Application of induced pluripotent stem (iPS) cells in intractable childhood disorders

Lessons from Dravet synd. patient-derived iPSCs

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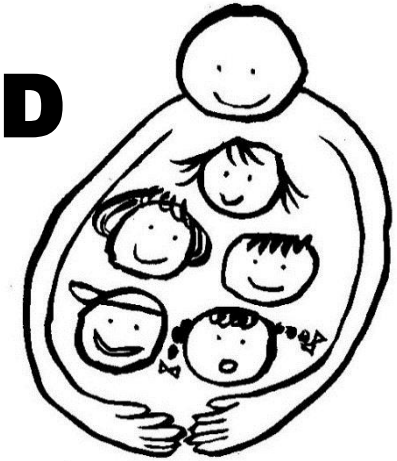


Summary

iPS cells invented by Prof. Shinya Yamanaka

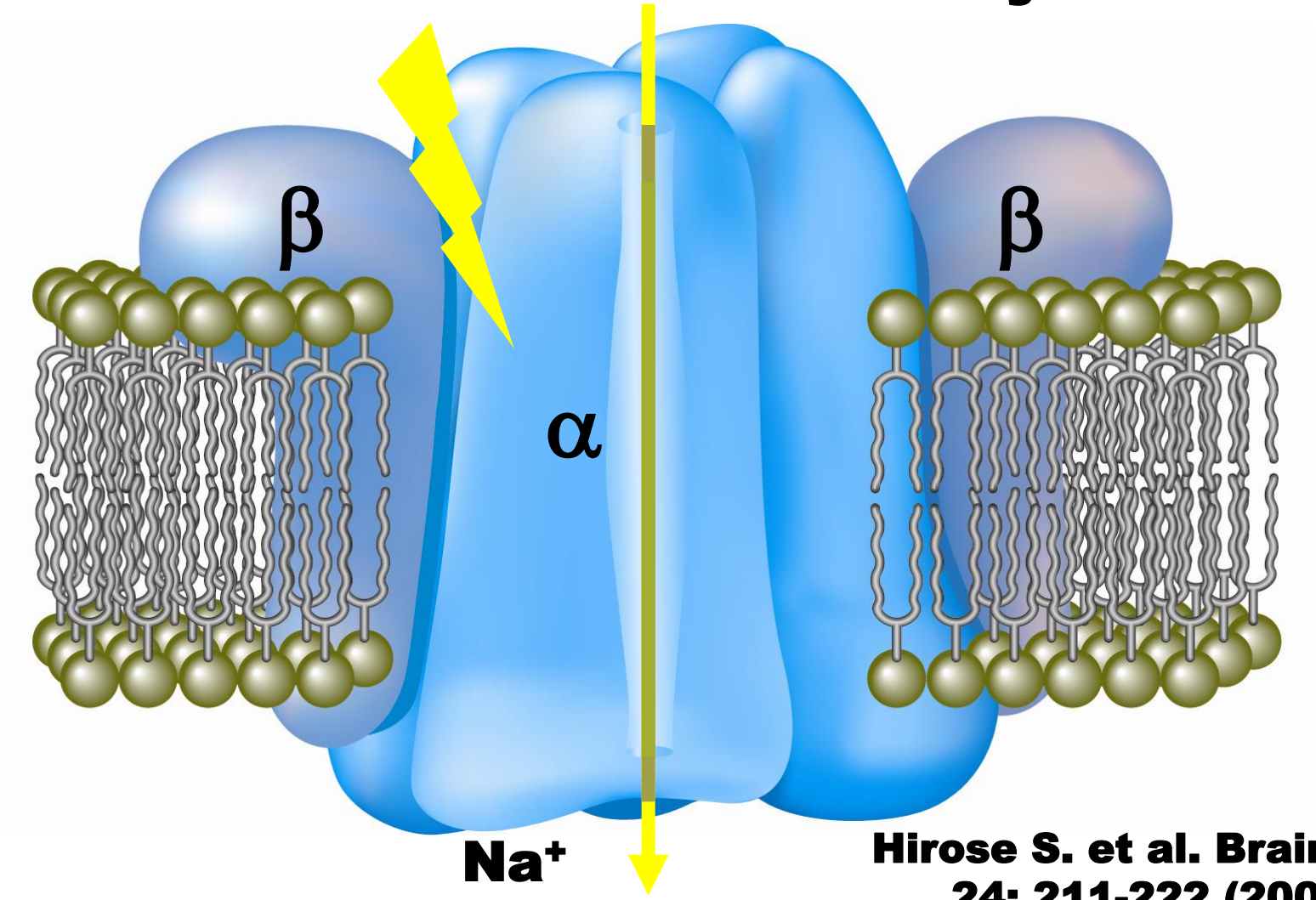
The Nobel Prize in Medicine 2012

DRAVET syndrome
(Severe Myoclonic Epilepsy in Infancy)

- Incidence : 1 in 40,000.
- Refractory epilepsy
- Profound psychomotor delay
- Fever sensitive
- Na^+ channel abnormalities

Na^+ channel ($Na_v1.1$)

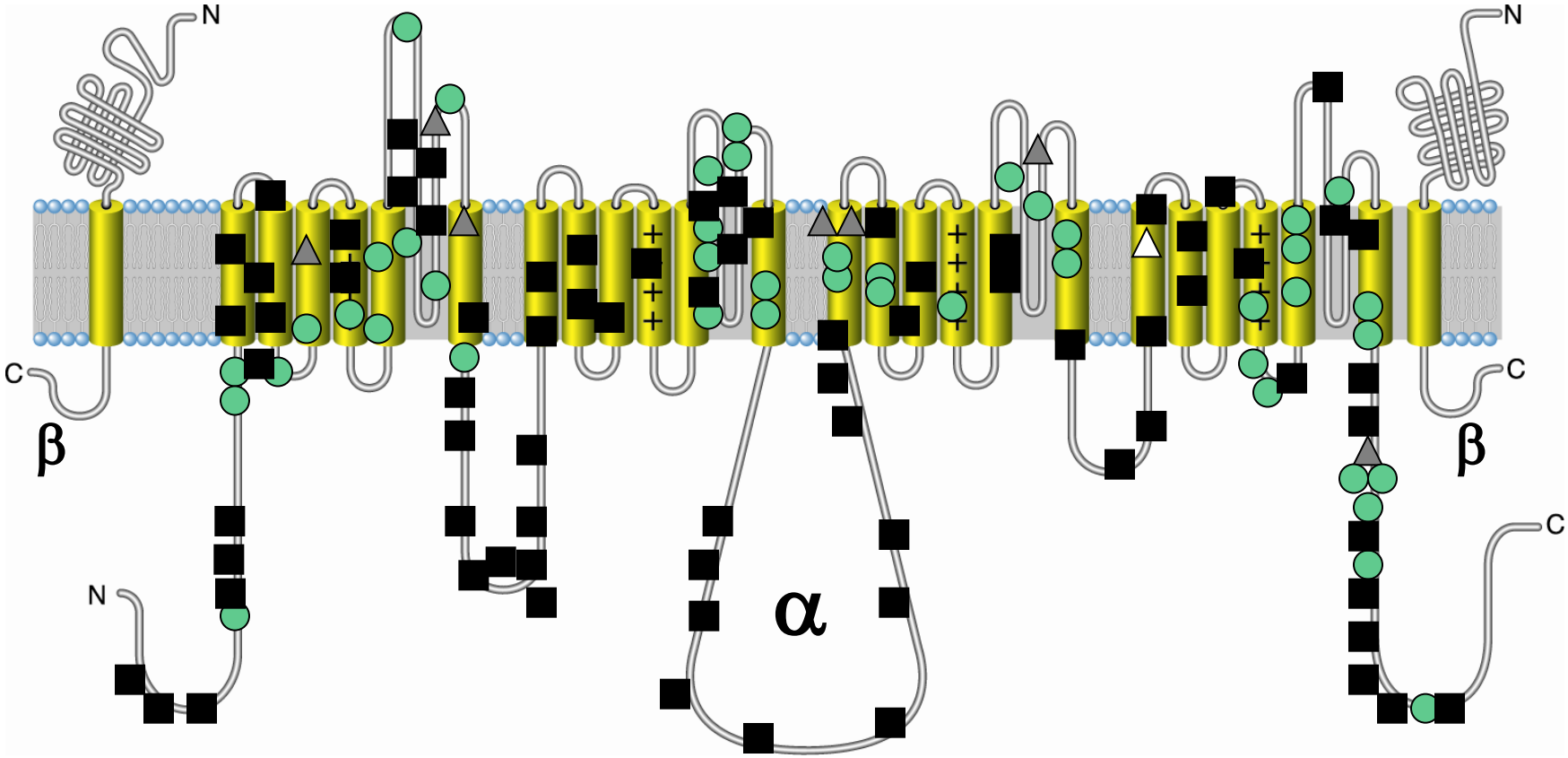
Mutations found in DRAVET syndrome



Hirose S. et al. Brain Dev 24: 211-222 (2002)

Mutations found in DRAVET

All are hetero and Most are *De Novo*



■ truncation ● missense ▲ splice site △ deletion

Lossin C Brain & Dev 31: 114-130 (2009)

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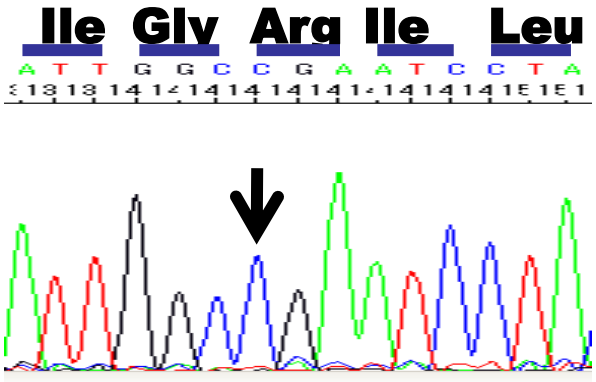
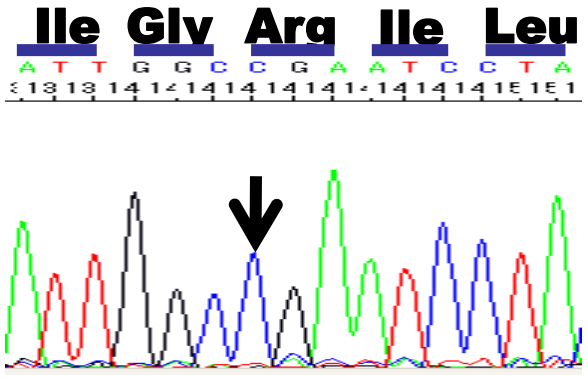
Patient whose skin was used to establish iPS cells

