Human Stem Cell Derived Neural Models for CJD Prion Propagation and Drug Discovery

Zuzana Krejciova

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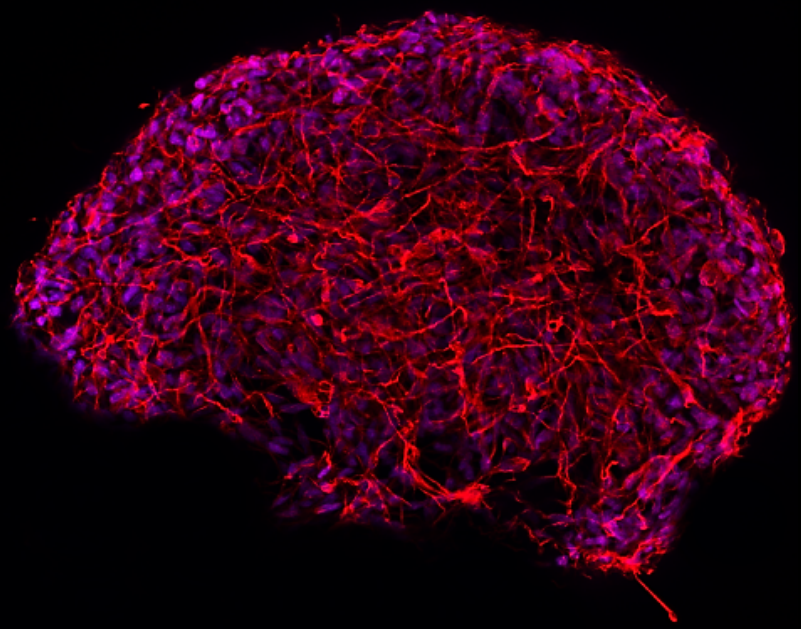
UCSF
University of California
San Francisco

IND
Institute for Neurodegenerative Diseases

Hallmarks of human PrP prion diseases

- PrP^{Sc} accumulation
- Gliosis
- Neuronal loss
- Spongiosis
- Amyloid and plaque formation



We still don't understand the underlying mechanism of neurodegeneration in human prion diseases due to the lack of relevant model system

GFAP
Hoechst

PRNP codon 129 genotype & CJD

	MM	MV	VV
Normal population	37%	51%	12%
sporadic CJD	71%	13%	16%
variant CJD	99%	< 1%	-
iatrogenic CJD	57%	20%	23%

Tg animal cell culture models for studying CJD

Cell line	PrP^{Sc}	Ref.
Cerebellar granule cells from Hu tg mice	vCD, sCJD, iCJD	Hannaoui et al., 2013
Mouse immortalized hypothalamic GT-1	rodent-adapted CJD	Arjona et al., 2014

- Adaptable for high throughput screening

Caveats:

- **May not faithfully recapitulate disease conditions of human neurodegenerative diseases**
- **Small molecules extending survival in mice infected with mouse-adapted prions are ineffective against CJD prions**

(*Berry et al., PNAS 2013)*

Human cell culture models for studying CJD and drug discovery

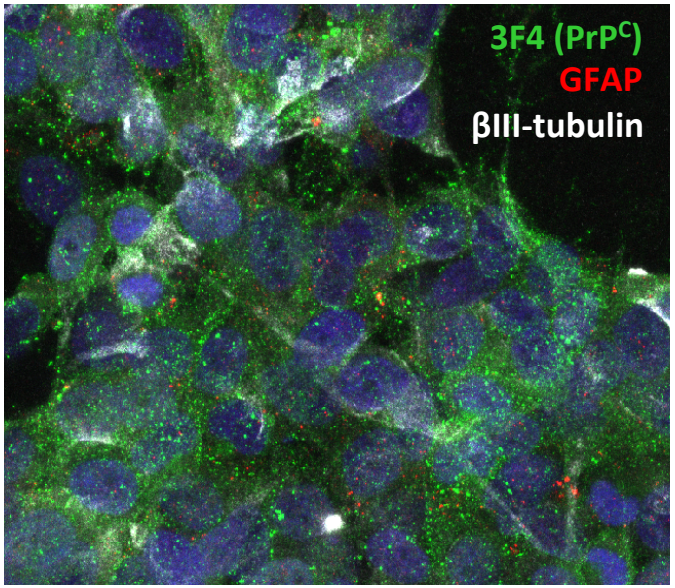
Cell line	PrP^{Sc}	Ref.
Human neuroblastoma SH-SY5Y	sCJD	Ladogana et al., 1995

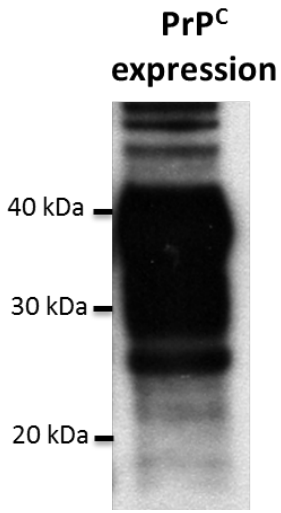
There is no human cell culture model in which human PrP^{Sc} prions replicate

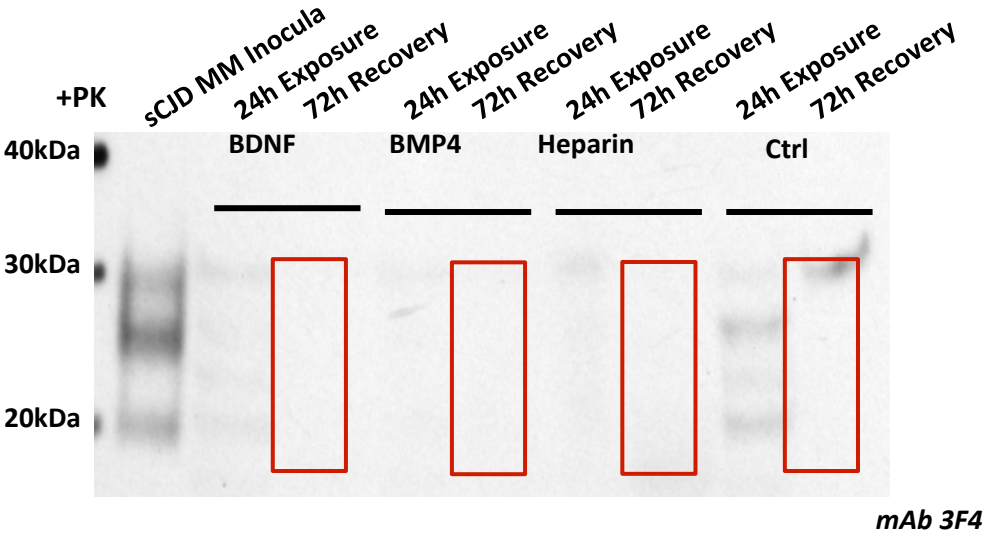
Possible reasons for failure:

- Transformed cells do not represent the phenotypic environment of human prion diseases
- Incompatibility between *PRNP* codon 129 genotype between inocula and cultured cells
- Low PrP^{C} expression
- Protein X
- PrP^{Sc} clearance rate is higher than PrP^{Sc} production
- High rate of cell division
- Tagging of PrP^{C} compromise PrP^{Sc} formation and infectivity *in vivo* & *in vitro*
- Culture conditions

