

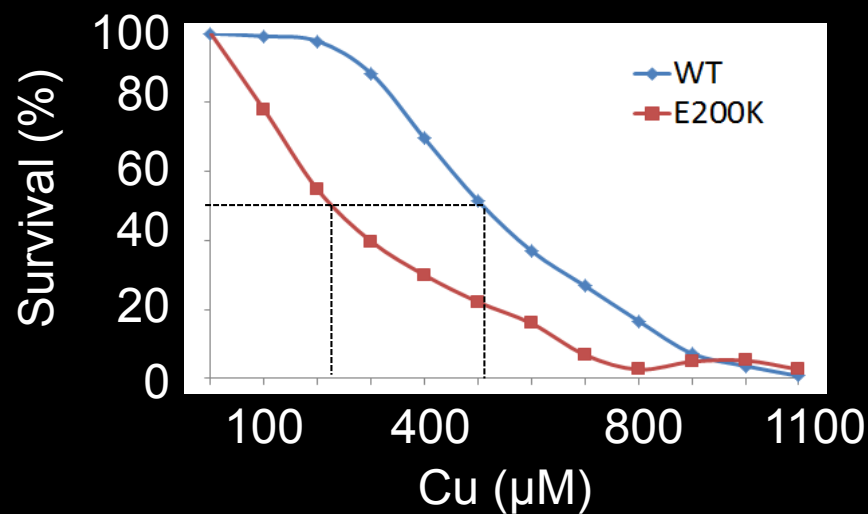
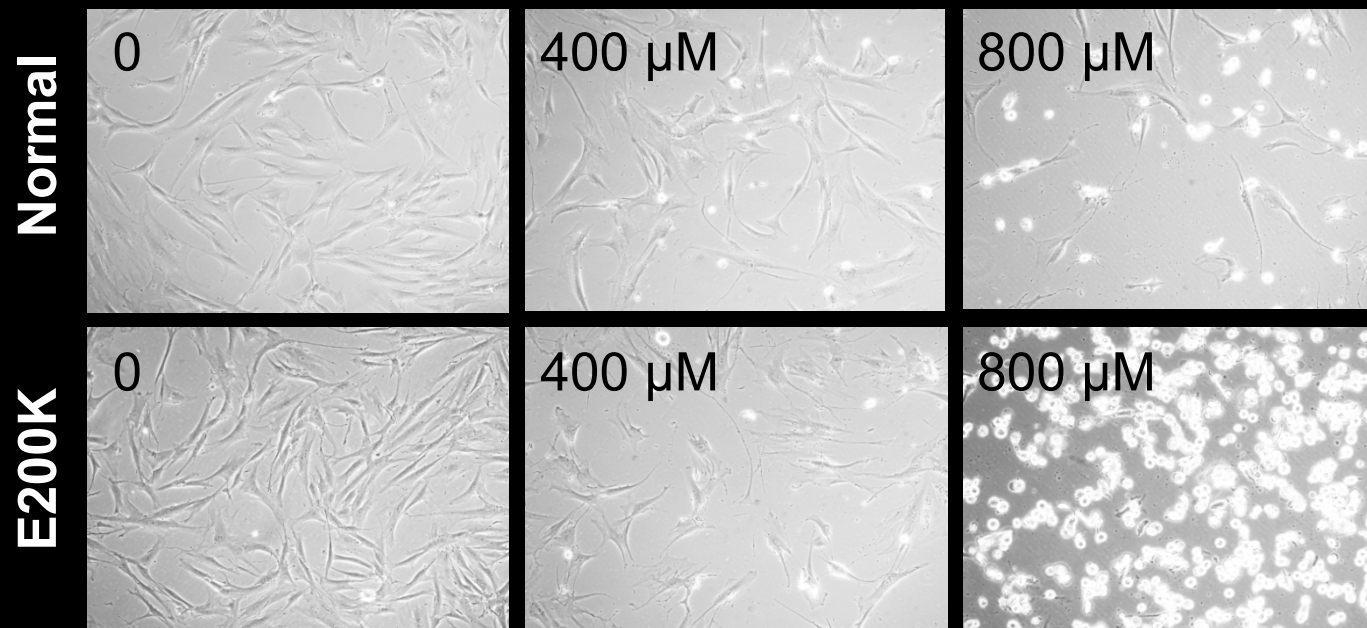
Developing therapeutics for CJD using patient-specific iPSC-derived neurons