- 29-year-old woman
- No perinatal abnormality
- Normal development till 6mo.
- First GTC at 7mo.
- Frequent seizures thereafter
- Non-convulsive status epilepticus

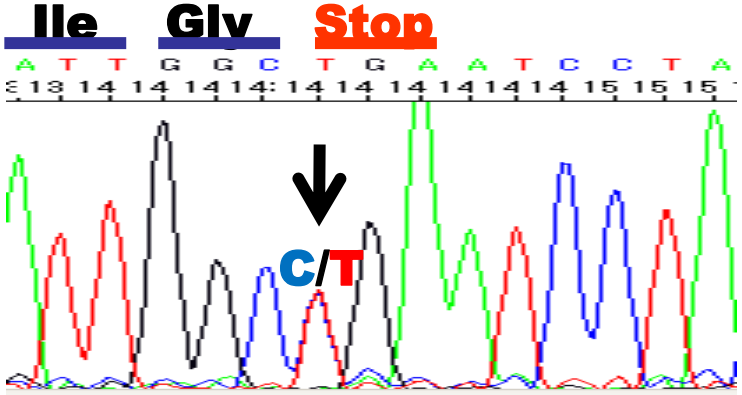
Typical DRAVET syndrome

SCN1A mutation of the Patient





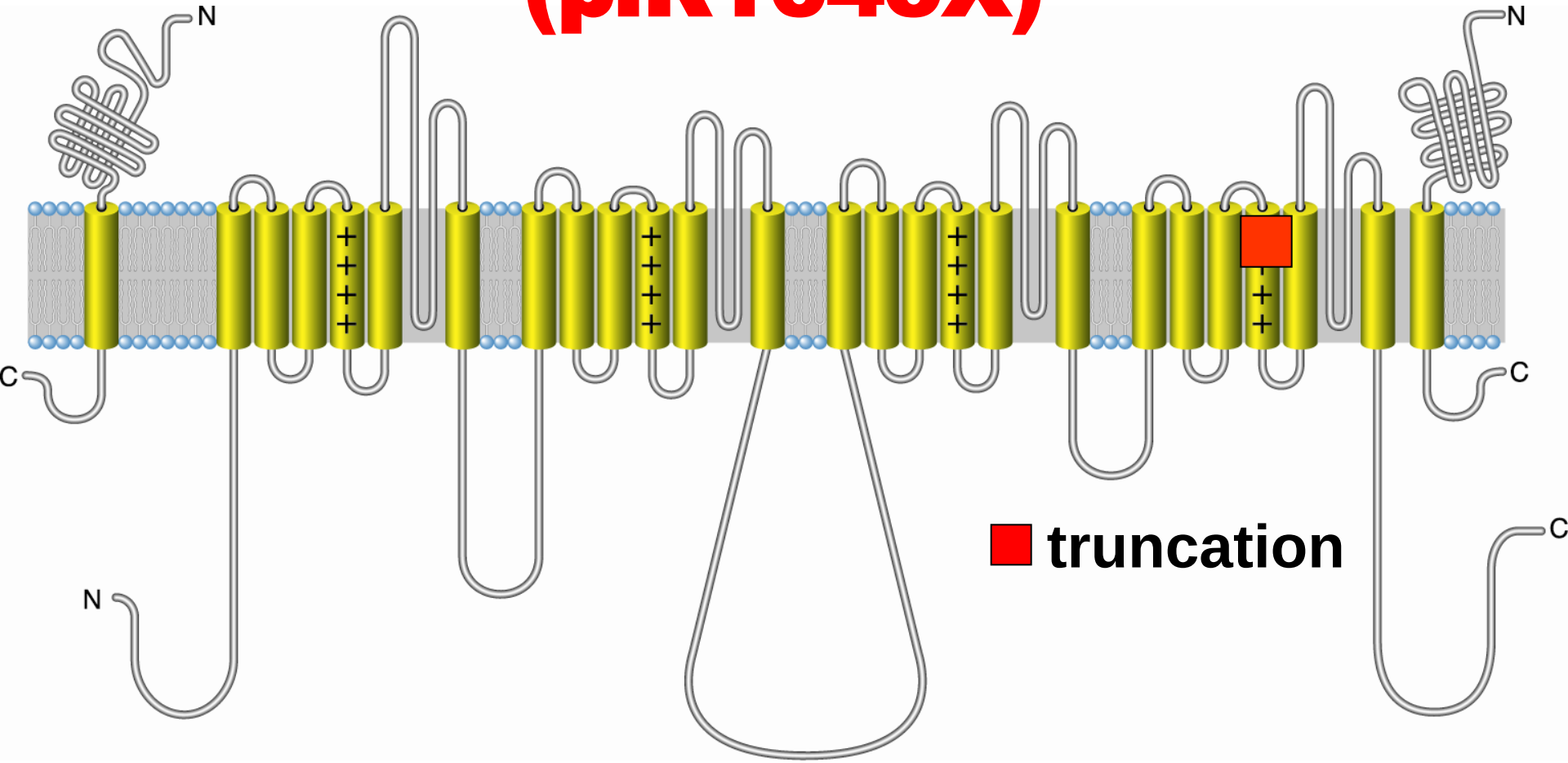
c.4933 C>T **p.R1645X**



Fukuma et al.
Epilepsia
25: 535-542 (2005)

SCN1A mutation of the Patient

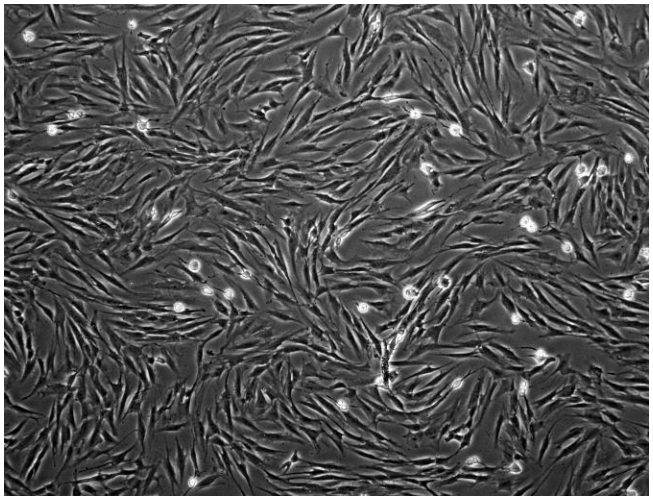
Non-sense mutation (p.R1645X)



Fukuma et al. Epilepsia 25: 535-542 (2005)