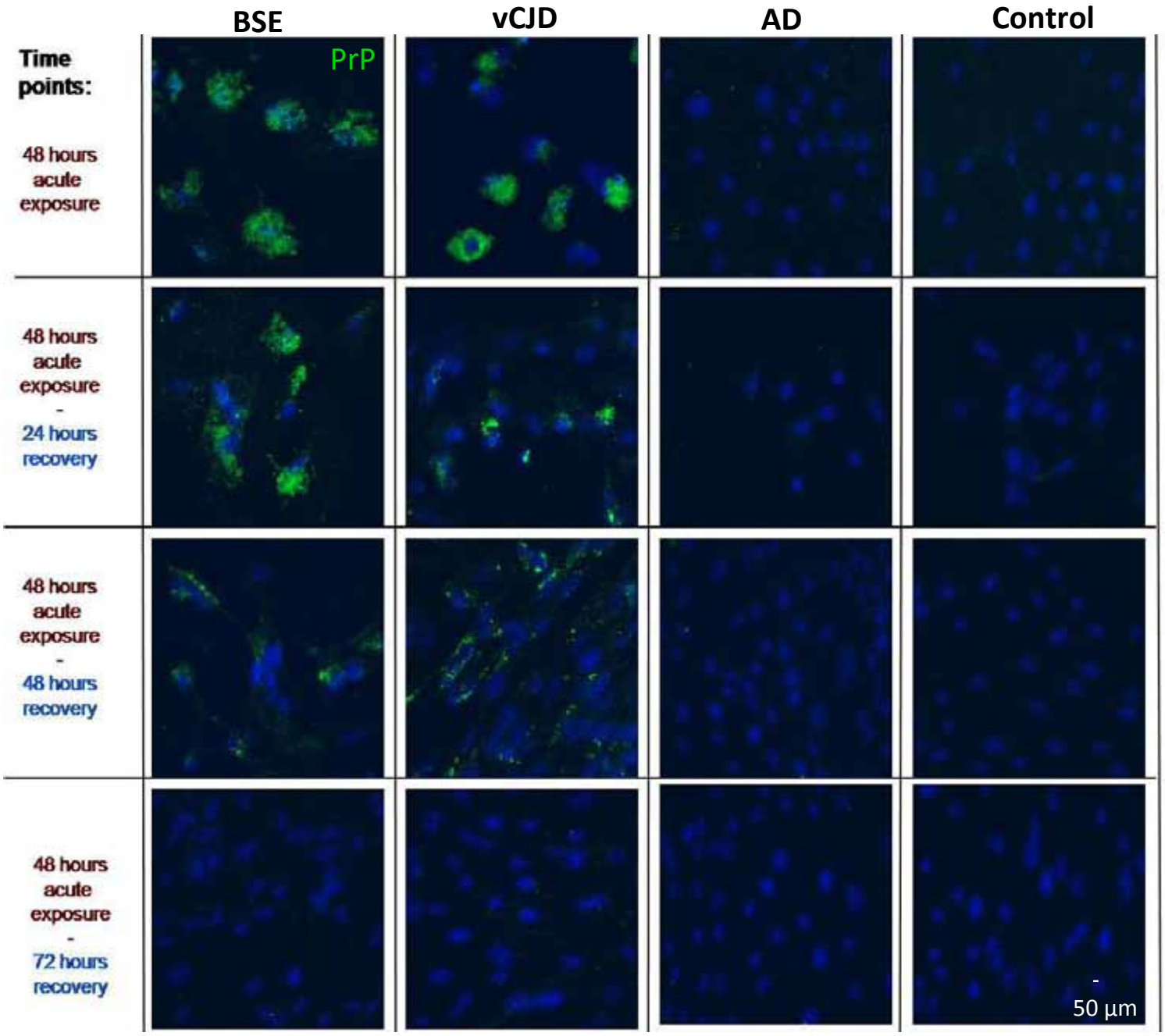
Human neuroglioma H4 line (MM)





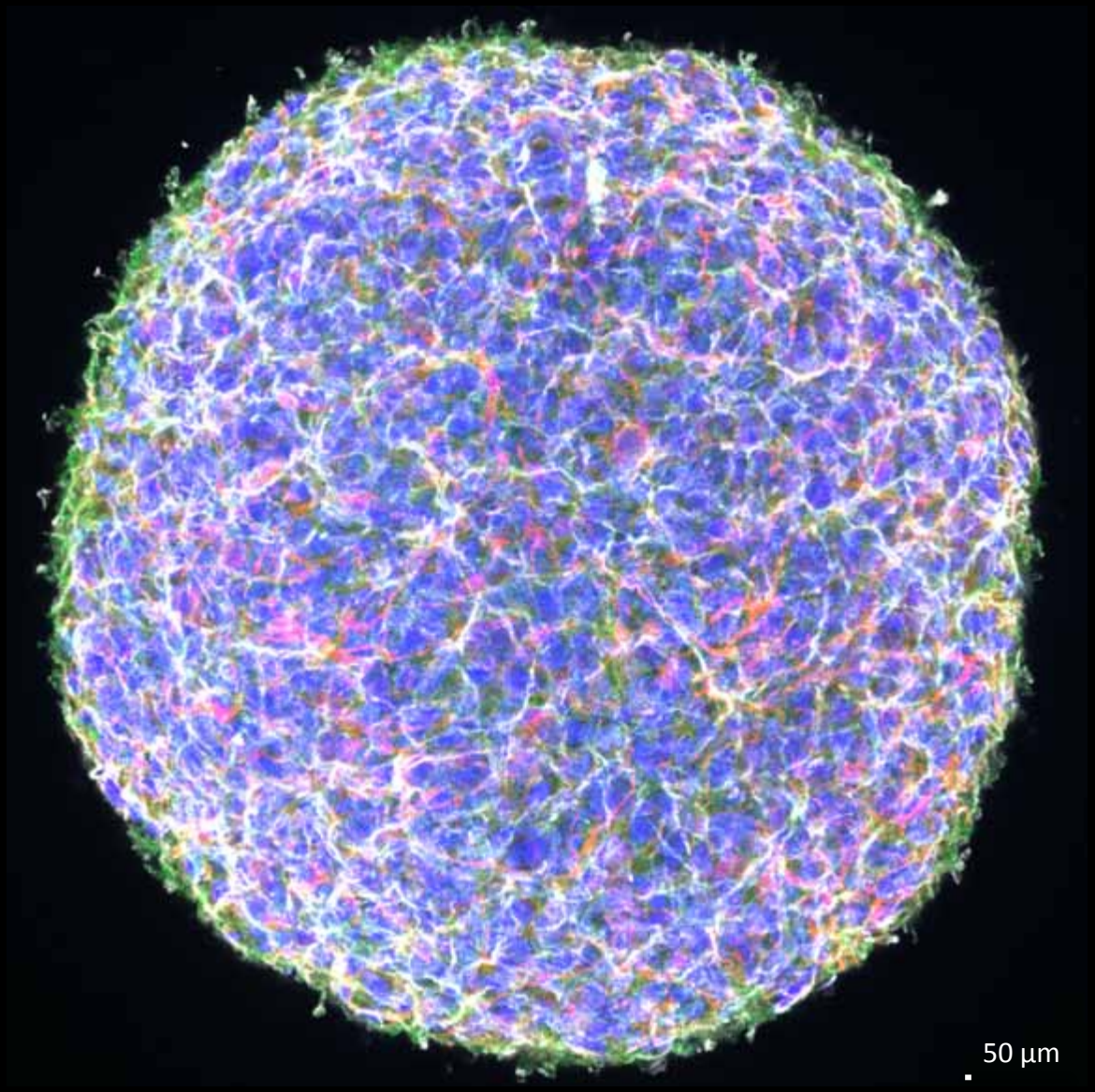


hESC H9 (MV) exposed to prion infection



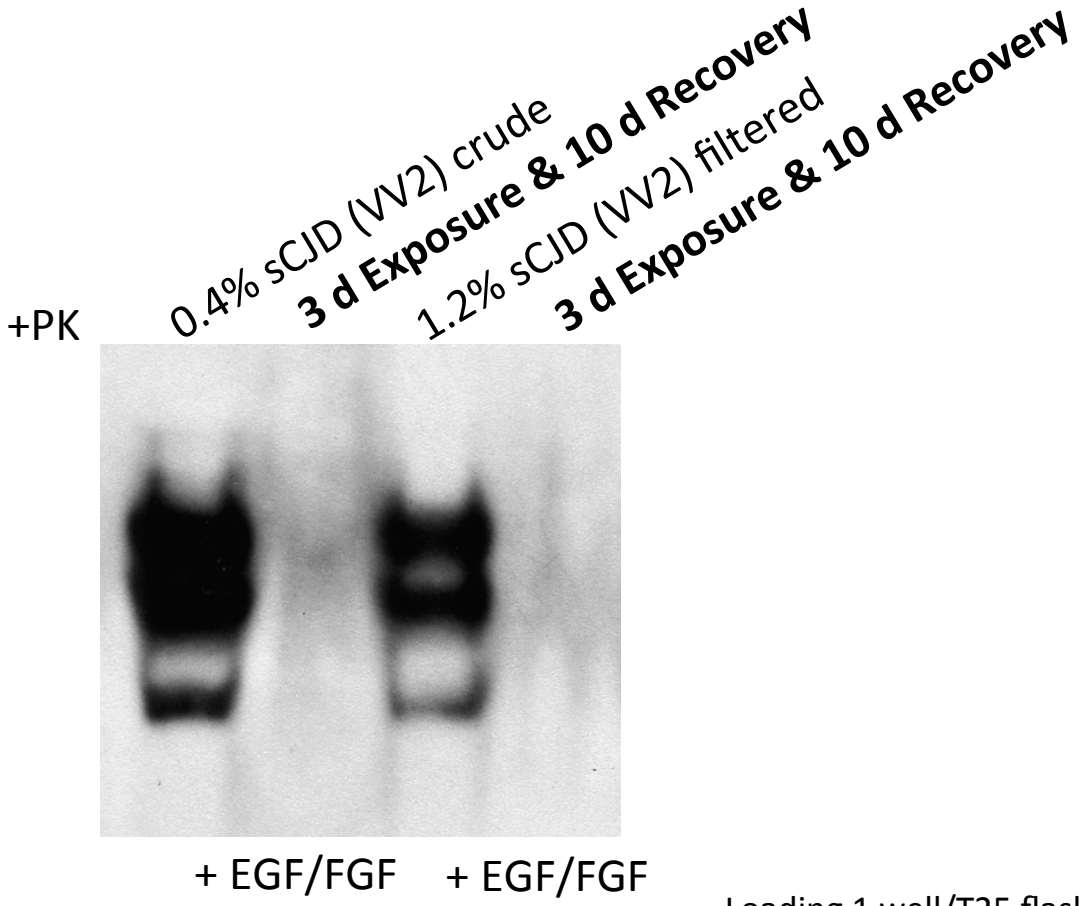
Krejciova et al., 2011

Human fetal brain-derived neurospheres (MV)