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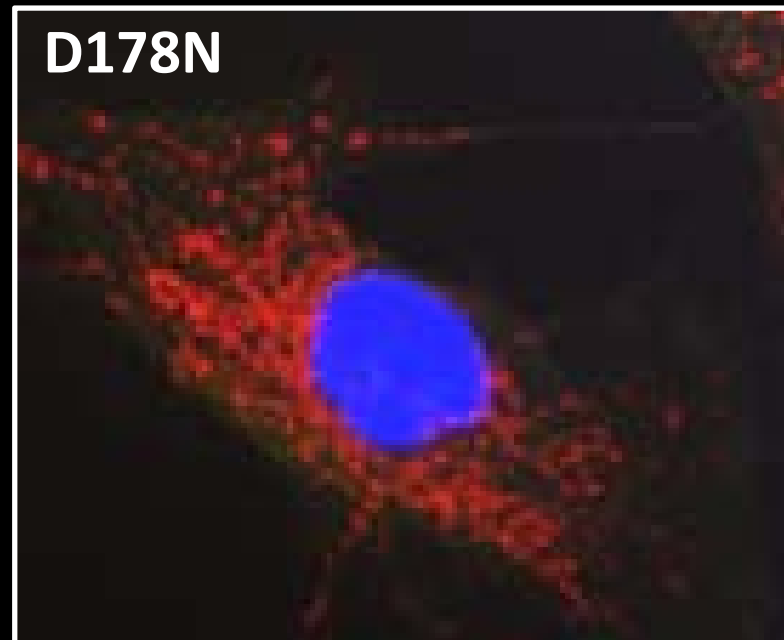
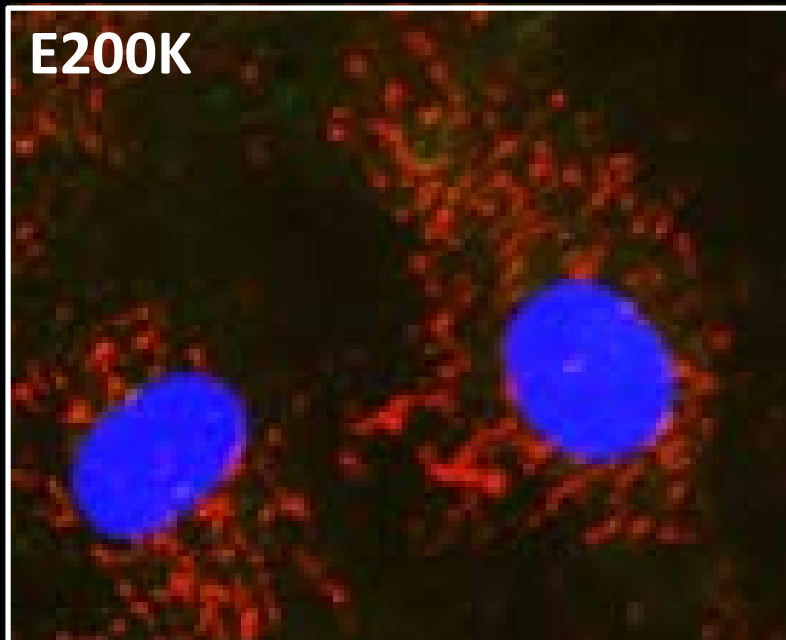
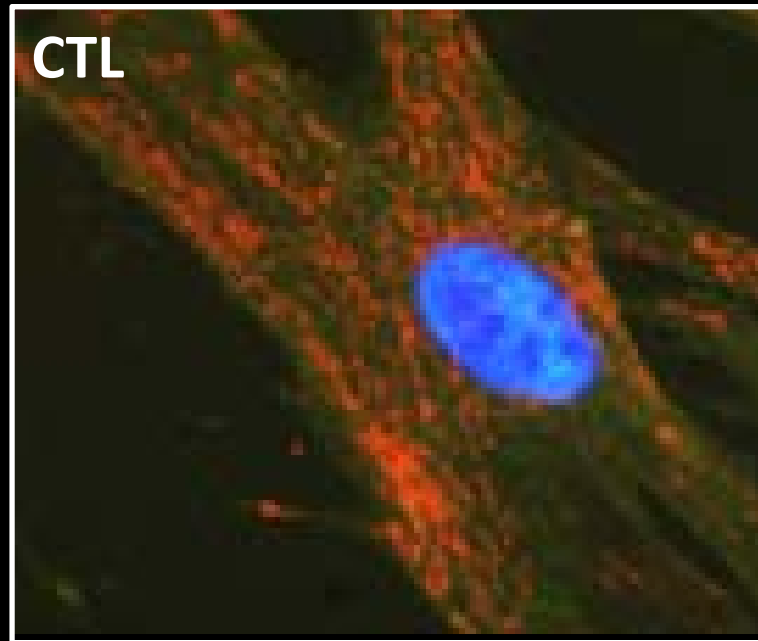
Code	Genotype	Asso. Diseases	Phenotype
1	E200K (MM)	fCJD	Carrier
2	D178N (MM)	FFI	Carrier
3	F198S (MV)	GSS	Carrier
4	E200K (MM)	fCJD	Carrier
5	WT (MM)	sCJD	sCJD
6	5-Oct ins(VV)	CJD	Carrier
7	WT (MM)	Normal	Normal
8	WT (MV)	Normal	Normal
9	WT (MV)	AD	AD
10	WT (MM)	Normal	Normal
11	E200G (MV)	fCJD	Carrier
12	Del24bp	Diabetes	Diabetes
13	2-Oct ins (MM)	fCJD	Carrier
14	WT (MM)	sCJD	sCJD
15	WT (MM)	Amputation	Normal
16	WT (?)	Panniculectomy	Normal
17	WT (?)	sCJD	sCJD
18	WT (?)	Normal	Normal
19	WT (?)	Normal	Normal
20	WT (?)	Normal	Normal
21	E200K (MV)	fCJD	Carrier
22	WT (?)	sCJD	sCJD
23	D178N (MM)	FFI	Carrier
24	E200K (MV?)	sCJD	sCJD