Patient iPS cells established

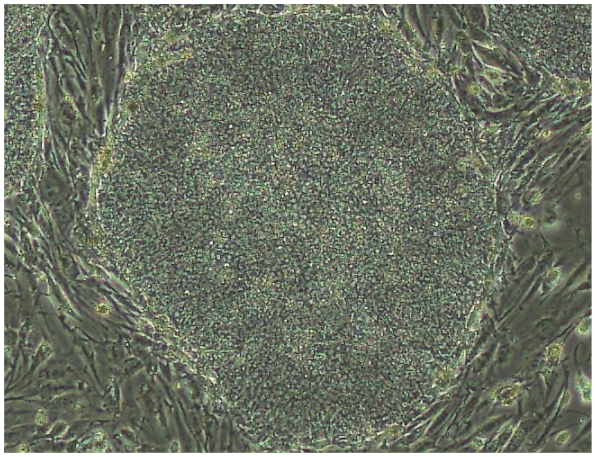
Patient fibroblast



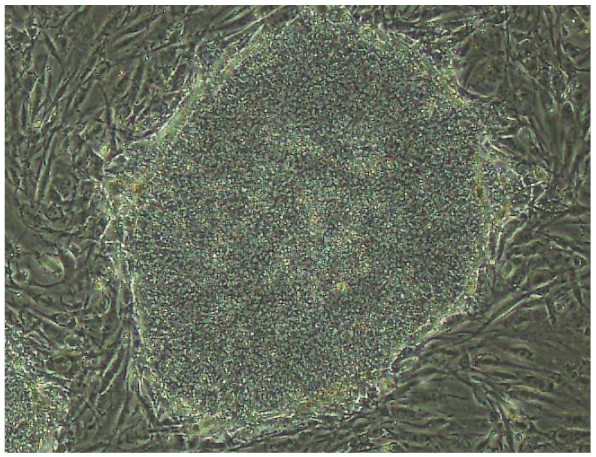
Yamanaka's 4 factors

Oct3/4 ·Sox2·Klf4·c-Myc

iPS cells Line A

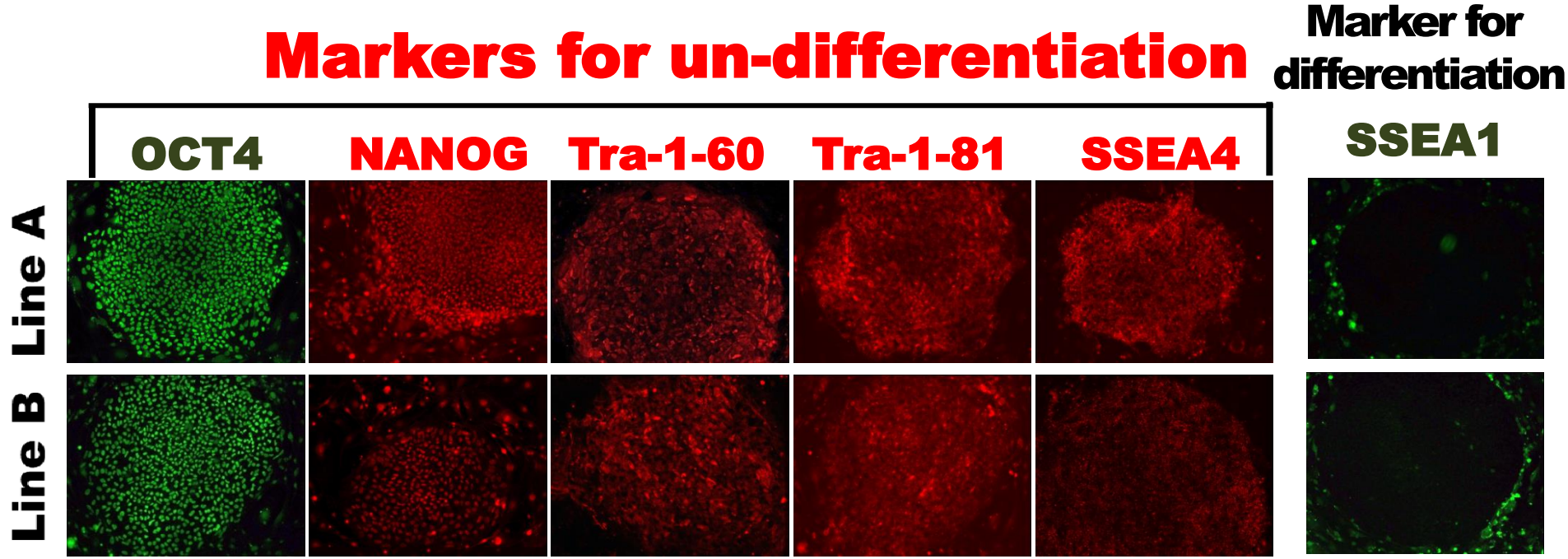


iPS cells Line B

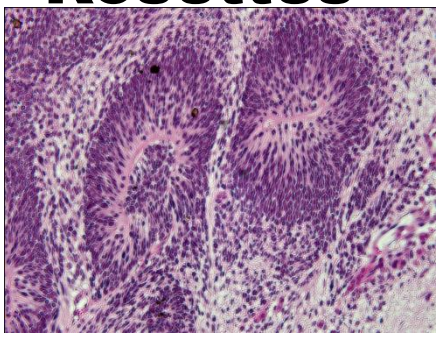
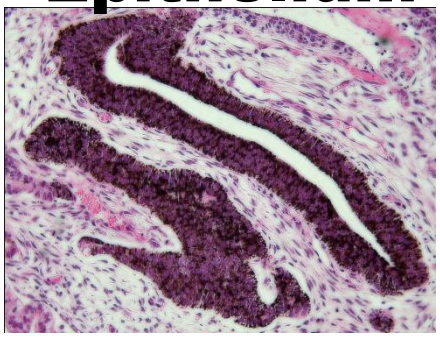
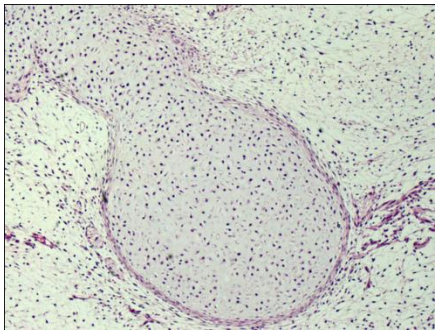
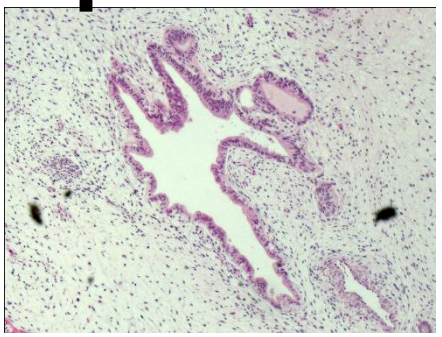
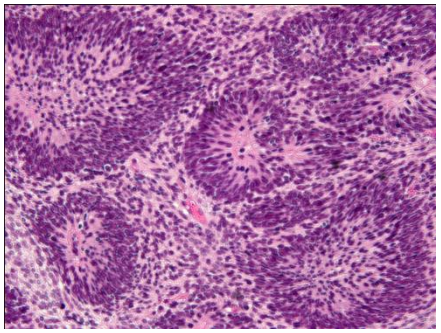
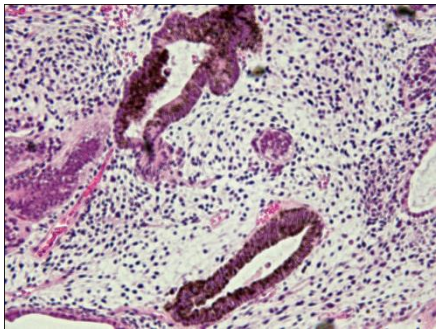
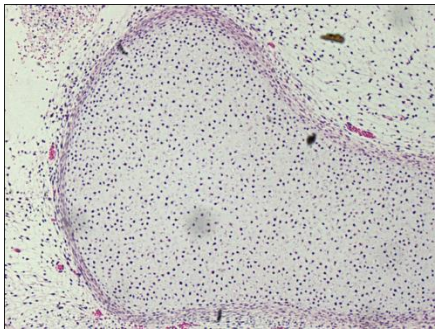
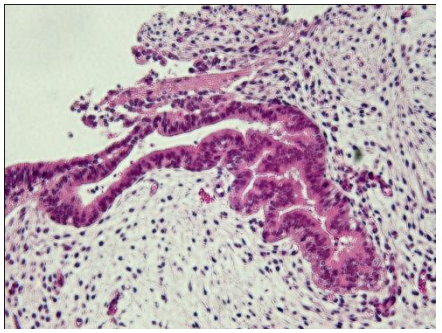


iPS cells remain un-differentiated

Immunostaining for iPS cells



iPS cells show Teratoma formation

	Ectoderm		Mesoderm	Endoderm
	Neuronal Rosettes	Pigmented Epithelium	Cartilage	Respiratory Epithelium
Line A				
Line B				