Human fetal brain-derived spheres (MV)

Exposure to sCJD (VV_2)



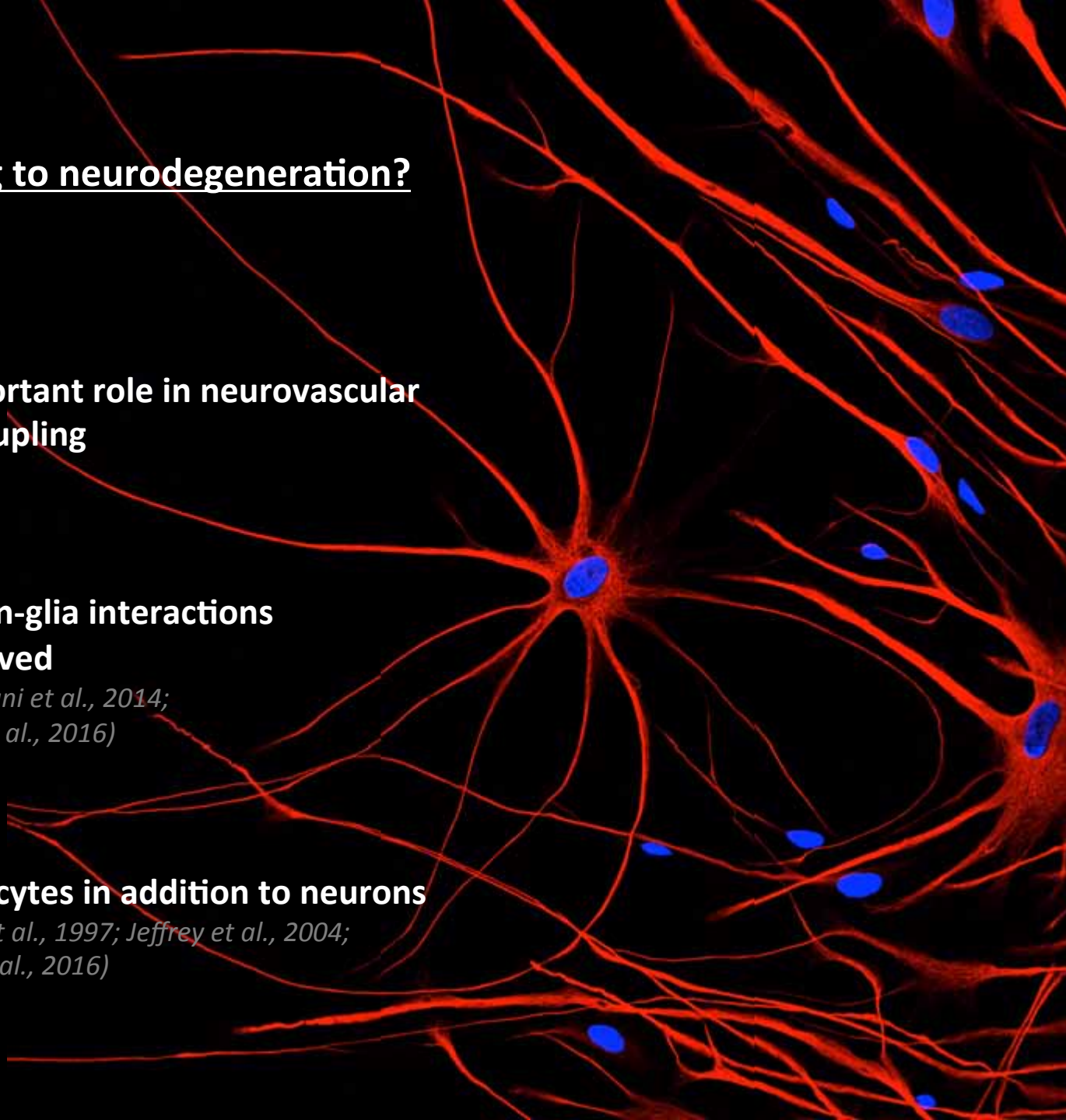
Loading 1 well/T25 flask
mAb 3F4

Astrocytes contributing to neurodegeneration?

- **Astrocytes play an important role in neurovascular and neurometabolic coupling**

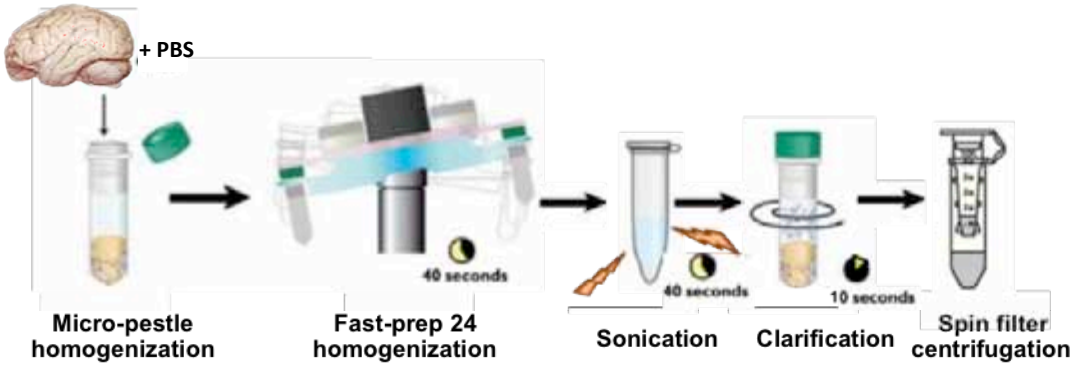
- **In prion diseases neuron-glia interactions are thought to be involved**
  (Gomez-Nicola et al., 2013; Asuni et al., 2014; Hennessey et al., 2015; Fang et al., 2016)

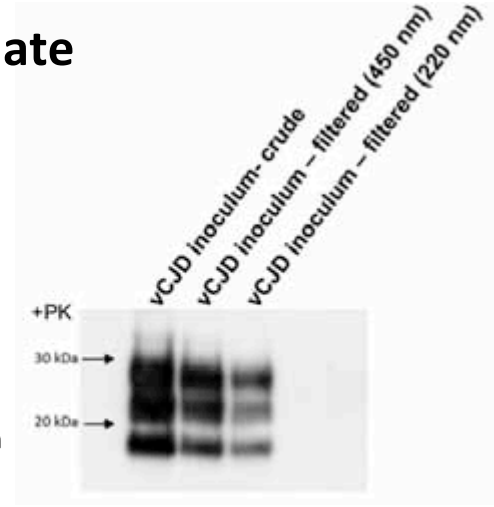
- **PrP^{Sc} replicates in astrocytes in addition to neurons**
  (Diedrich et al., 1991; Raeber et al., 1997; Jeffrey et al., 2004; Cronier et al., 2004; Victoria et al., 2016)



