	2.15

Down-regulated

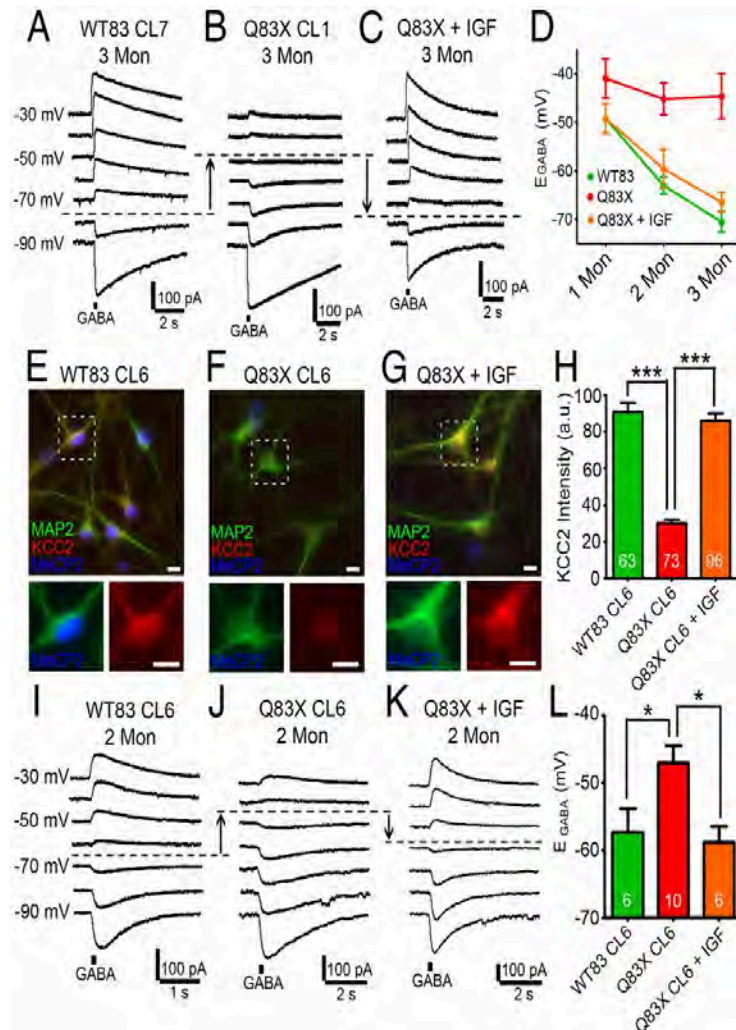
Symbol	Fold dif.
BMP2	0.15
BMP3	0.01
BMP4	0.5
CD70	0.06
IL10	0.17
IL17B	0.32
IL18	0.2



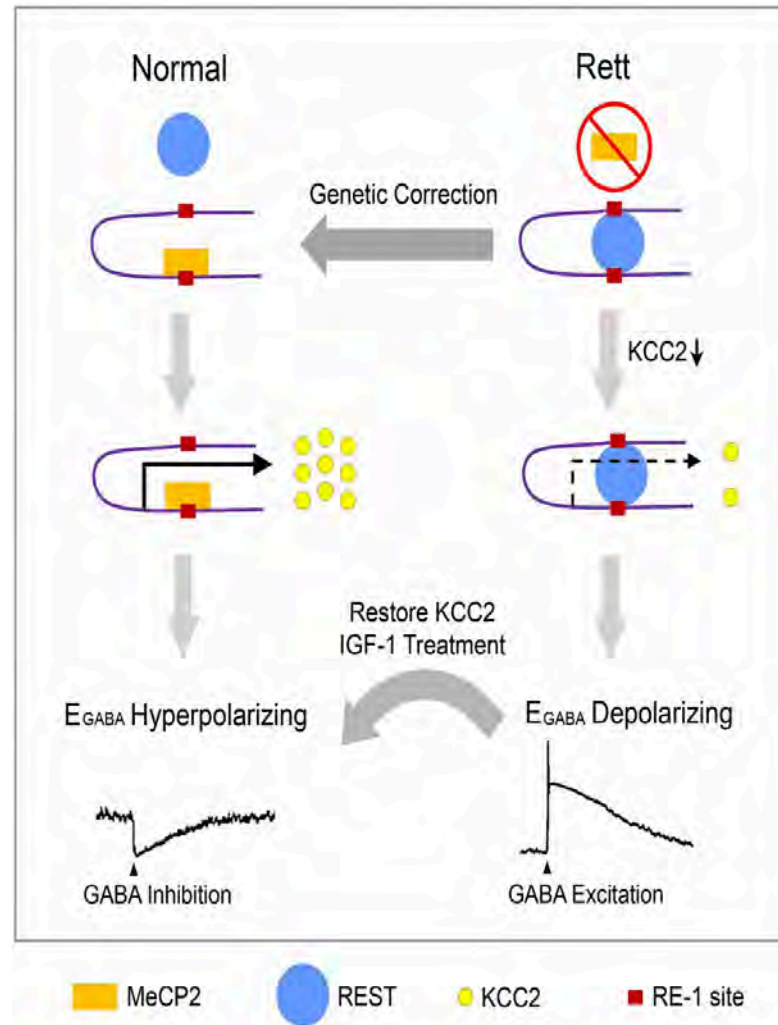
RTT neurons show deficits in KCC2 expression



