Examining Enzyme Replacement Therapy of CLN2 Disease Using a Human Neural Progenitor Cell Model

Diane-Marie Brache-Smith

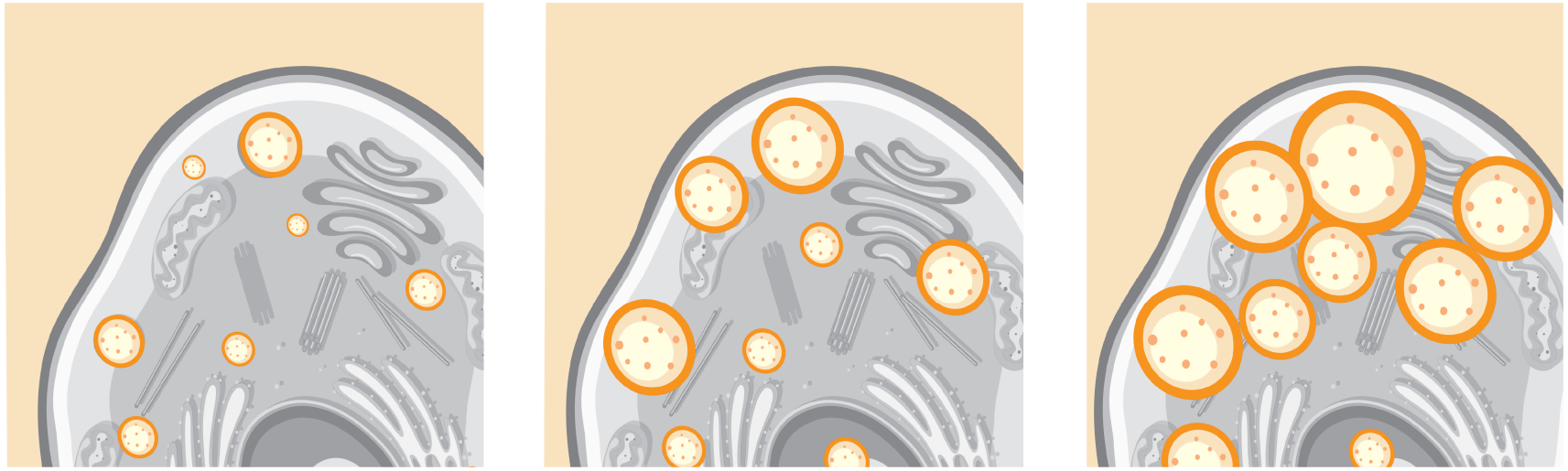
[1]*Miami Dade College North Campus Miami, Fl*

[2]*Nova Southeastern University, Davie, FL*

Ceroid Neuronal Lipofuscinosis Type 2 (CLN2)

a lysosomal storage disease

How CLN2 disrupts normal cell behavior



Normal Cell lysosomes remove waste

Without TPP-1 waste accumulates over time

Cells lose function

- Autosomal recessive, neurodegenerative lysosomal storage disorder.
- Deficiency of digestive enzyme tripeptidyl peptidase (TPP-1).
- This deficiency is associated with the accumulation of lysosomal lipopigment storage material.
- Children diagnosed have a life expectancy of 8-10 years