Exposure of human astrocytes to CJD brain homogenate

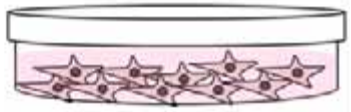
CJD inoculum

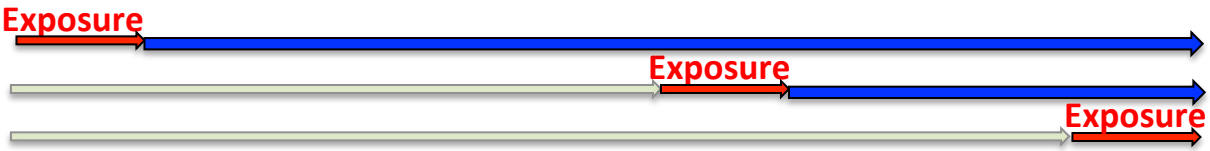




Exposure regime

1% spin filtered brain homogenate





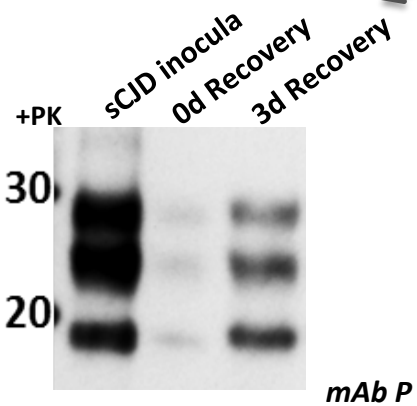
Time points

Recovery 8d

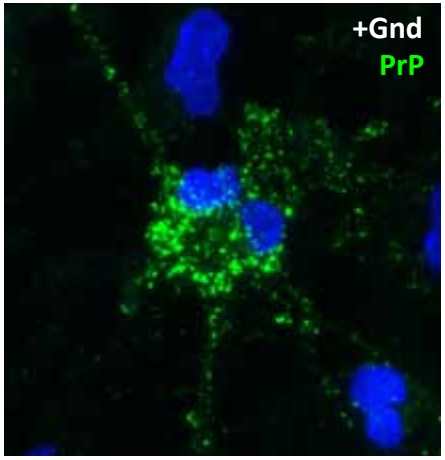
Recovery 3d

Recovery 0d

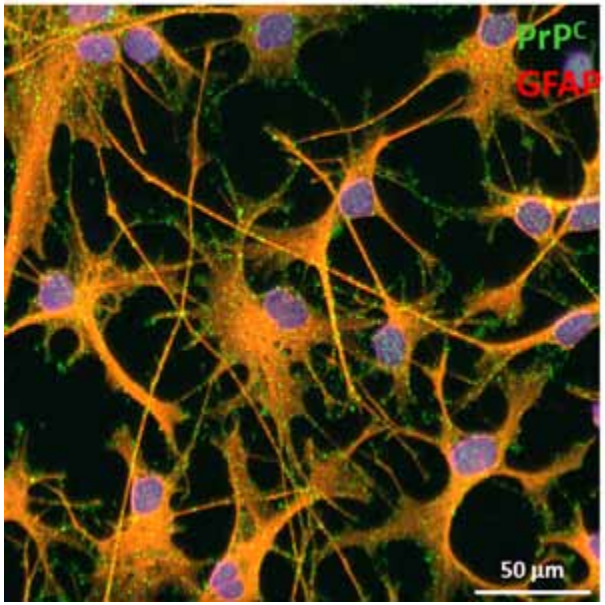
Analysis



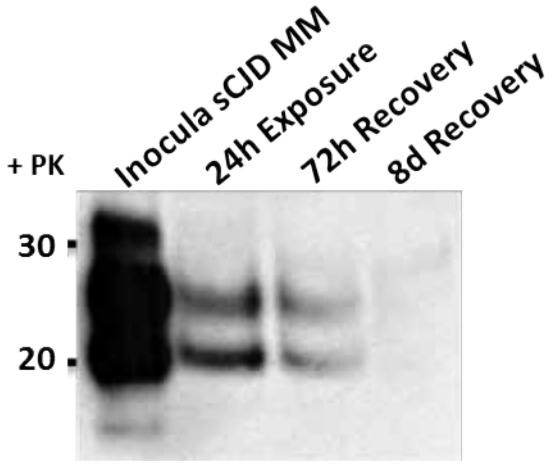
mAb P



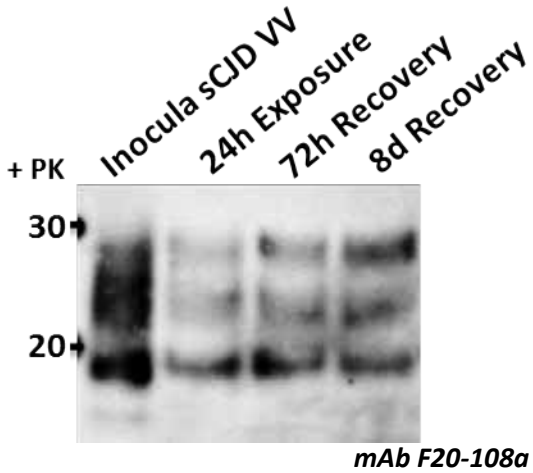
hESCs derived astrocytes (MV)



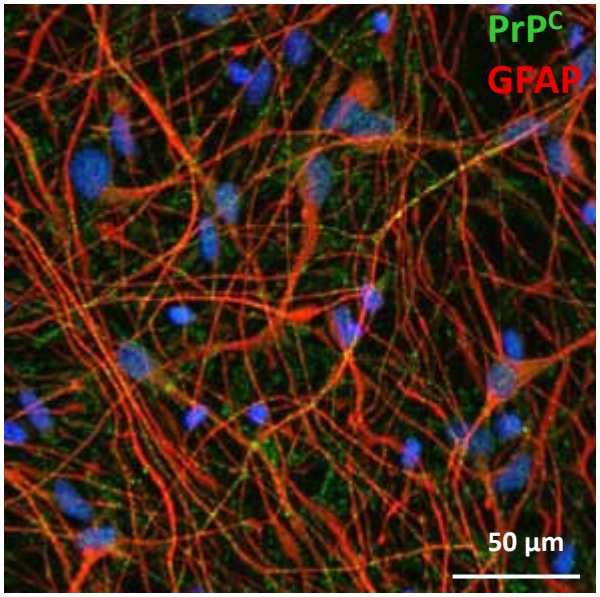
Exposure to sCJD (MM)



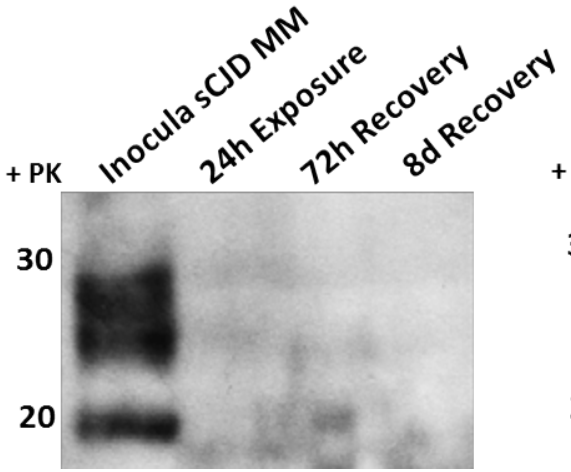
Exposure to sCJD (VV_2)



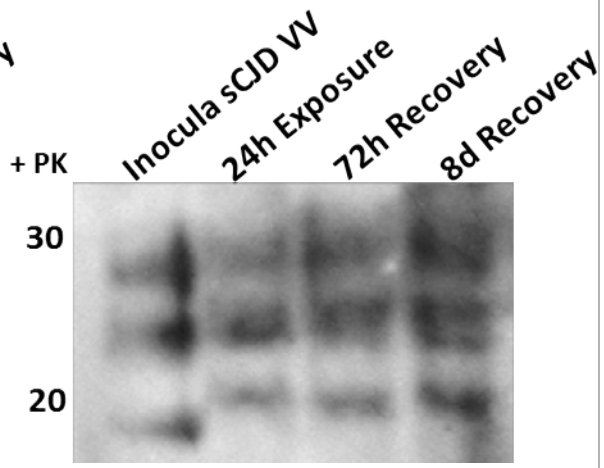
mAb F20-108a



Exposure to sCJD (MM)



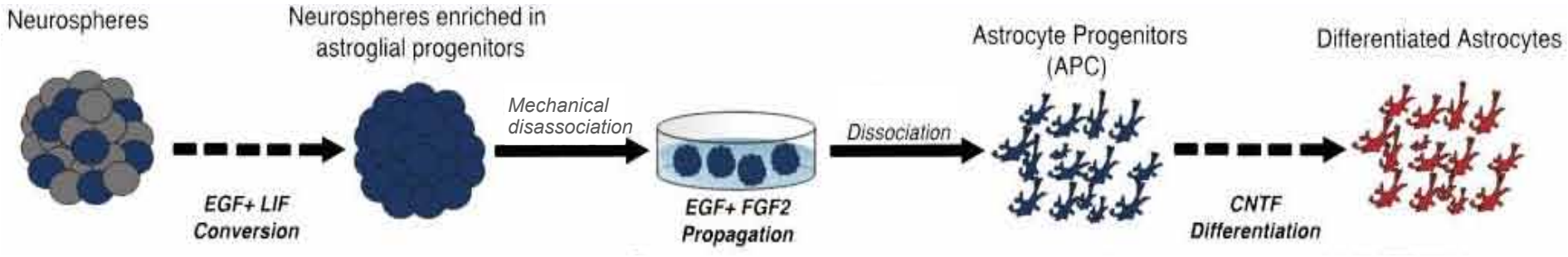
Exposure to sCJD (VV_2)