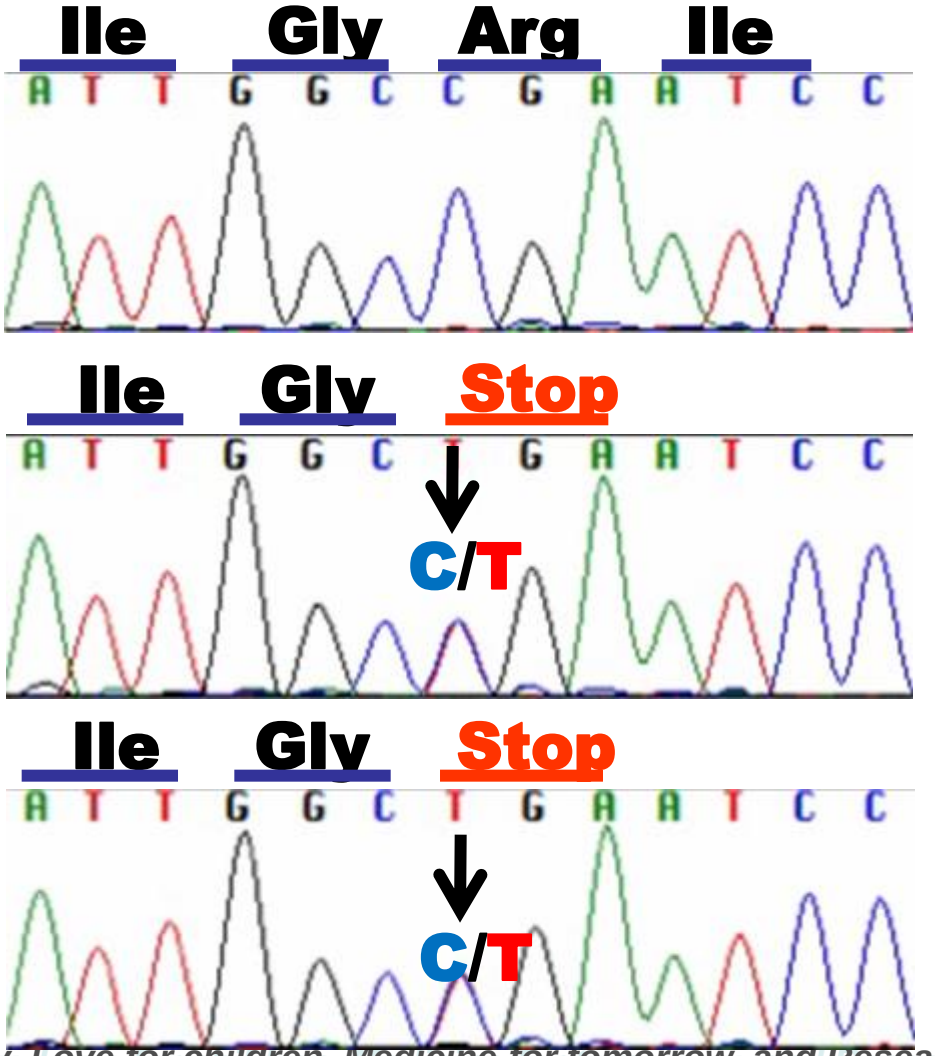
HE

iPS cells retain *SCN1A* mutation

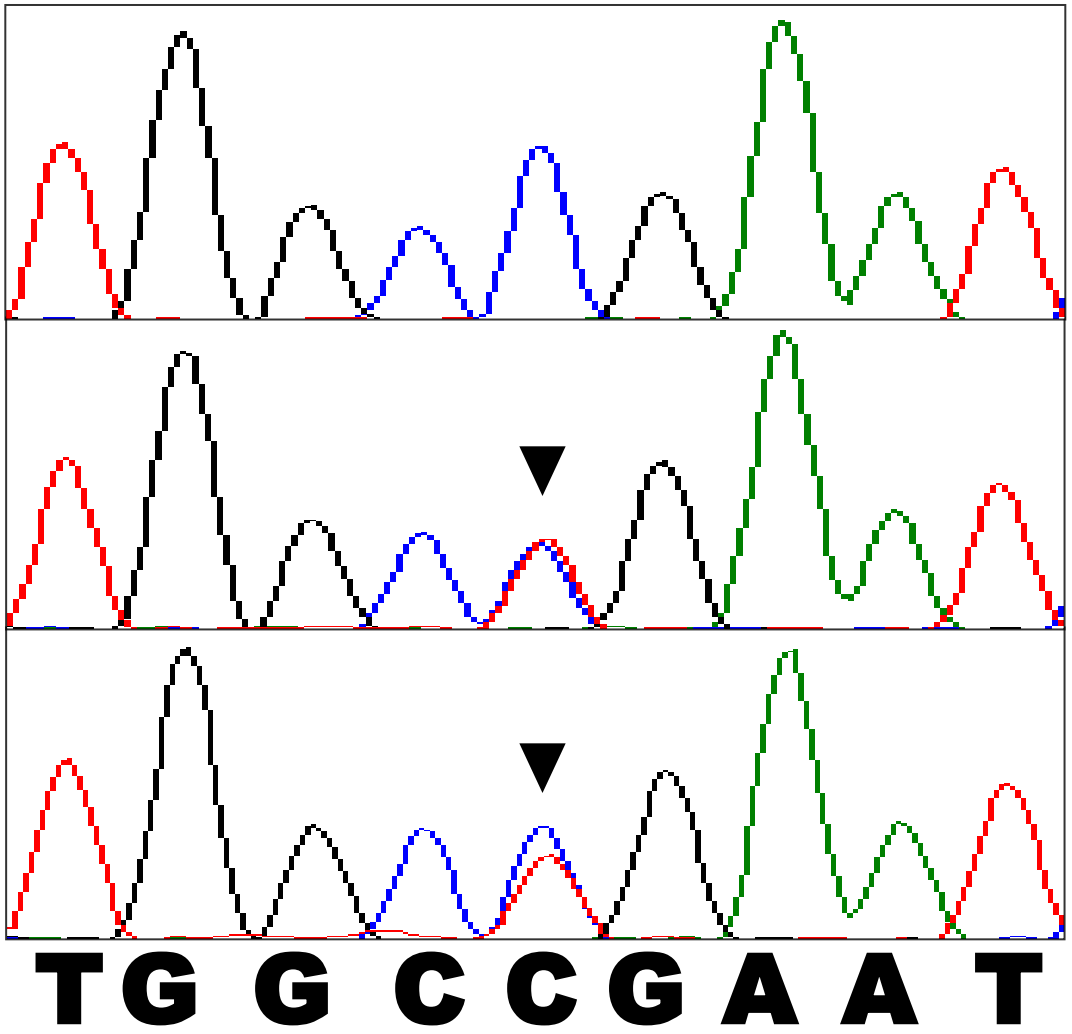
Control iPS

p.R1645X (Hetero)
Patient iPS cells
Line A

Patient iPS cells
Line B

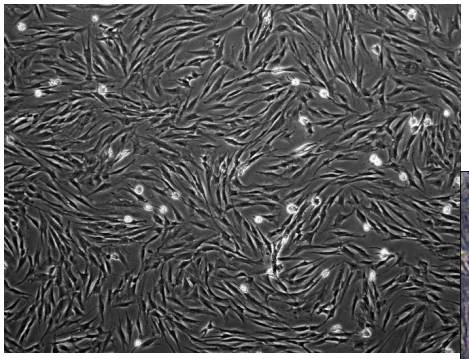


SCN1A mRNA of Neurons



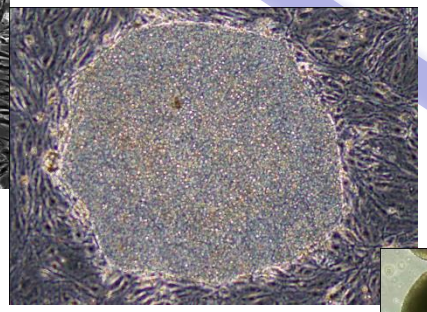
Higurashi N et al. Mol Brain 6:19 (2013)

Neurons derived from iPS

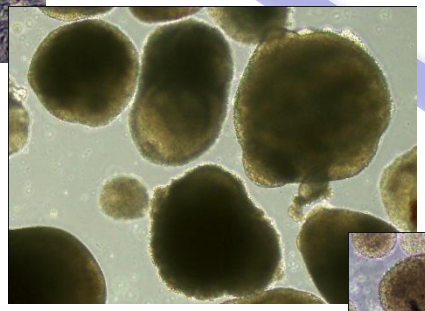


Fibroblasts

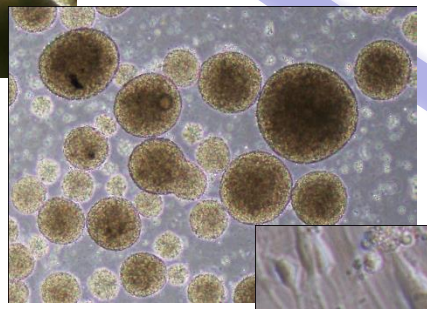
Yamanaska's
4 factors



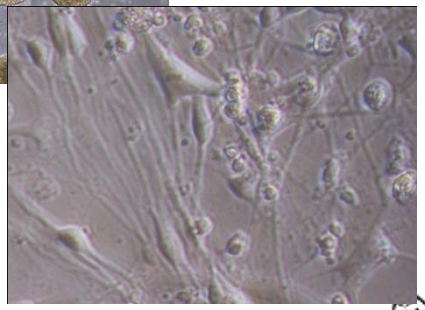
iPS cells



Embryoid body



Neurosphere



Neurons

Higurashi N et al. Mol Brain 6:19 (2013)

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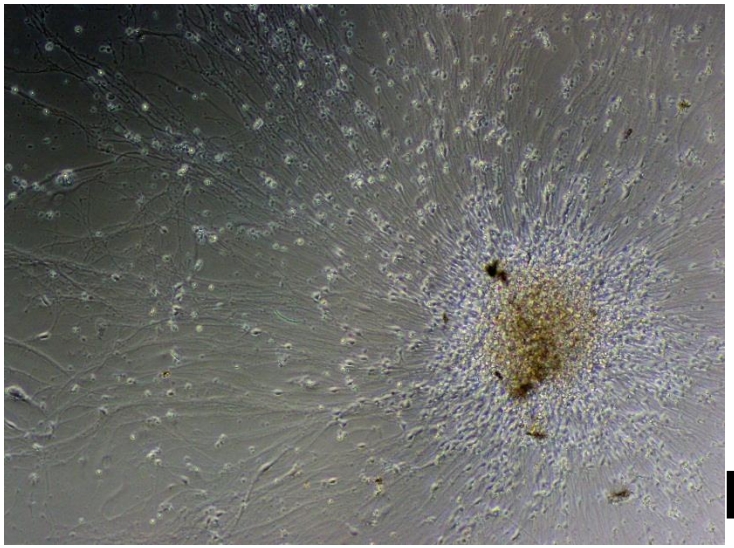
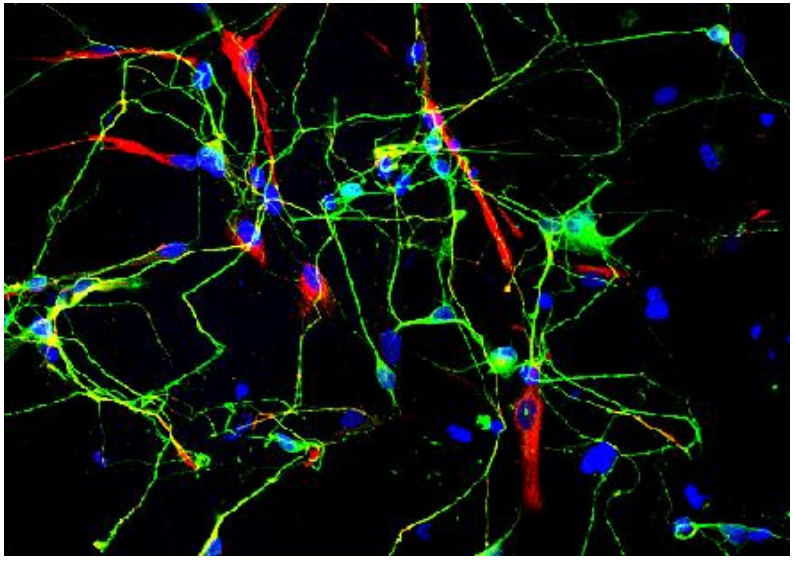
iPS cells develop to neuronal cells



Day 10



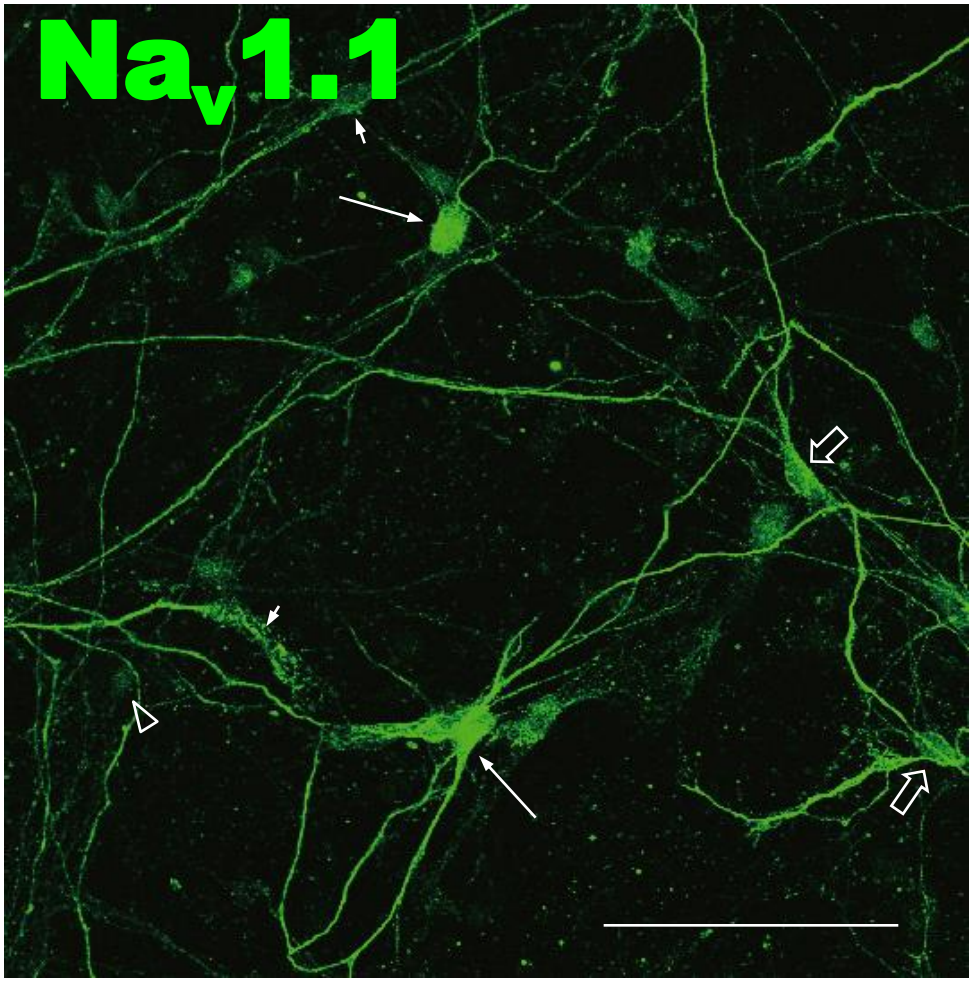
βIII-tubulin neurons

GFAP glias

Hoechst 33258 nucleus

Higurashi N et al. Mol Brain 6:19 (2013)