TPP-1 Enzyme Deficiency and CLN2

1998 — TPP1 was confirmed as the missing enzyme in CLN2

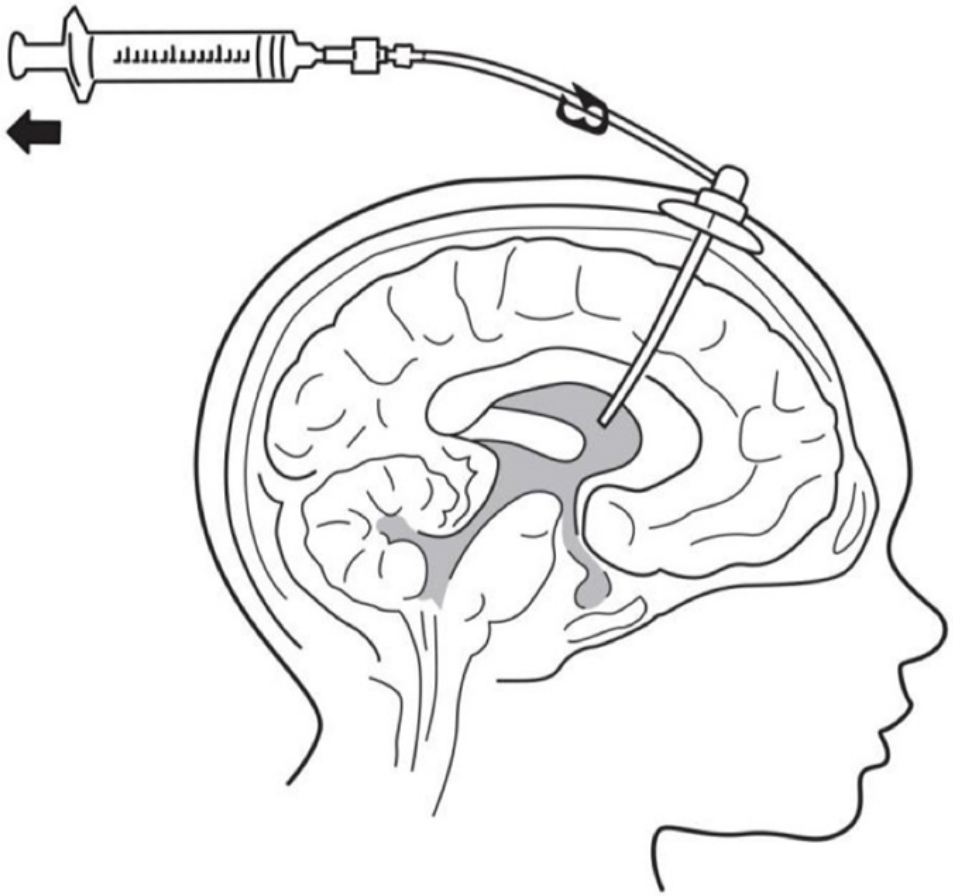
2000 — TPP1 first isolated

2007 — ICV delivery of TPP1 in mouse model of CLN2

2016 — FDA approves ICV delivery of TPP1 in humans as treatment for CLN2

Intracerebroventricular Infusion of Brineura® (TPP-1)