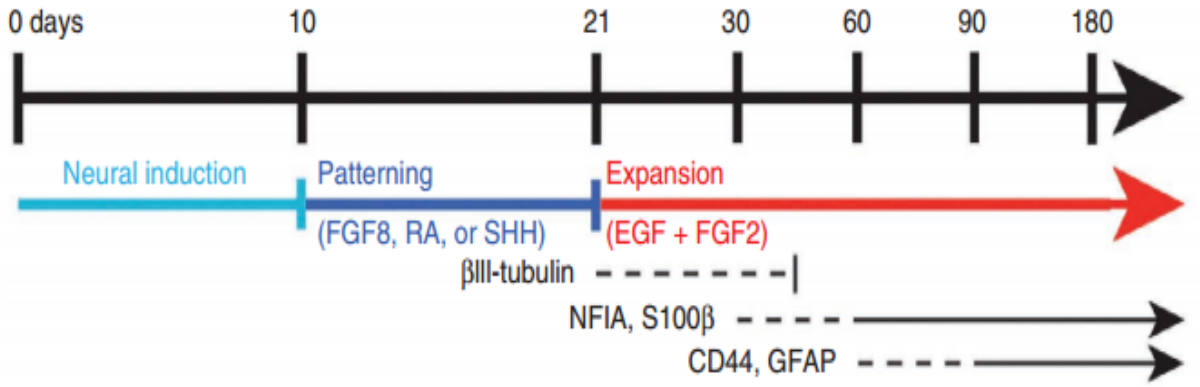


mAb 3F4

Generation of human cell culture model for studying CJD pathogenesis & drug discovery

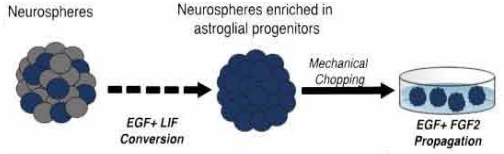
Astrocyte progenitor cells (APC) generation from human iPS cells



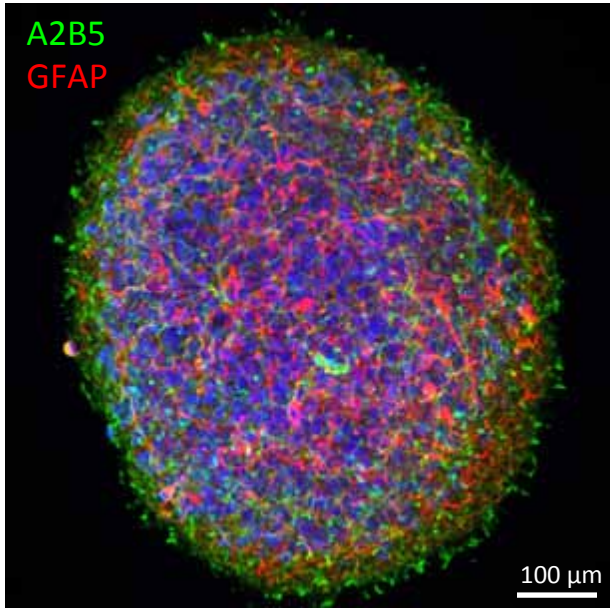


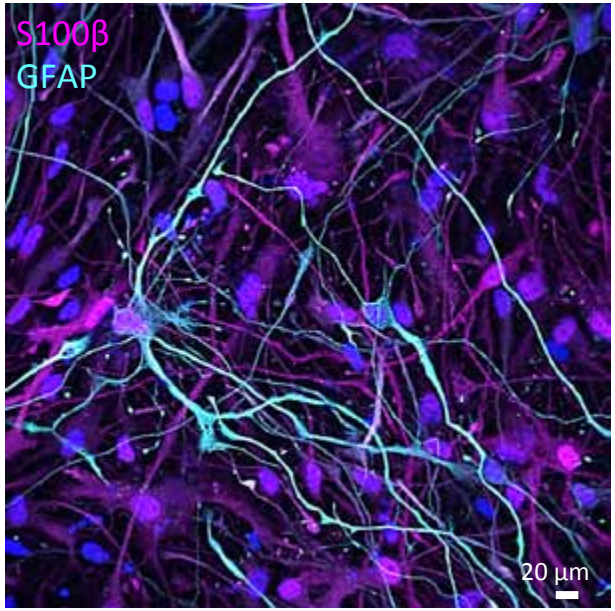
Krencik & Zhang, Nat Protoc, 2011
Serio et al., PNAS, 2013

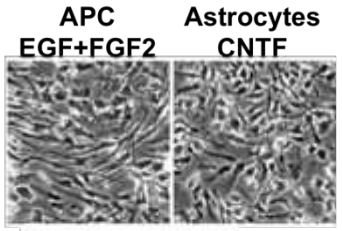
Astrocyte Differentiation

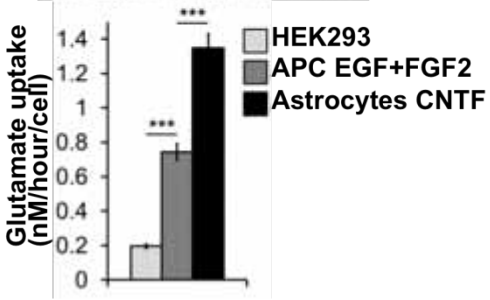


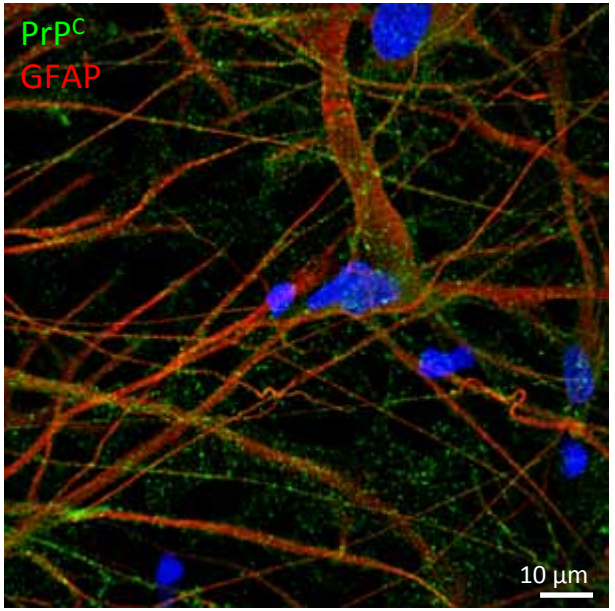
Astrocyte progenitors → **Differentiation in CNTF media 2 wks**

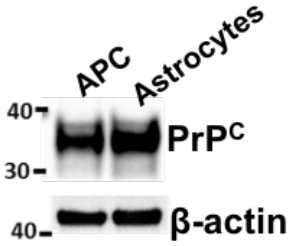






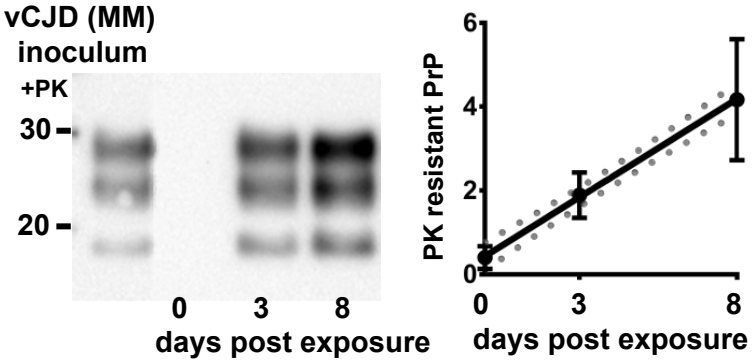




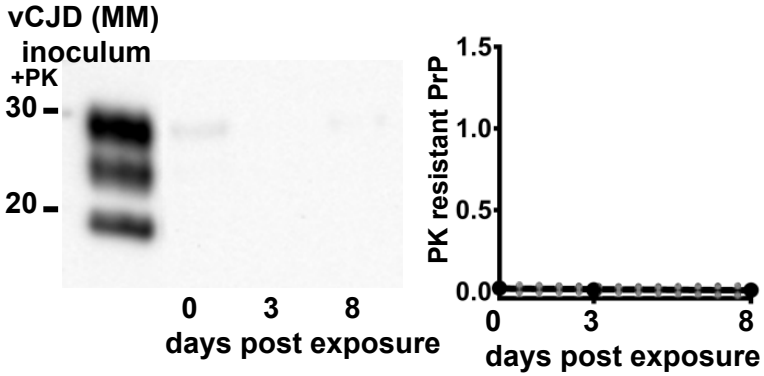


Human astrocytes replicate CJD PrP^{Sc} *in vitro* in a *PRNP* codon 129-dependent manner