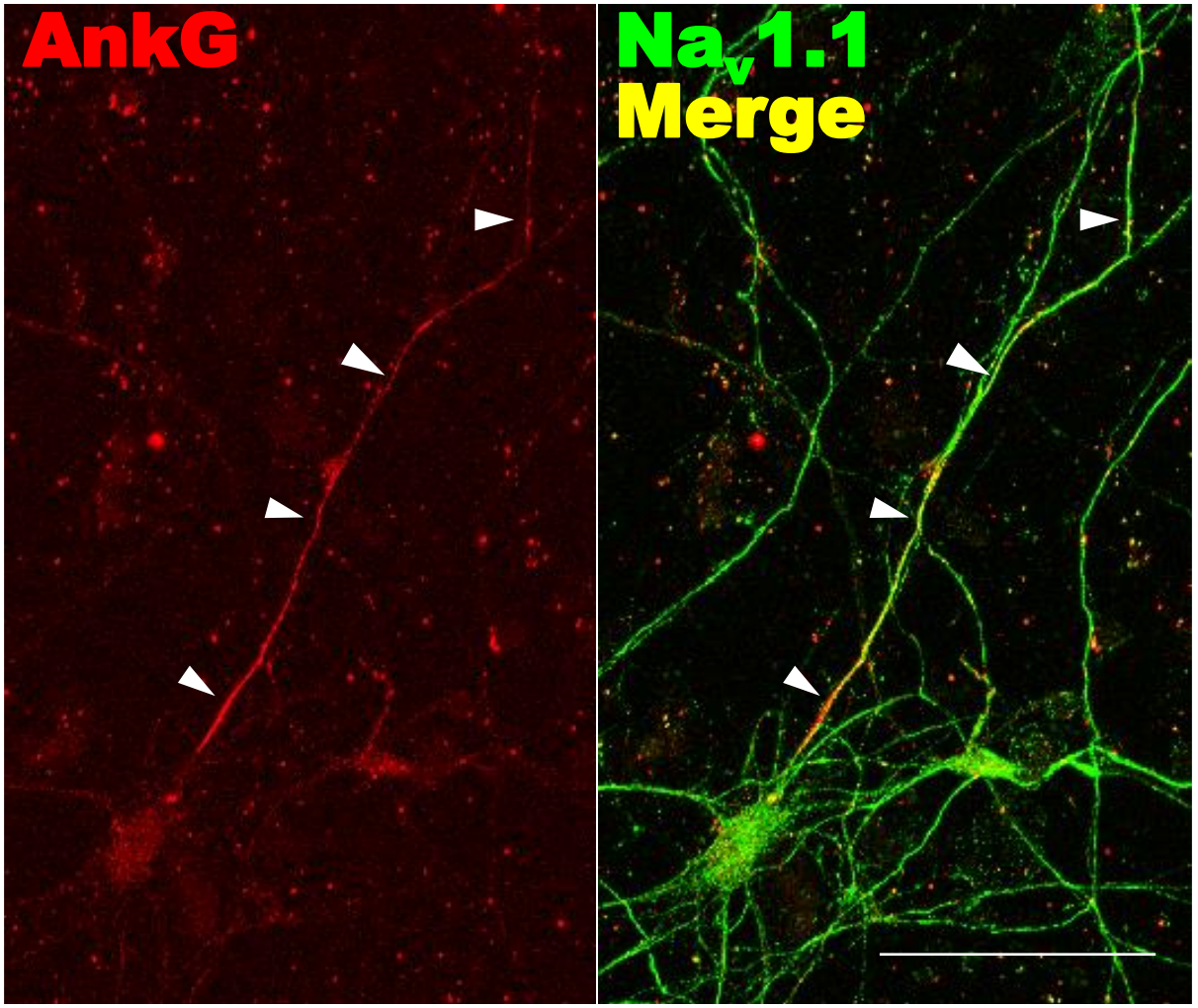
$Na_v1.1$ Expression



Control (day 36)

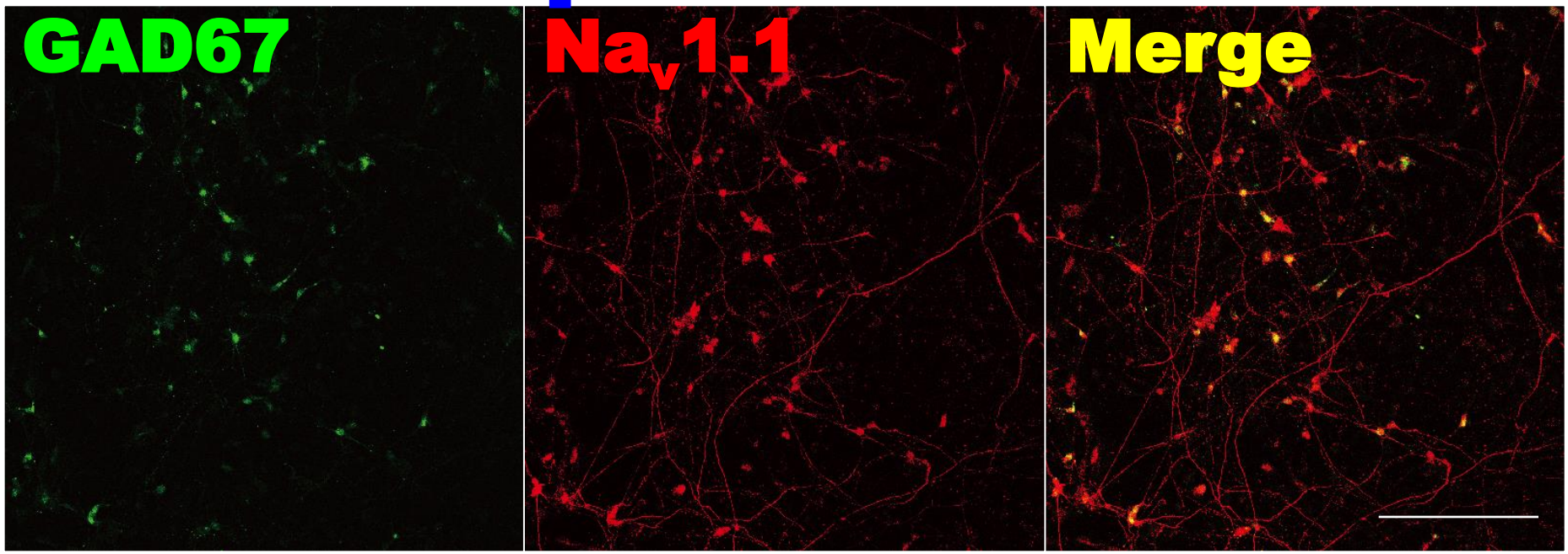
50~60% of Neurons express $Na_v1.1$

$Na_v1.1$ is expressed at Axon Initial Segment (AIS)



Control (day 30)

GABAergic Neurons are predominant

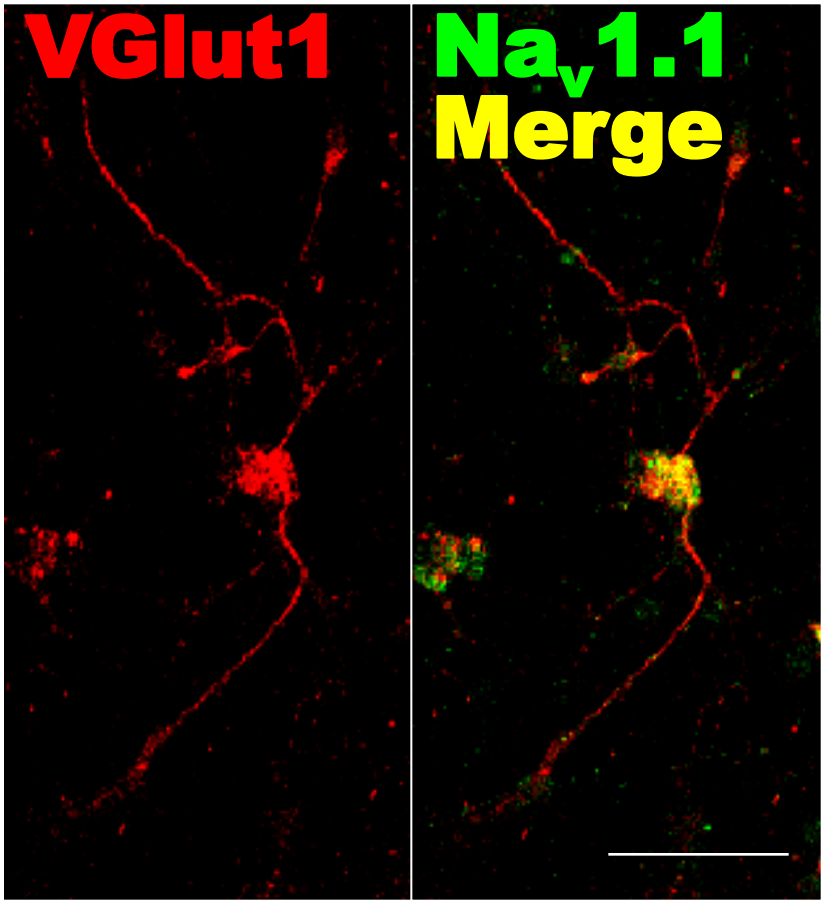


Control (day 29)

50～60% of Na$_v$1.1 positive neurons are GABAergic

Higurashi N et al. Mol Brain 6:19 (2013)

Few Glutamatergic Neurons



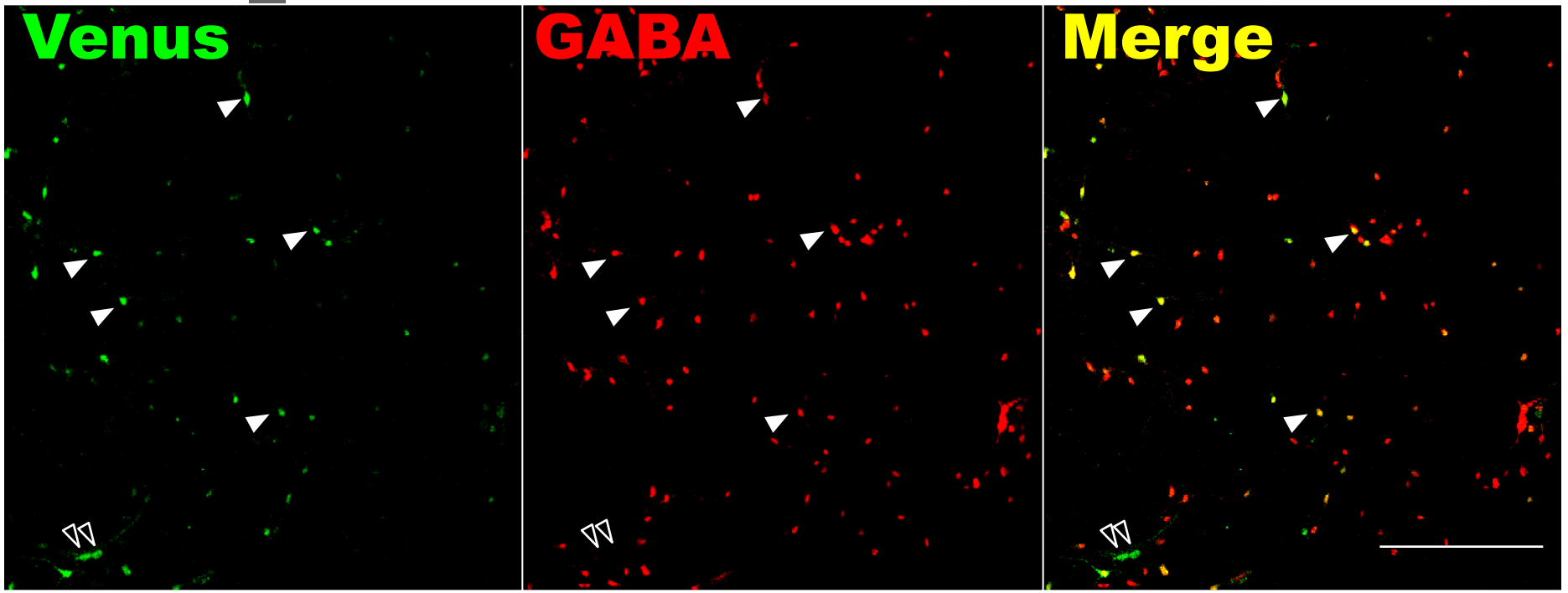
control
(day 30)