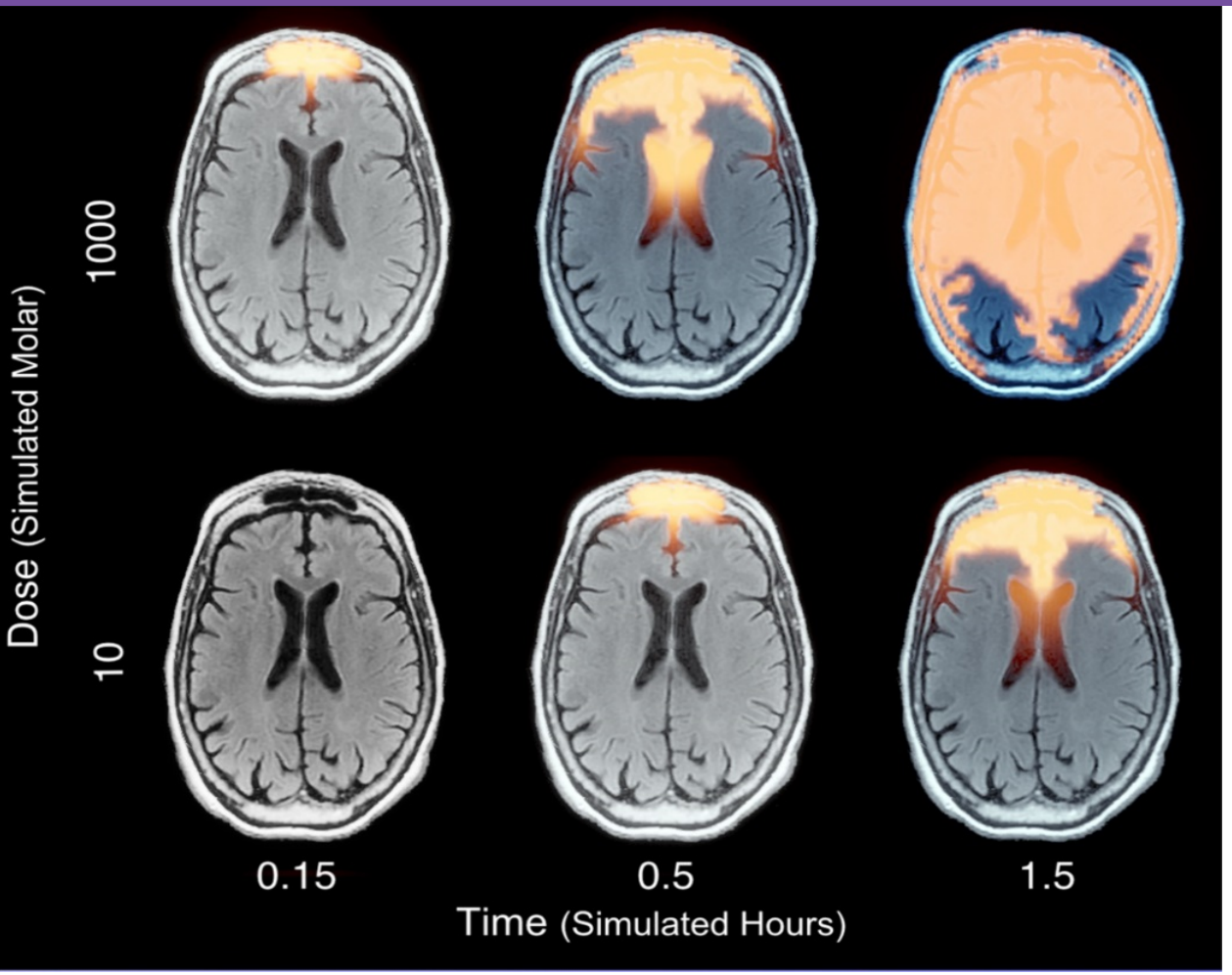
Treatment for CLN2

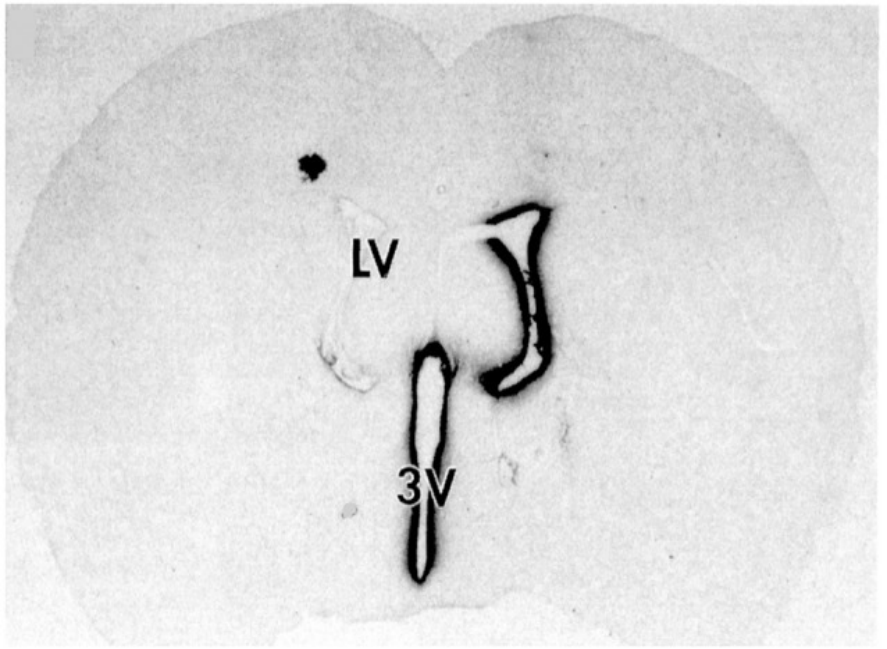


An intracranial canula is surgically inserted and 300mg dose of recombinant human TPP1 is delivered biweekly.