Vulnerability to copper oxidative stress



LC₅₀
WT \approx 500 μM
E200K \approx 200 μM

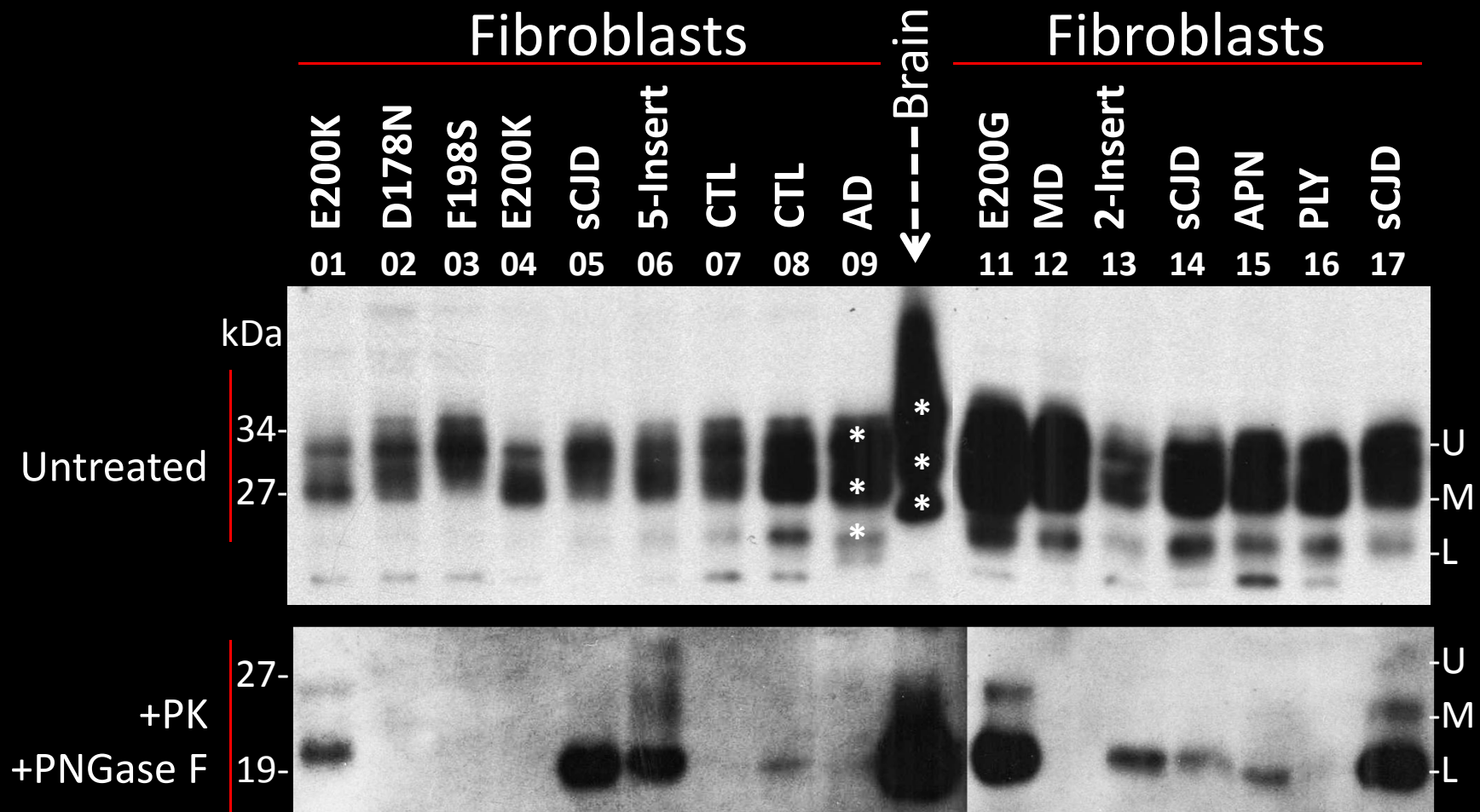
Mitochondria from normal and mutant fibroblasts