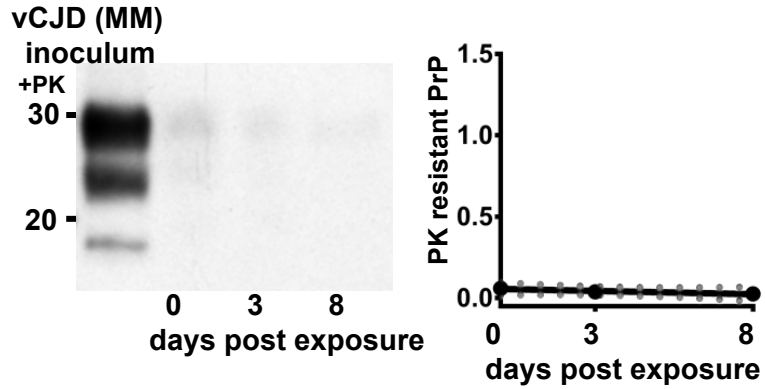
Astrocytes (MM)



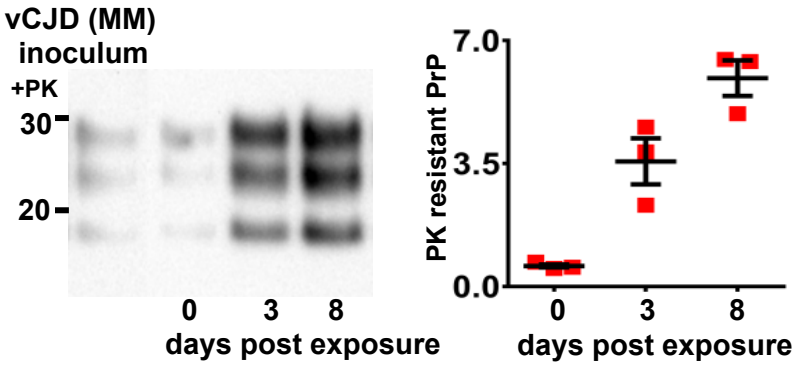
Astrocytes (MV)

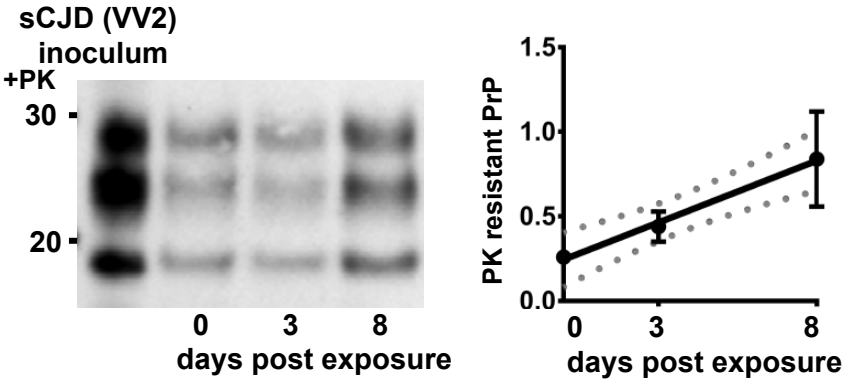


Astrocytes (VV)