<1%of $Na_v1.1$ positive neurons are Glutamatergic

Detection of $Na_v1.1$ positive neurons

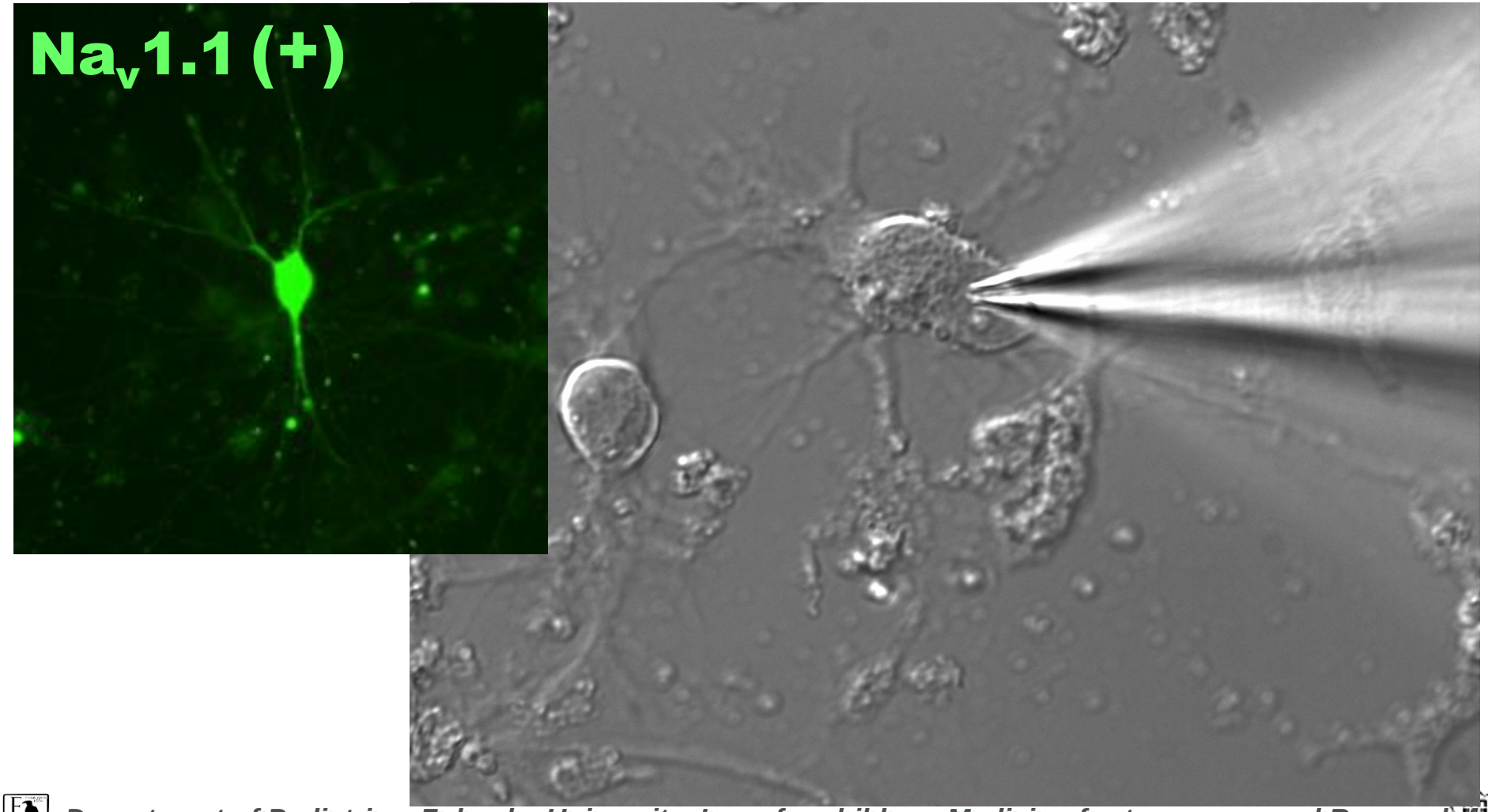
: 5'-Untranslated Exon

: 5'-Untranslated sequence of the first coding exon

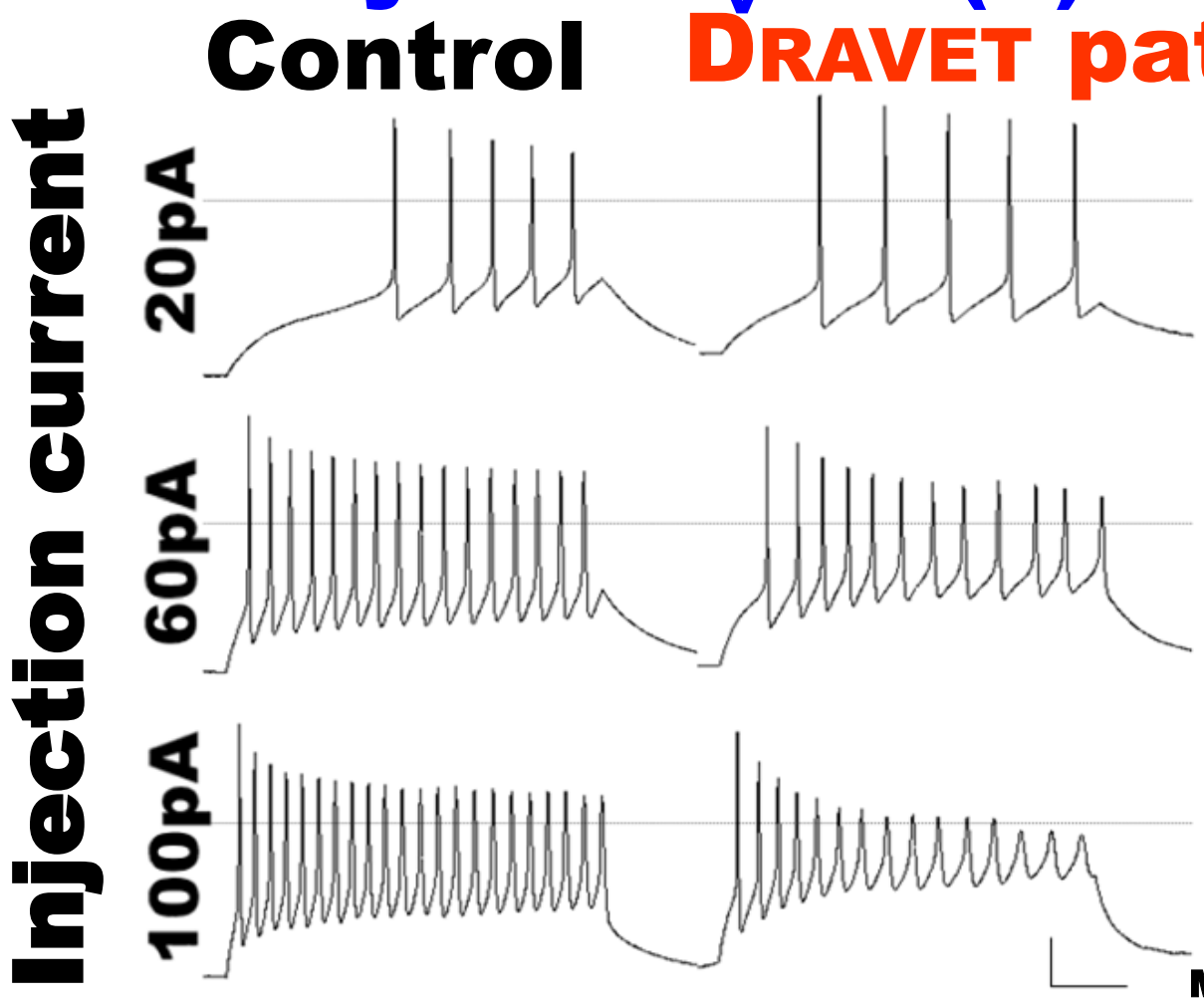


(day 35)

Patch clamp
(current clamp)