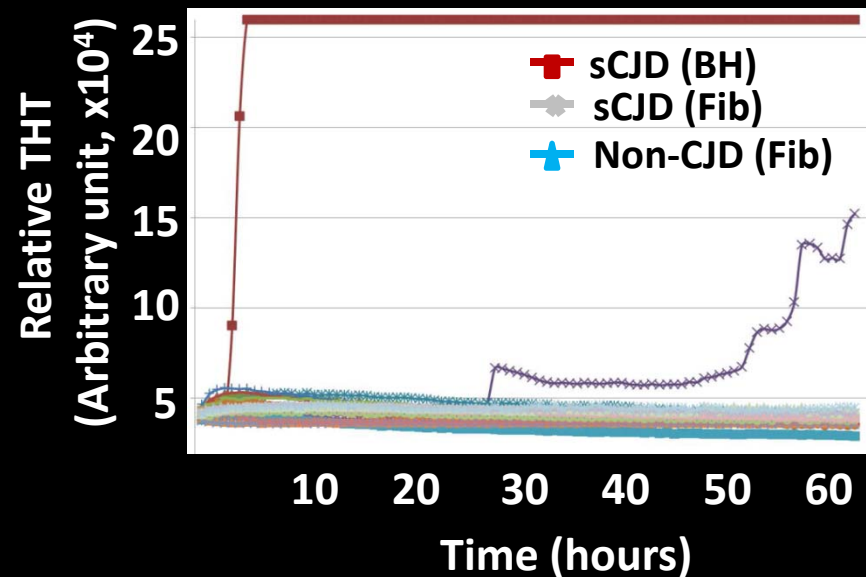
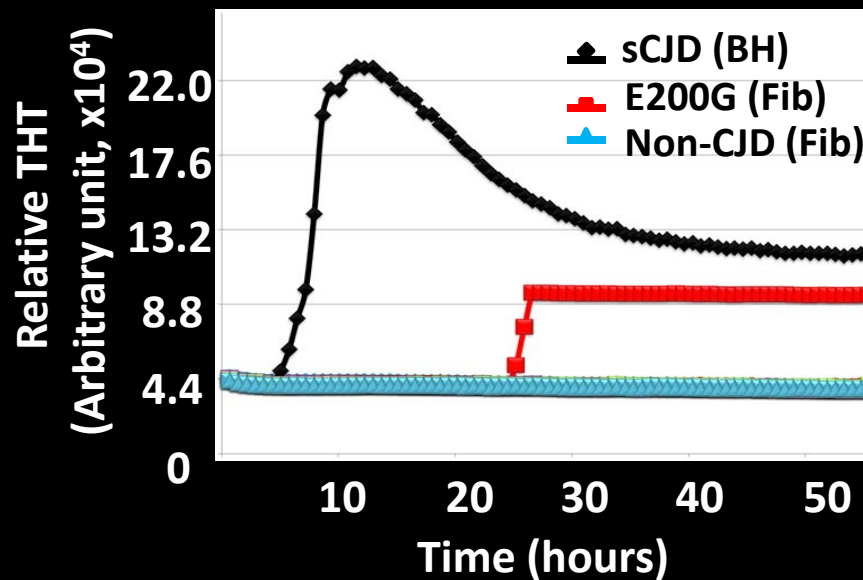
Electron microscopy of patient-specific fibroblasts



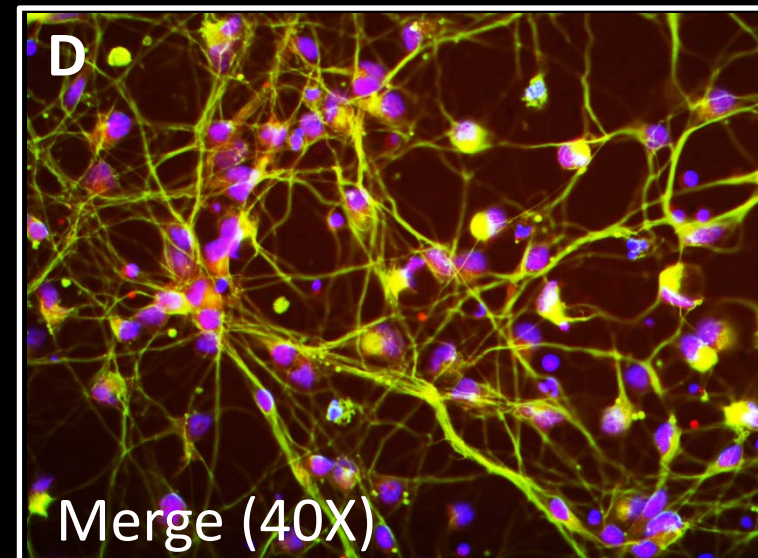
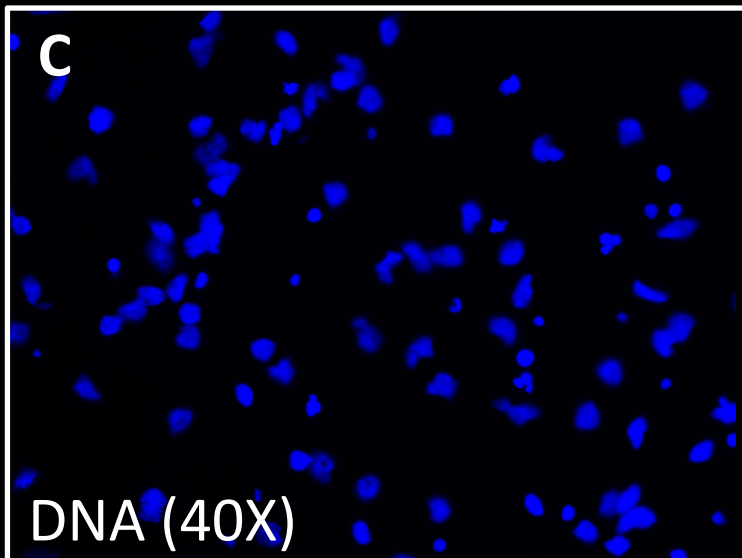
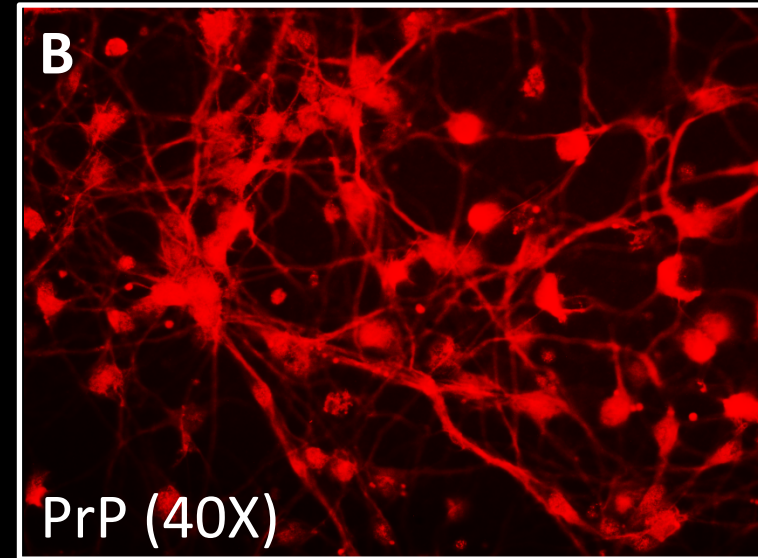
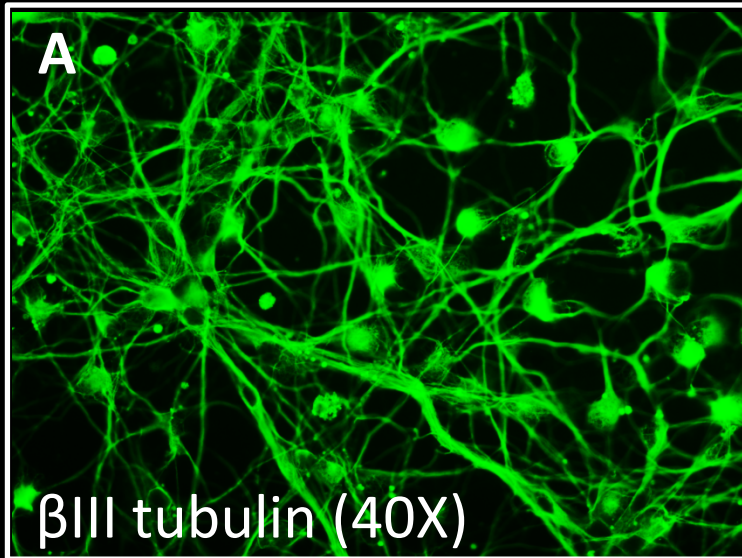
PrP in patient-specific fibroblasts