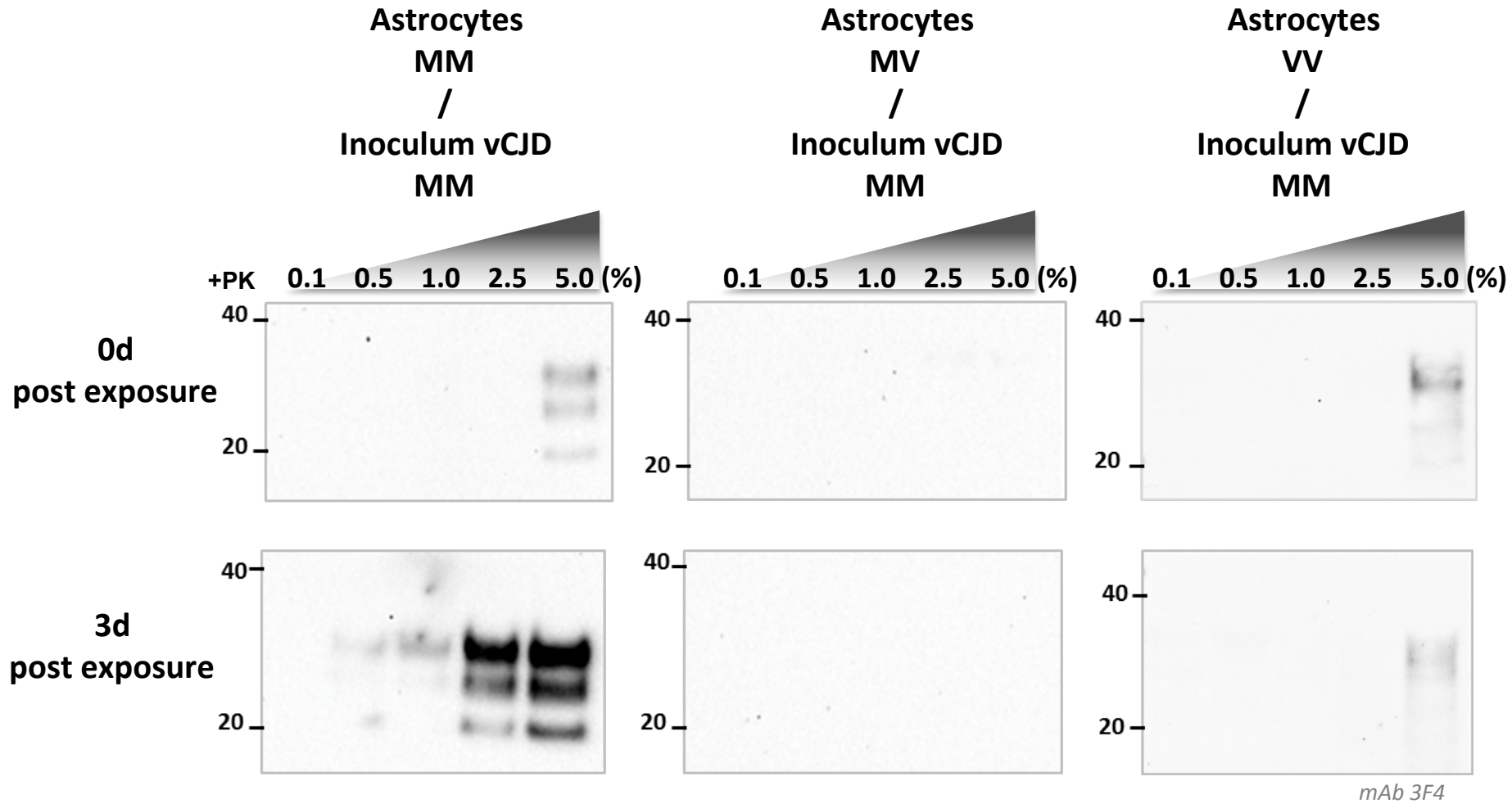
Additional astrocyte (MM) line





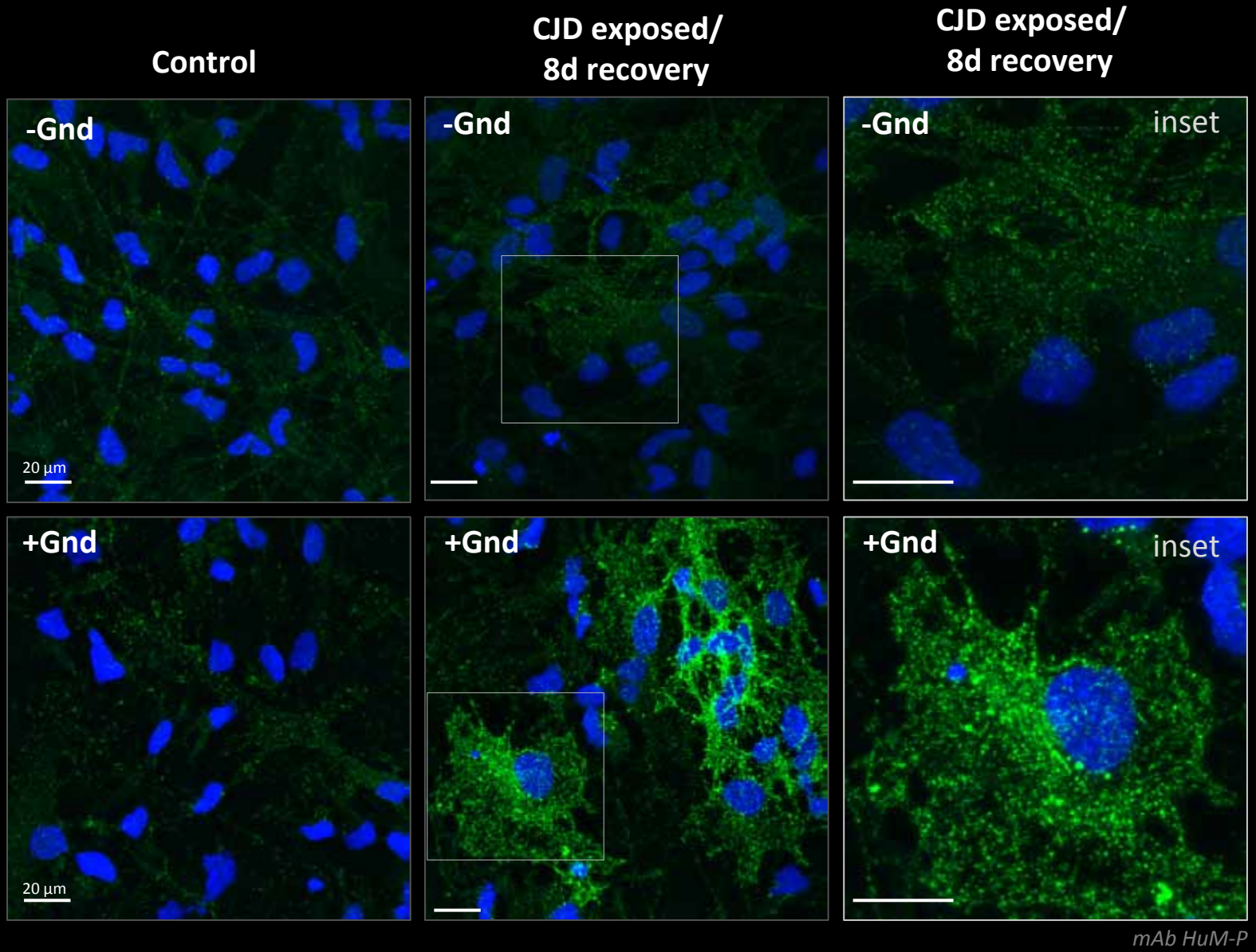
Krejciova Z., Alibhai J., et al., (submitted)

Replication of vCJD PrP^{Sc} in human astrocytes is *PRNP* codon 129- and concentration-dependent



Krejciova Z., Alibhai J., et al., (submitted)

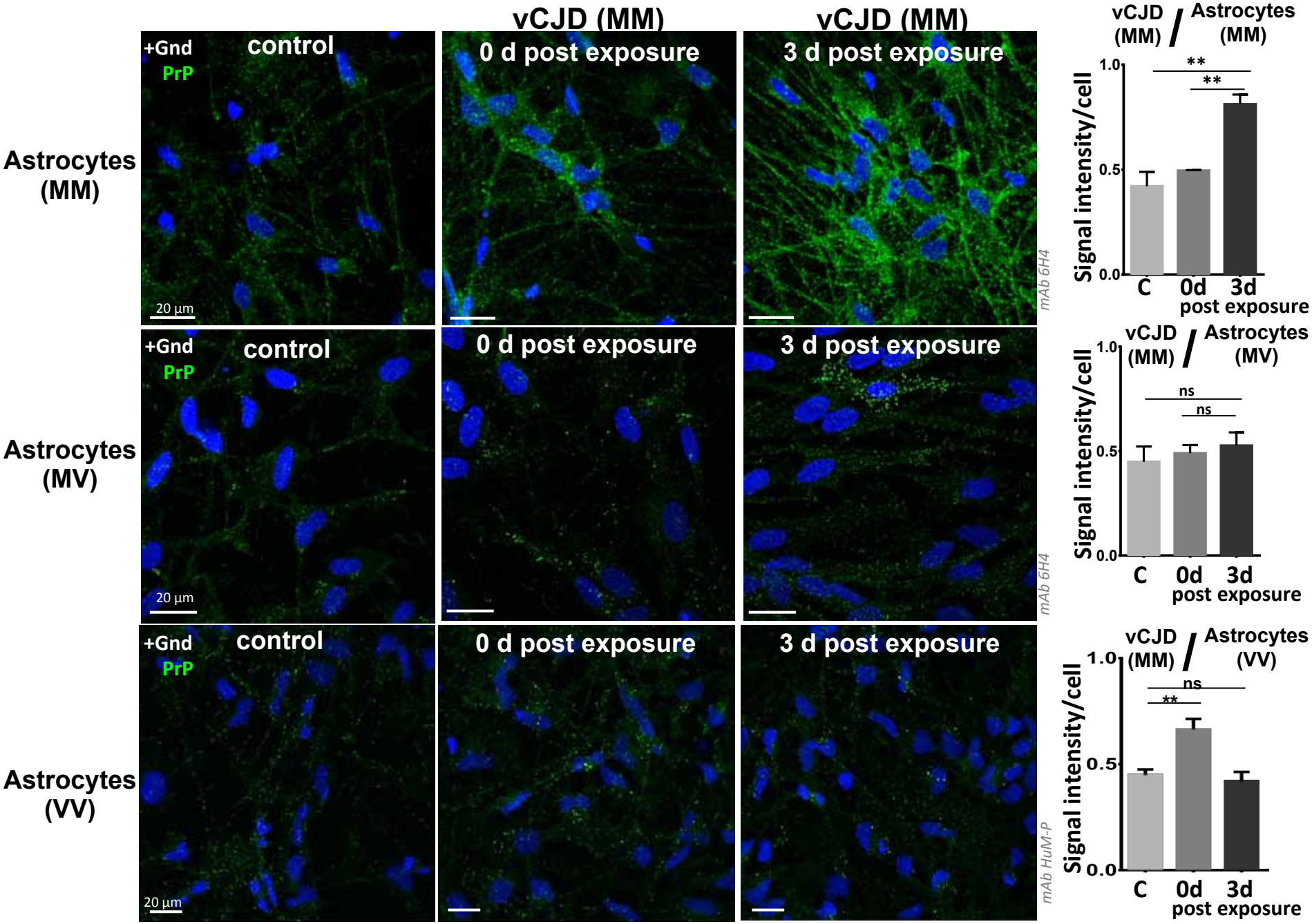
Guanidine mediates cryptic PrP^{Sc} epitope retrieval