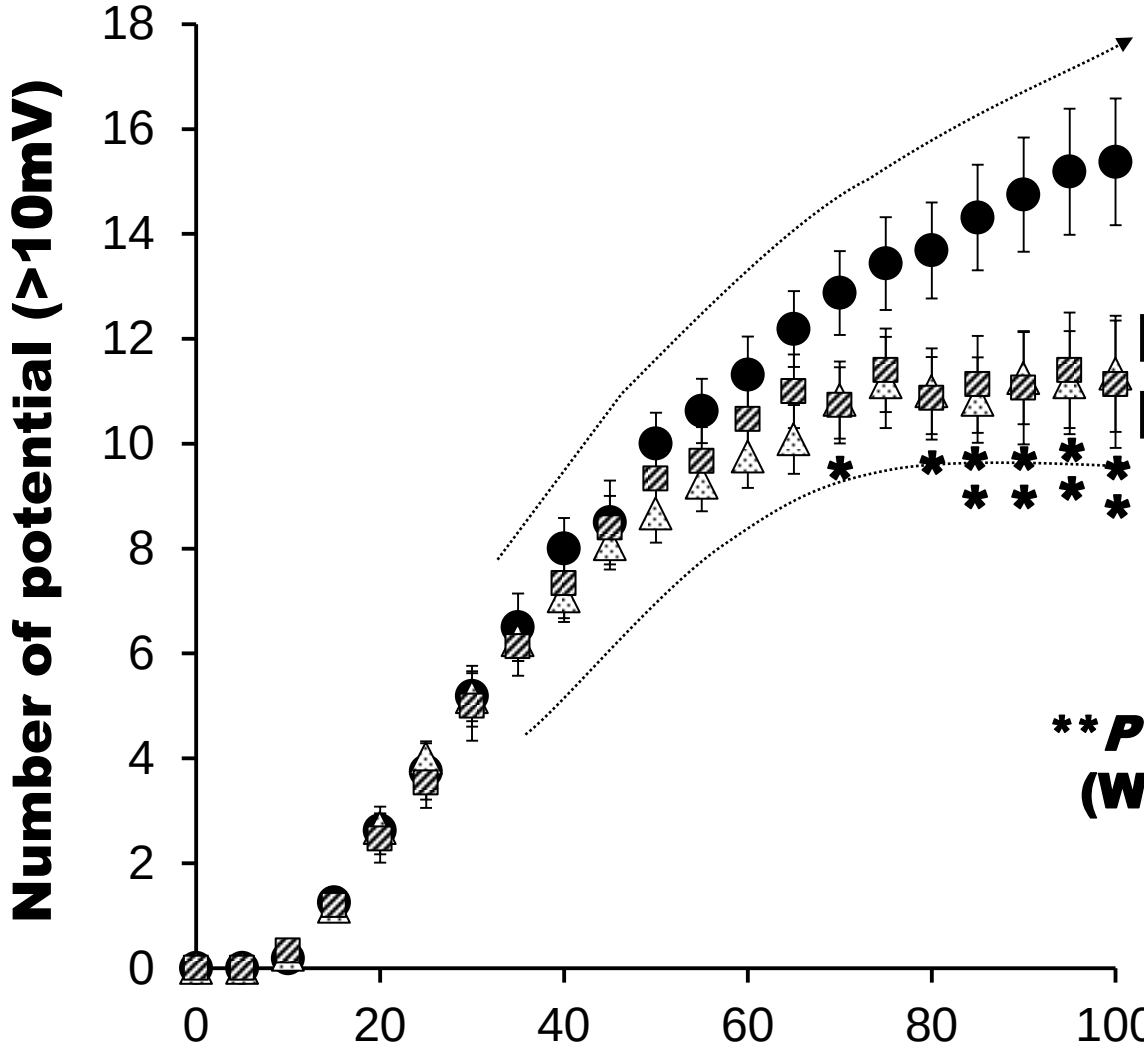
Abnormality in $Na_v1.1$ (+) neurons



Higurashi N et al.
Mol Brain 6:19 (2013)

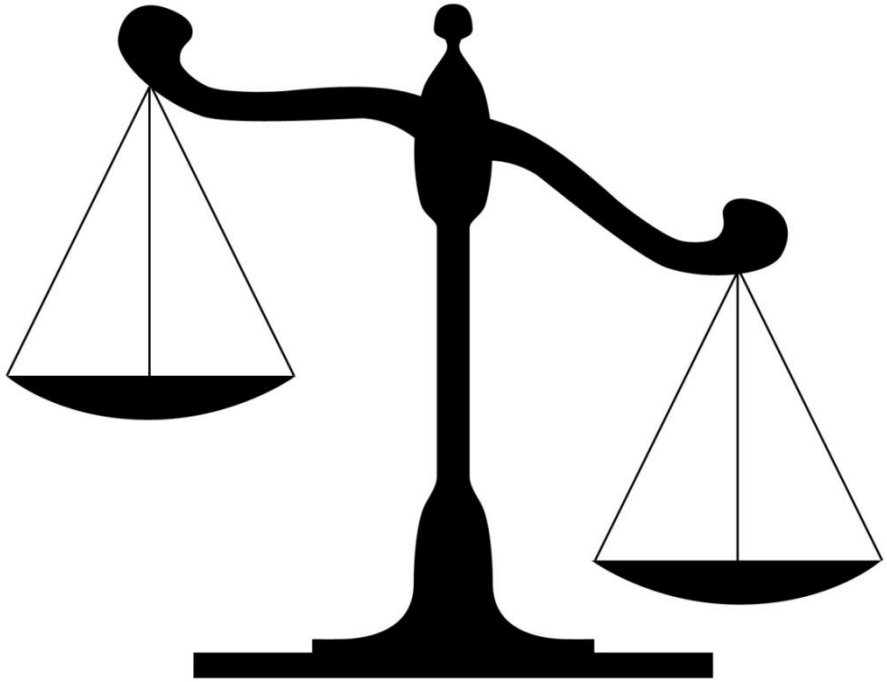
Depolarization spike attenuated in inhibitory interneuron

Input-output relationship



Higurashi N et al.
Mol Brain 6:19 (2013)

Dysfunction of inhibitory interneuron



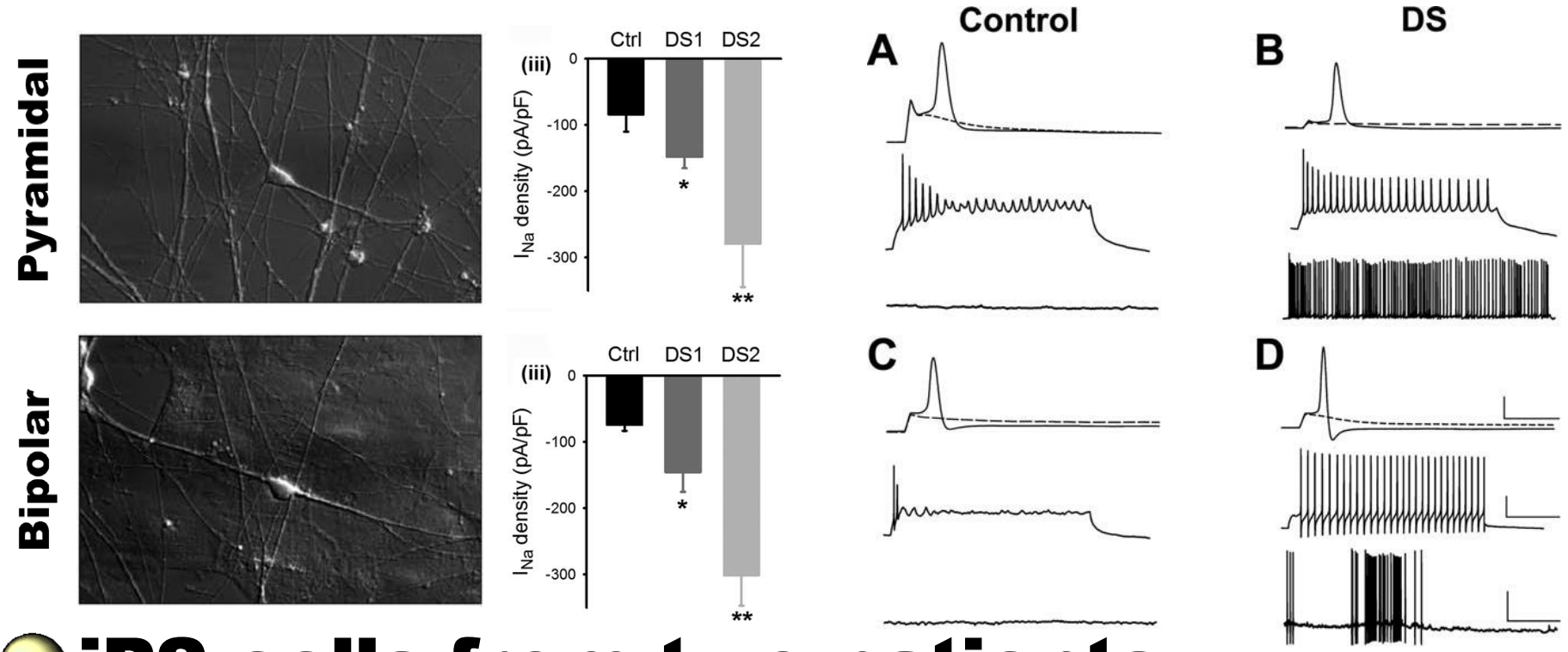
Relative hyper-excitation (seizures)

Na^+ inhibitors aggravate the unbalance

Other iPS cells studies on Dravet syndrome

Other iPS study #1

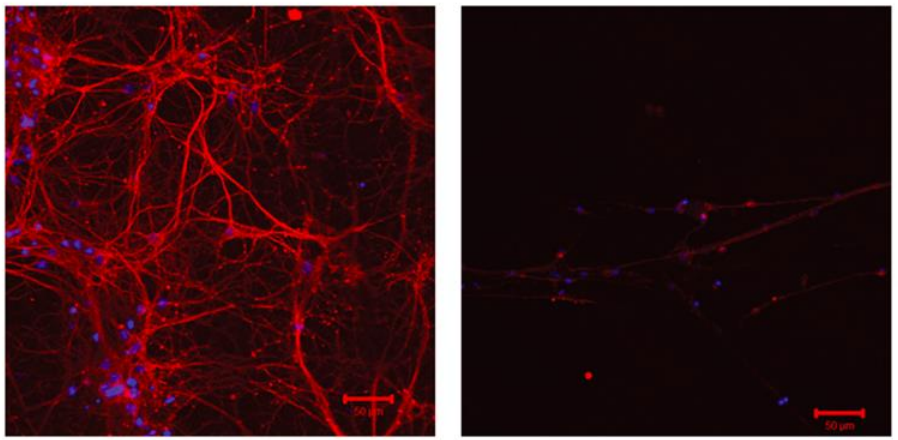
Liu et al., Ann Neurol 2013;74:128-139

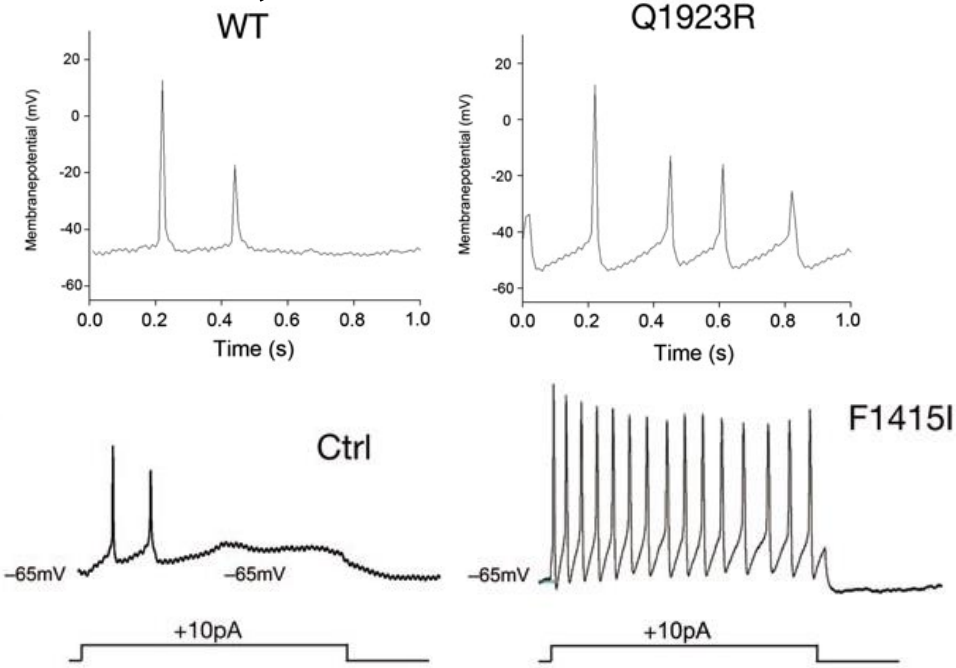


- **iPS cells from two patients (IVS14+3A>T and p.Y325X)**
- **Pyramidal and bipolar cell like neurons are both hyper-active (excitatory)**