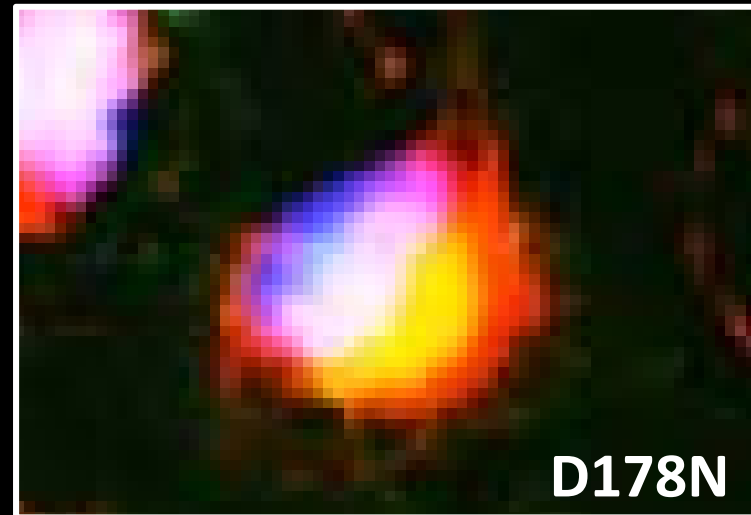
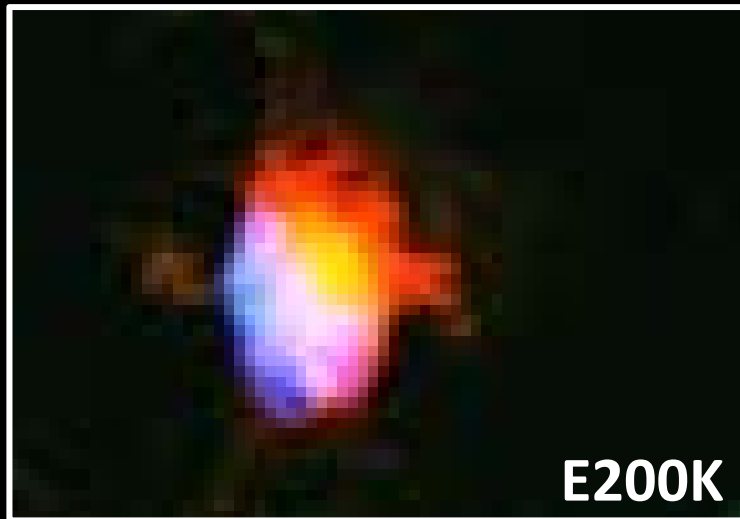
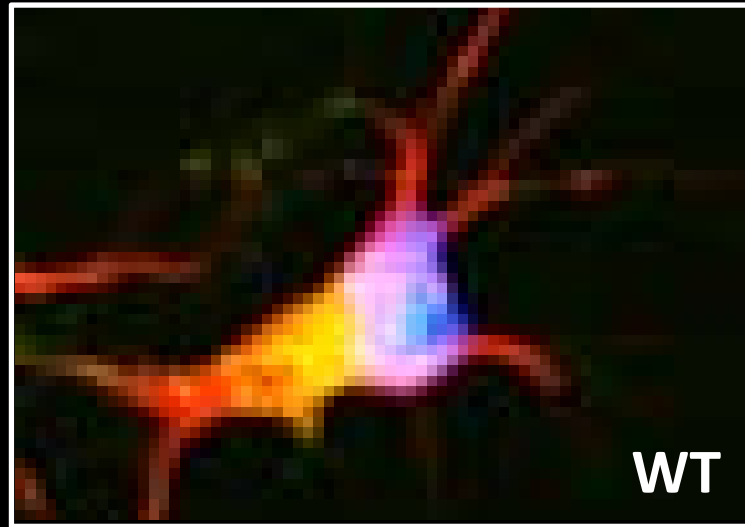
RT-QuIC analysis of PrP seeding activity with patient-specific fibroblasts