Intracerebroventricular Infusion of Brineura® (TPP-1)

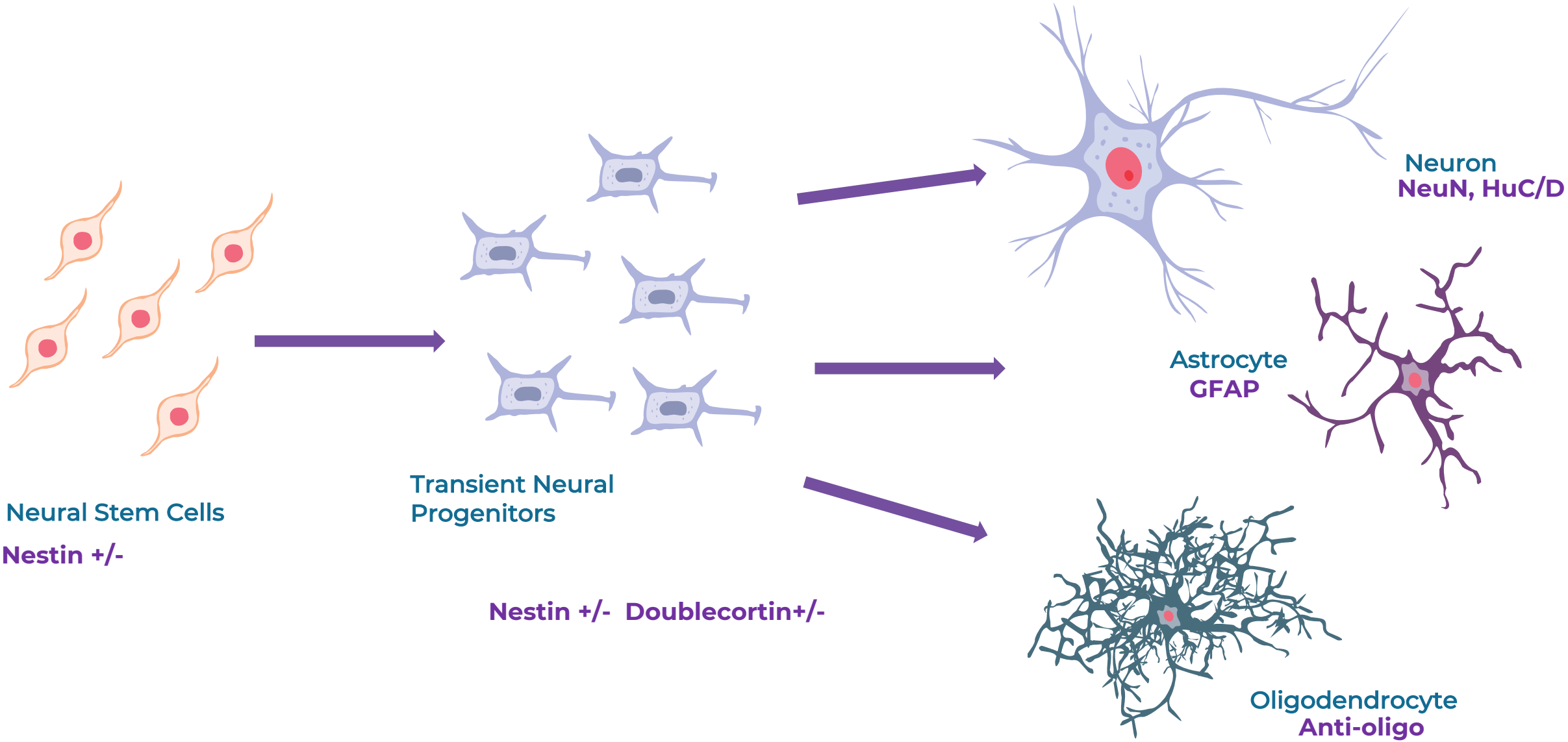
Treatment for CLN2





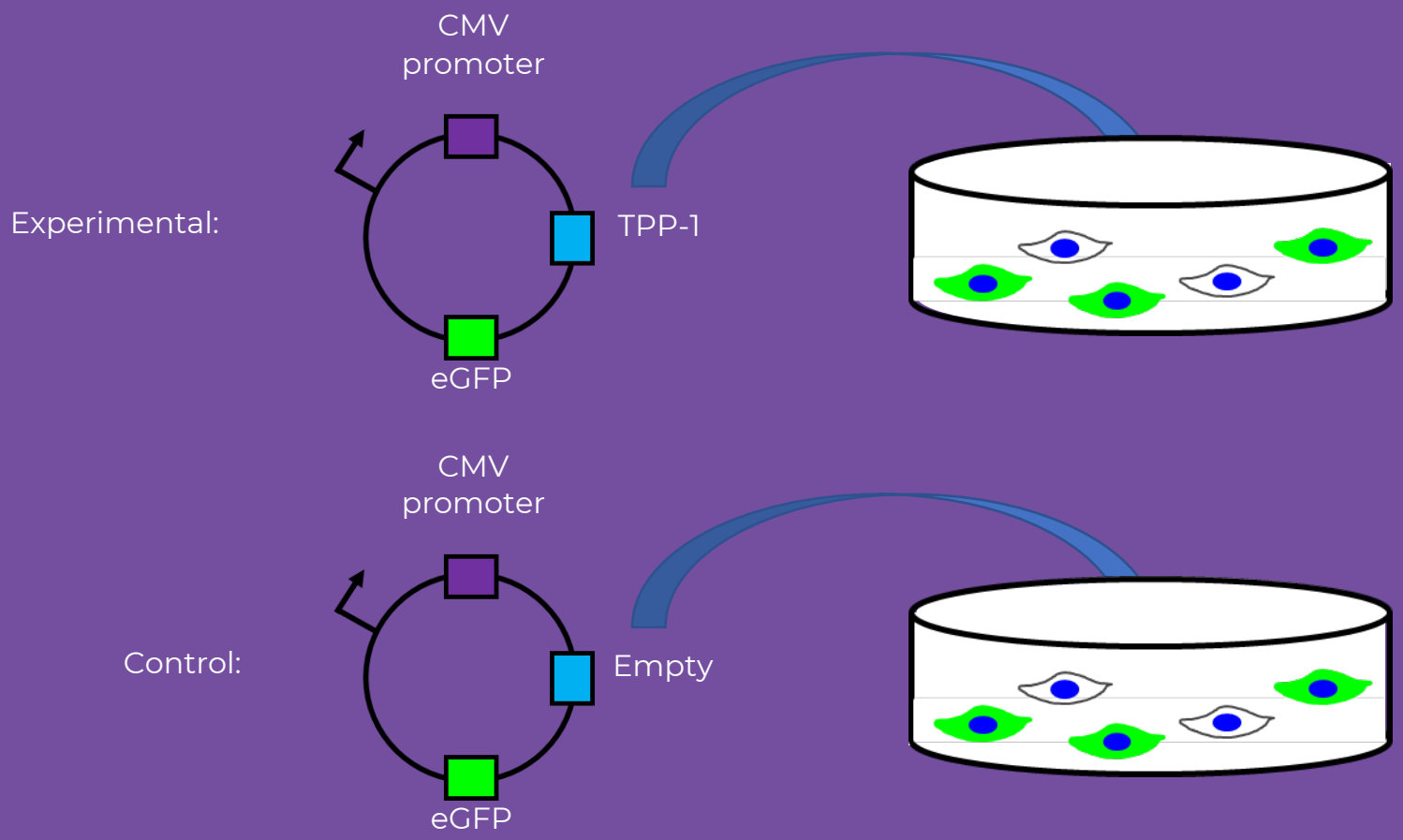
Diffusion models suggest TPP-1 will diffuse out from the LVs along a concentration gradient.

Do altered levels of TPP-1 affect how neural cells grow?



Do increased levels of TPP-1 alter neurogenesis?