Other iPS study #2

Jiao et al., Hum Mol Genet 201;22:4241-4252





- **iPS cells from two patients (p.Q1923R and p.F1415I)**
- **Almost all derived neurons are glutamatergic**
- **Glutamatergic neurons are hyper-active (excitatory)**


iPS Cells

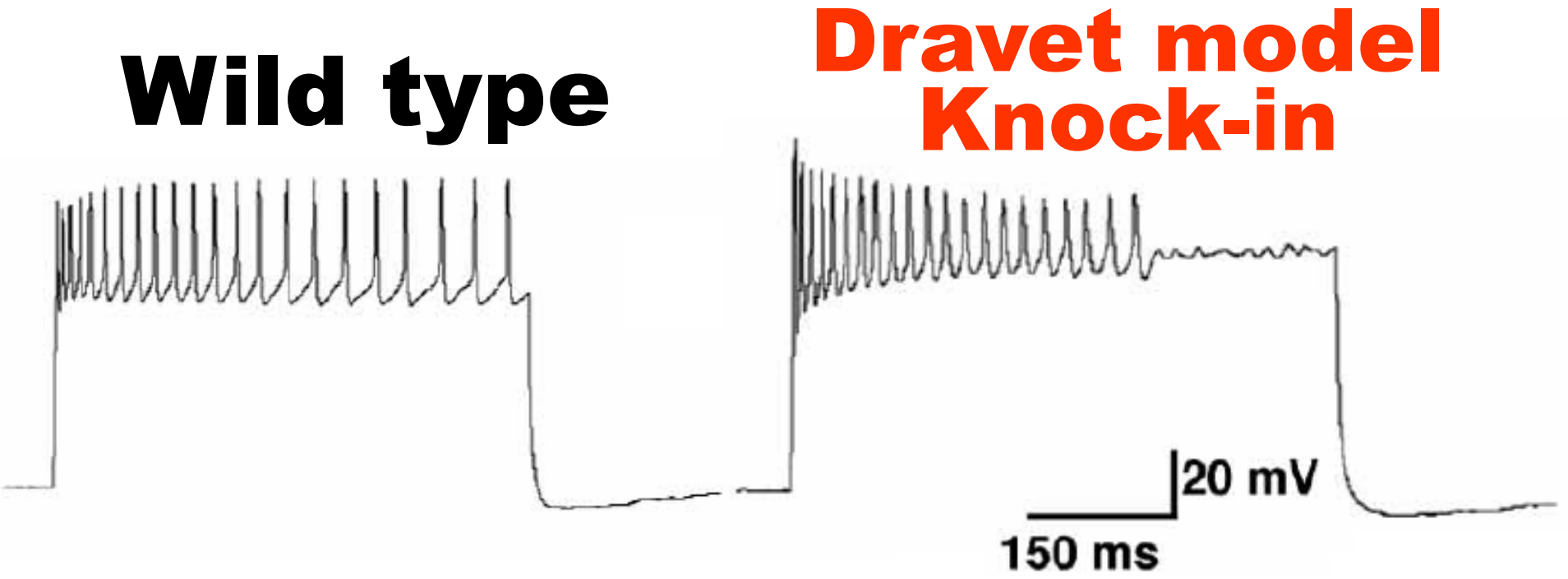

Findings with Model mouse for Dravet syndrome

Department of Pediatrics, Fukuoka University, Love for children, Medicine for tomorrow, and Research for

Model mouse for Dravet syndrome

Our unpublished data

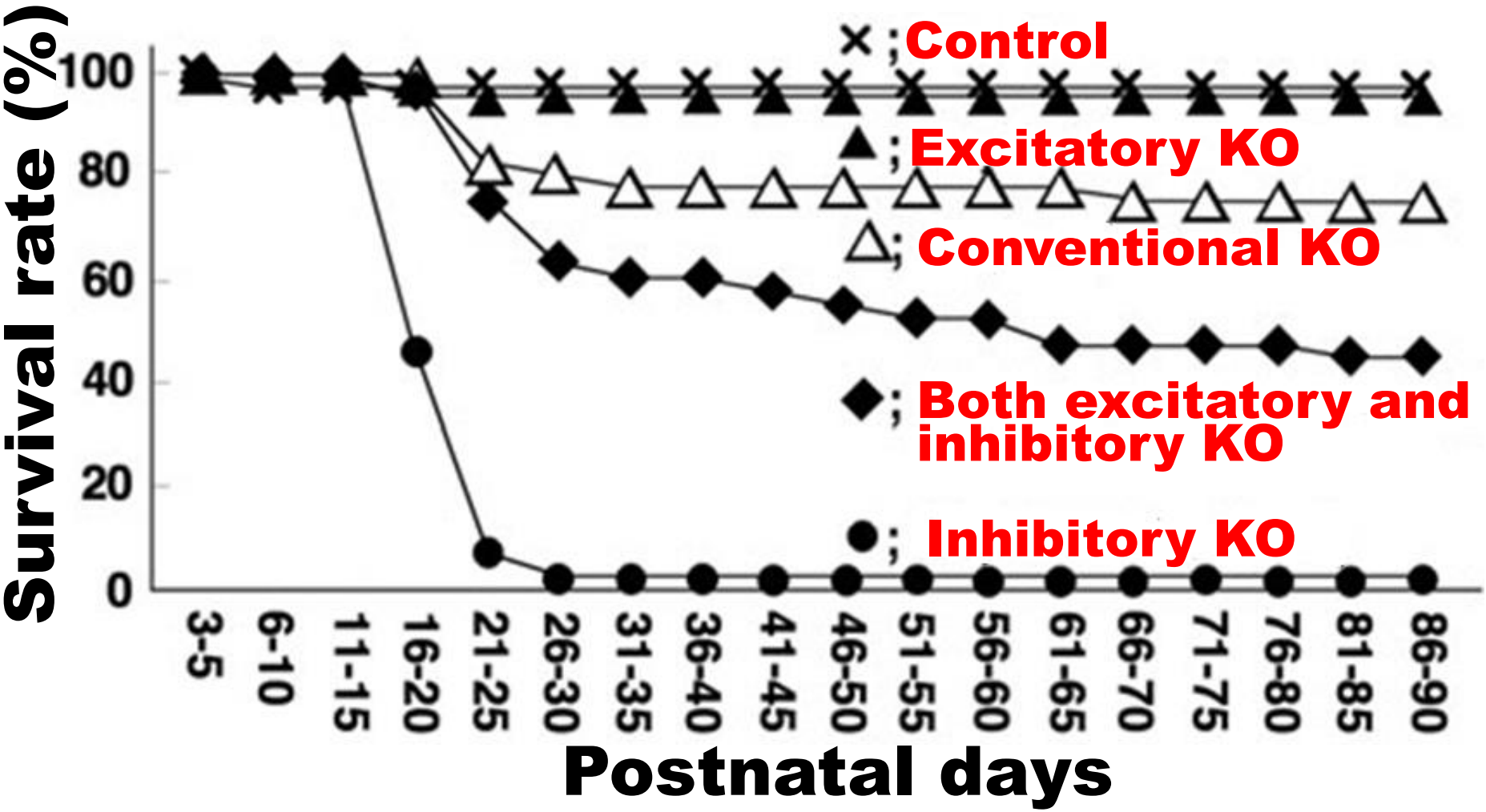
Dravet Syndrome model mouse showed similar results to ours



Depolarization spike attenuated in inhibitory interneuron

Ogiwara I, et al. J Neurosci;27:5903–5914 (2007)

Scn1a knock out mice



Ogiwara I. et al. Hum Mol Genet (2013)


iPS Cells


The pathomechanisms of Dravet syndrome

Lessons from patient-derived iPSCs and animal models

- $Na_v1.1$ channel expressed mainly in GABAergic inhibitory neurons
- Dravet syndrome mutations cause dysfunction of inhibitory interneurons
- This finding accords with clinical findings with CBZ and LTG

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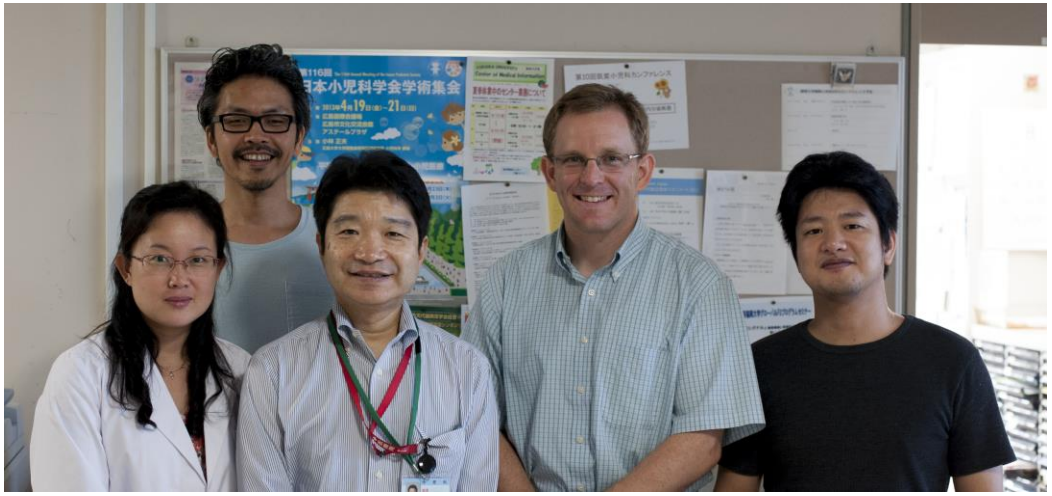
Dr. Christoph Lossin (UC Davis)



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Prof. Hideyuki Okano

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