Immunofluorescent staining of iPSC-derived neurons (WT) with β III tubulin and PrP (Tohoku2)

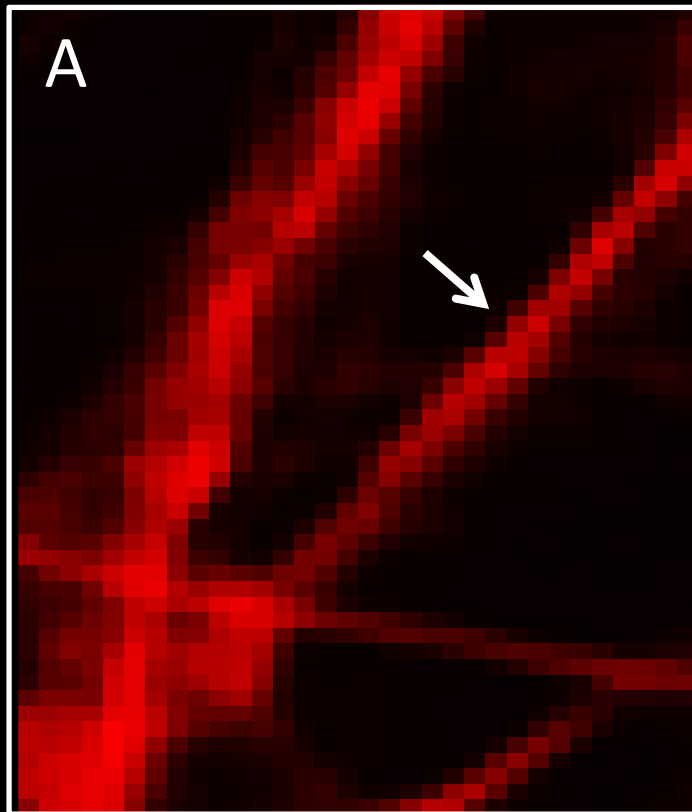


Comparison of iPSC-derived neurons carrying WT and mutant PrP

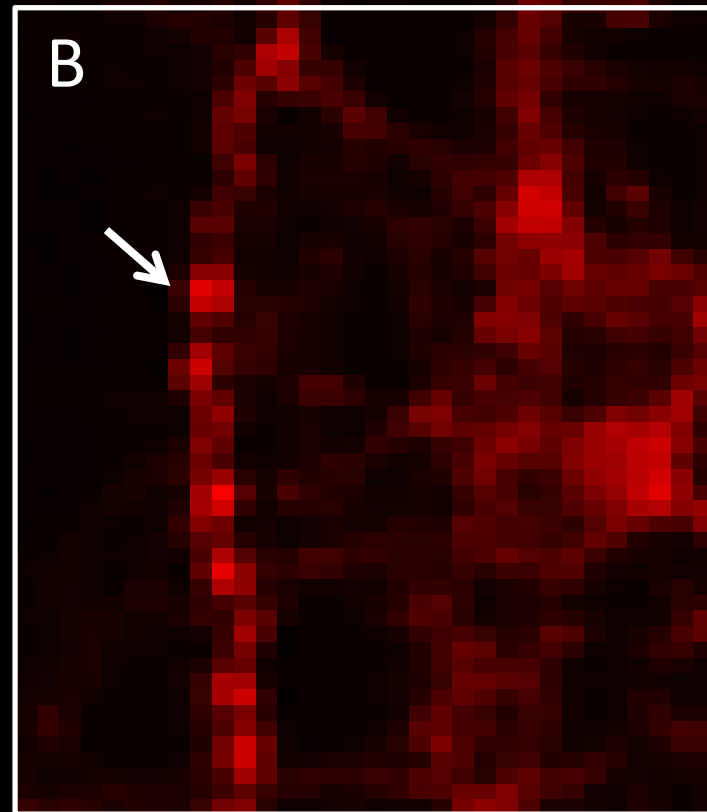


Effect of prion on iPSC-derived neurons

untreated



prion-treated



Summary of previous study

- Fibroblasts have been generated from asymptomatic mutation-carriers, sCJD patients, and controls
- Fibroblasts exhibit some prion-related phenotypes
- iPSC lines and iPSC-derived neurons have been generated from normal controls and two mutations
- Neurodegeneration-like changes were found in mutant and prion-challenged WT iPSC-derived neurons

Aim of new study

- Employ the newly-generated authentic human brain cells to investigate cellular mechanism of the anti-prion activity of the GSK compound, an inhibitor of protein kinase RNA-like ER kinase (PERK) that has been reported to effectively prevent neurodegeneration in prion-infected mice