mAb HuM-P

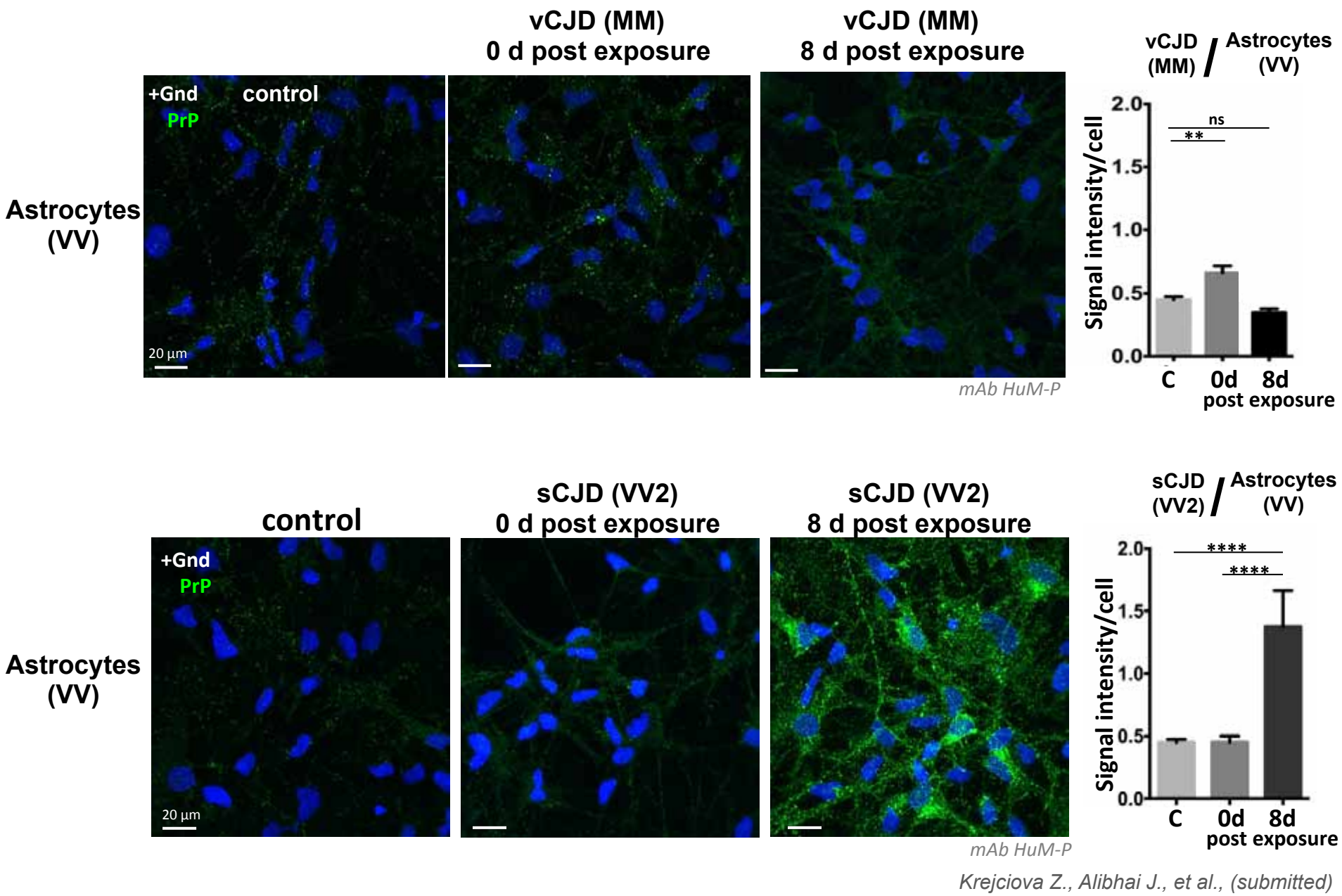
Krejciova Z., Alibhai J., et al., (submitted)

Human astrocytes replicate PrP^{Sc} when the CJD inoculum & cell genotype are matched



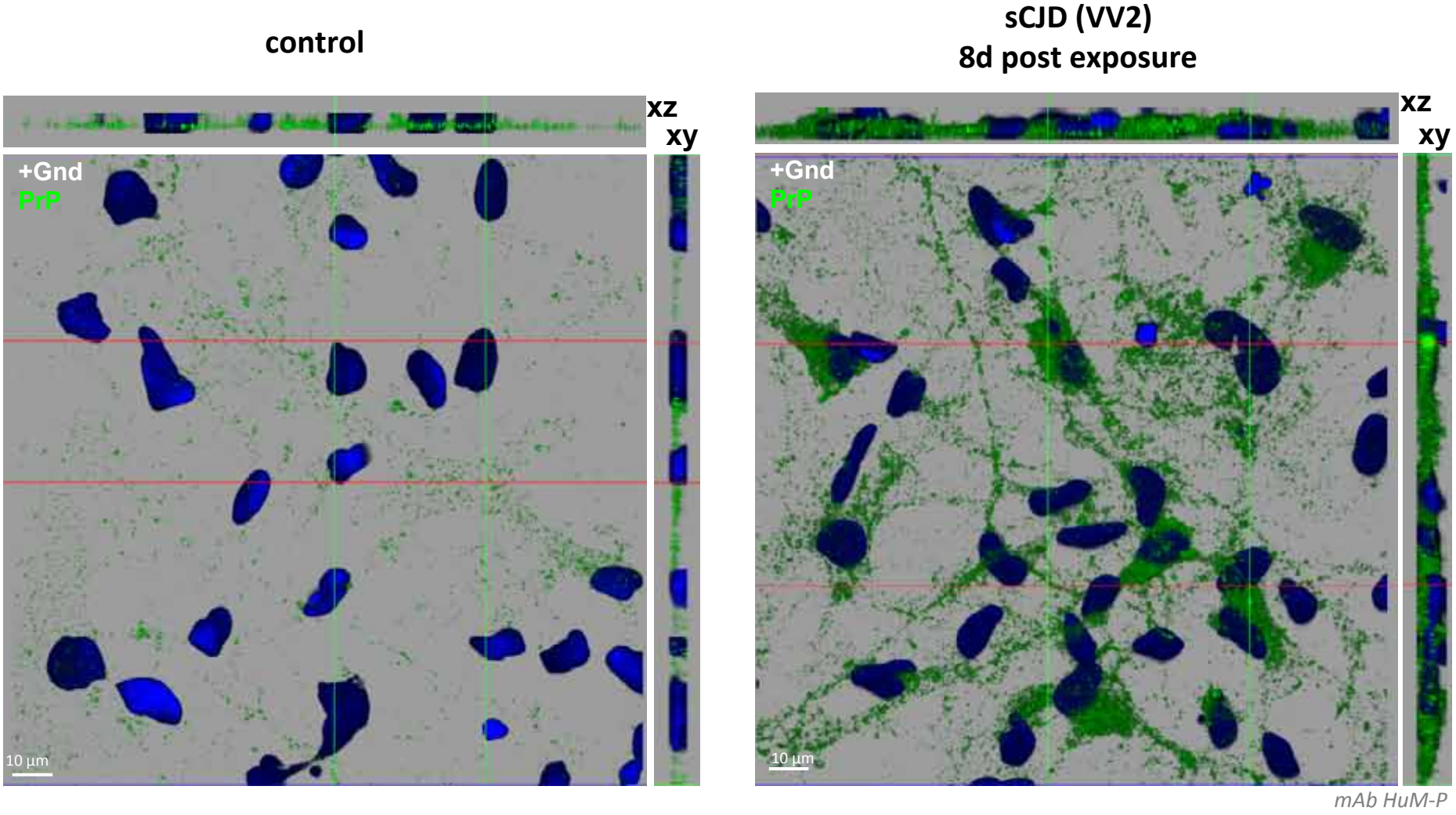
Kreiciova , Alibhai, et al., (submitted)

Human astrocytes (VV) replicate sCJD (VV2) PrP^{Sc} prions



Krejciova Z., Alibhai J., et al., (submitted)

3D reconstruction of PrP^{Sc} accumulation in astrocytes (VV) exposed to sCJD (VV2)



Krejciova Z., Alibhai J., et al., (submitted)

Summary

Proliferating ES, FDC-like and transformed human cells failed to propagate CJD PrP^{Sc} *in vitro*

Human iPS cell-differentiated astrocytes are susceptible to CJD PrP^{Sc} replication *in vitro* in a *PRNP* codon 129 dependent manner

We hypothesize that astrocytes might contribute to prion-induced neurodegeneration in a non-cell autonomous manner, either by impairment of neuroprotective function or gain of neurotoxic function by generating neurotoxic signals in response to prion infection

Our model is providing a new *in vitro* system for accelerated mechanistic studies and drug discovery

Acknowledgements

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Centre for Regenerative Medicine

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Dr Nina Rzechorzek
Dr Chen Zhao

Ullian lab

Dr Robert Krencik

The Roslin Institute

Prof Jean Manson

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NC3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

DH Department of Health

NIH National Institute on Aging

Daiichi-Sankyo

Creutzfeldt-Jakob Disease Foundation, Inc.

Non-Expert Summary

Prions are infectious agents that cause neurodegenerative diseases such as Creutzfeldt-Jakob disease. For four decades, scientists have attempted to produce experimental models that approximate prion diseases – both to develop new therapies and to understand the mechanisms of the disease – but until now these efforts have been unsuccessful. For example, drugs developed using mice infected with prions have been shown to be ineffective against human prions.

Here we show that prions can infect brain cells (so-called astrocytes) derived from human stem cells, and determine which factors influence their susceptibility to prions. These include genetics and the cells' state of development. Our work thus addresses a long-standing gap, providing a more relevant tool for studying prion diseases and accelerating drug discovery.