RTT neurons show deficits in GABA functional switch



A model depicting the molecular mechanisms underlying KCC2 deficiency in Rett neurons

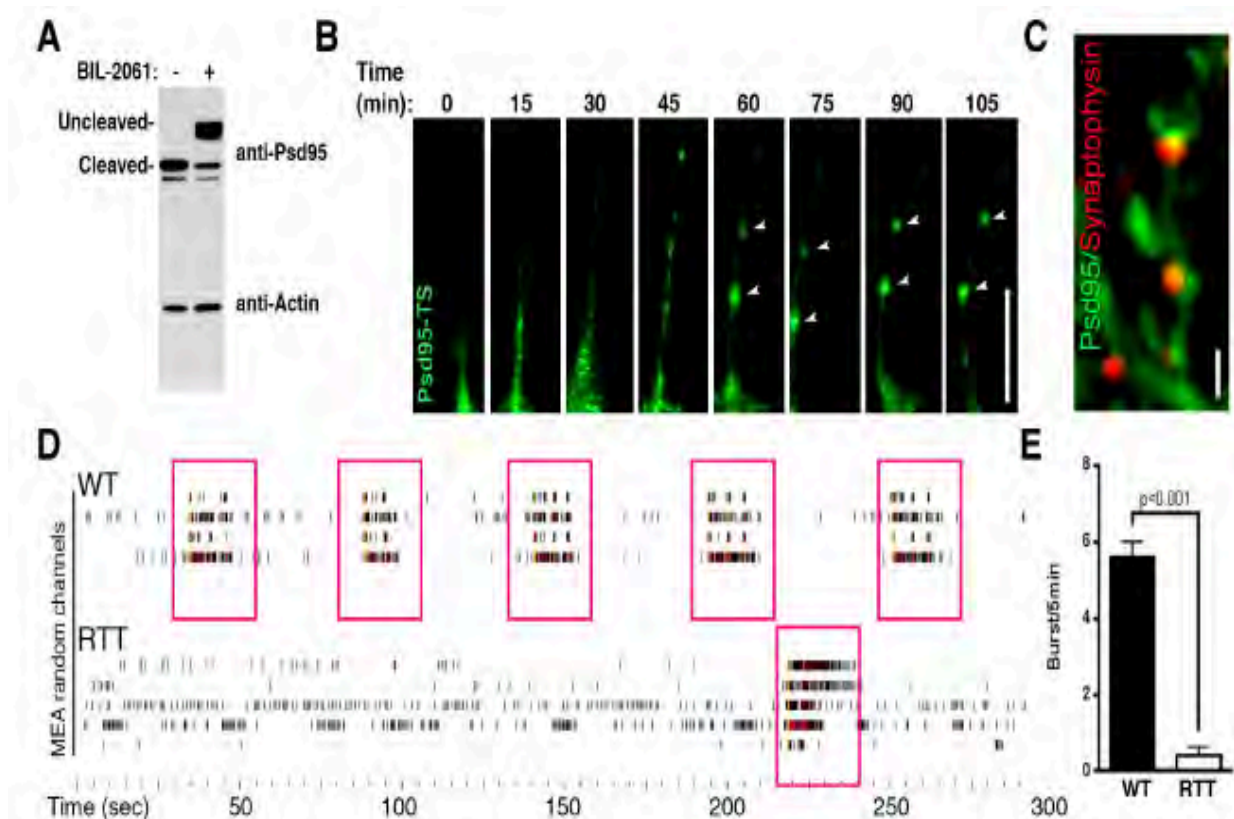


Take Home Messages:

- Loss of MeCP2 function is involved in glutamatergic synapses formation, neuronal morphology and defective network formation.
- RTT astrocytes have impaired metabolism and display an inflammatory cytokine signature. RTT neurons can be rescued by healthy astrocytes.
- RTT neurons are defective in KCC2 expression, resulting in a delayed GABA functional switch that might contribute to the late disease onset.

Ideas about “Regression”

- Use a Psd95-TS/MEA to distinguish between developmental failure or loss of synapses over time.



Ideas about “Regression”

- Study the RTT astrocyte-derived cytokine dynamics over time and the impact on neuronal networks (synaptogenesis/MEA)
- To restore KCC2 expression in the symptomatic RTT mouse model
- To explore a potential pruning defect in RTT neurons with microglia co-culture.



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**NIH Director's New Innovator Award Program
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