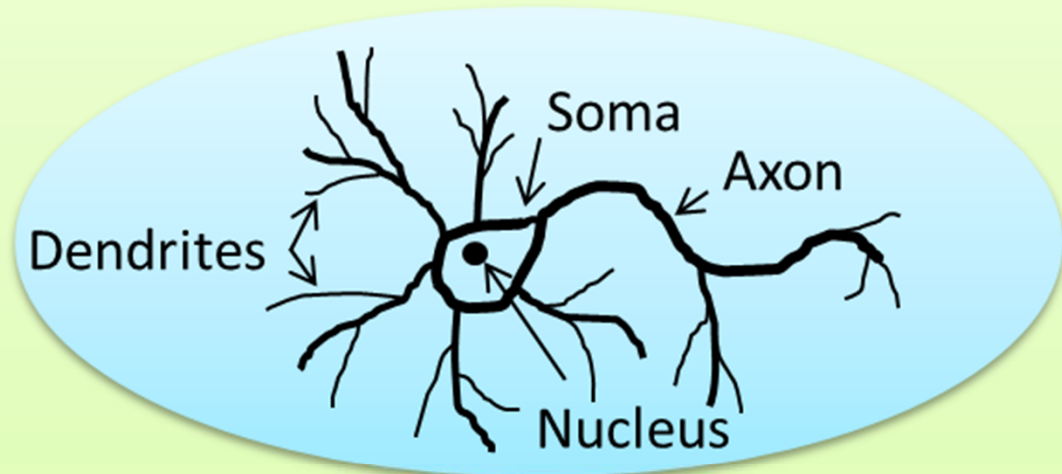
Brain homogenate



PrP^{Sc} purification



Biotinylation of purified PrP^{Sc}



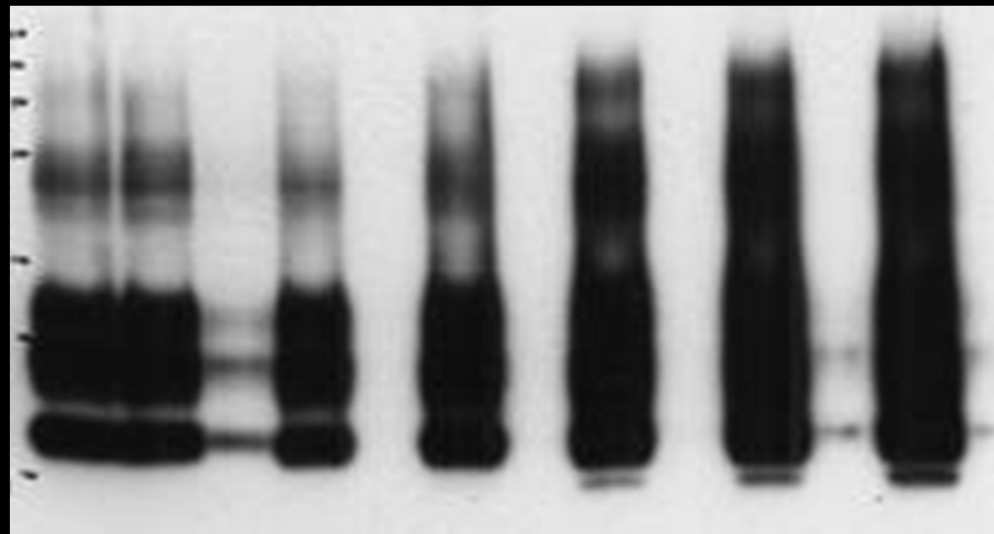
iPSC-neurons



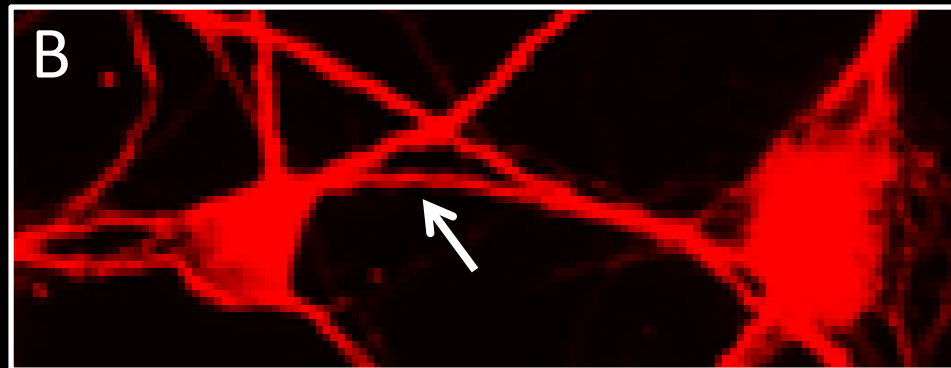
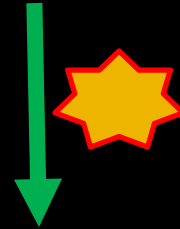
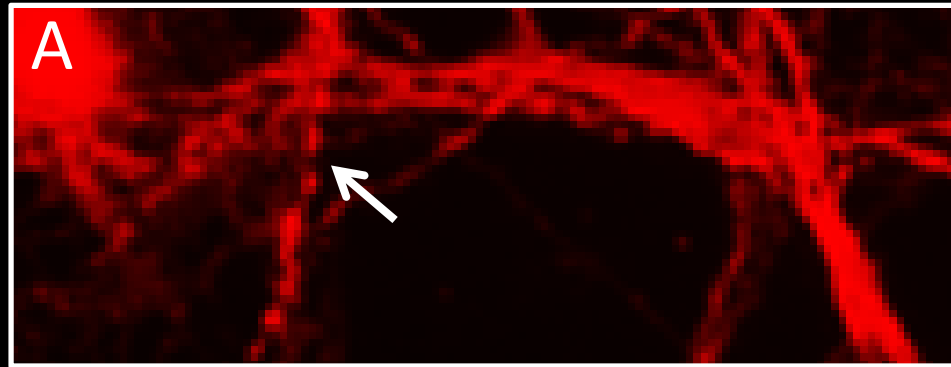
± GSK2656157

**Assessing neurodegeneration and inhibition of
PrP^{Sc} propagation in iPSC-derived neurons**

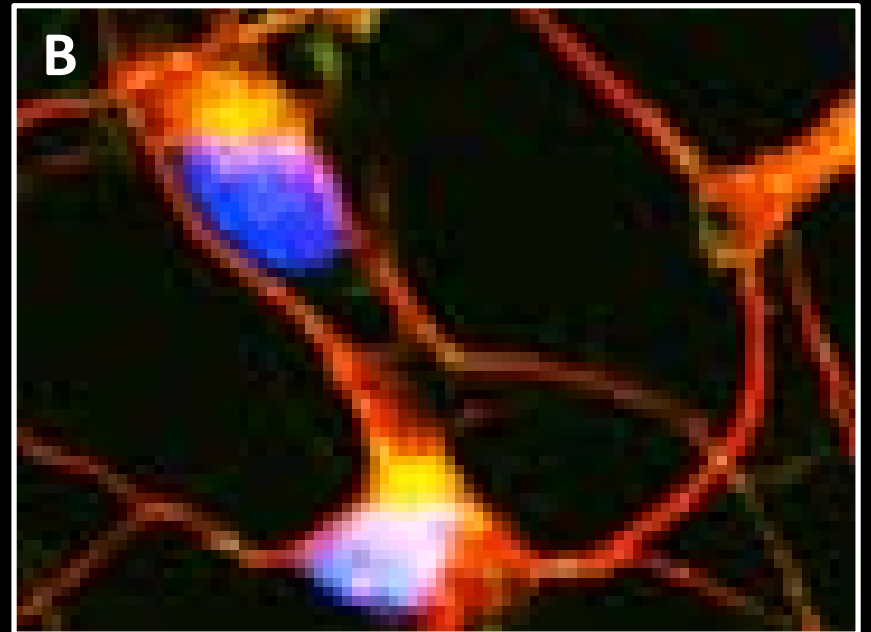
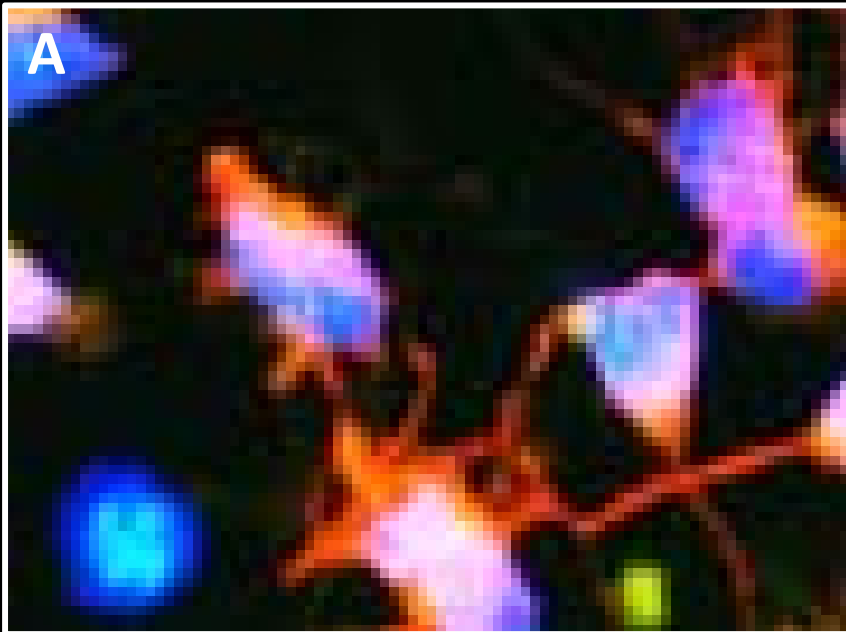
Purification of PrP^{Sc} from infected human brains



Treatment of infected iPSC-derived neurons with GSK compound



Treatment of iPSC-neurons with GSK compound



Summary of the current study

- PrP^{Sc} has been purified from infected human brains
- GSK compound may cure prion-induced neurodegeneration in WT iPSC-derived neurons
- GSK compound seems to improve neurodegeneration in iPSC-derived mutant neurons

Acknowledgements

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Conclusions

Our study suggests the therapeutic effect of GSK compound on prion-infected or mutant iPSC-derived human neurons, which is consistent with previous observations by other groups with animal models