Method: Expose hNPCs to either (1) adenovirus expressing TPP-1 and eGFP or (2) eGFP alone

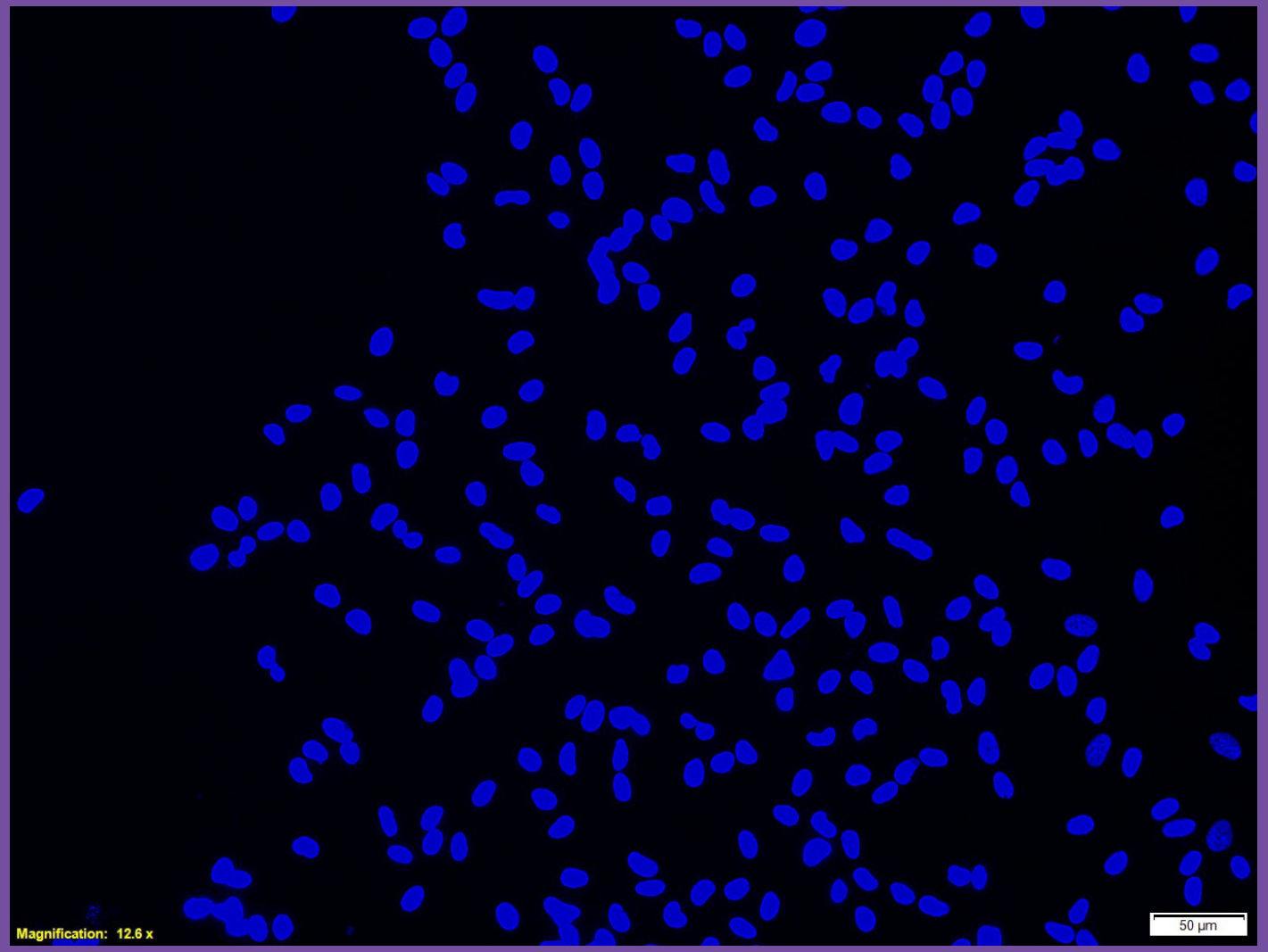


What are the affects on:

- Proliferation
- Differentiation
- Cell Death

The Cell Counting Problem

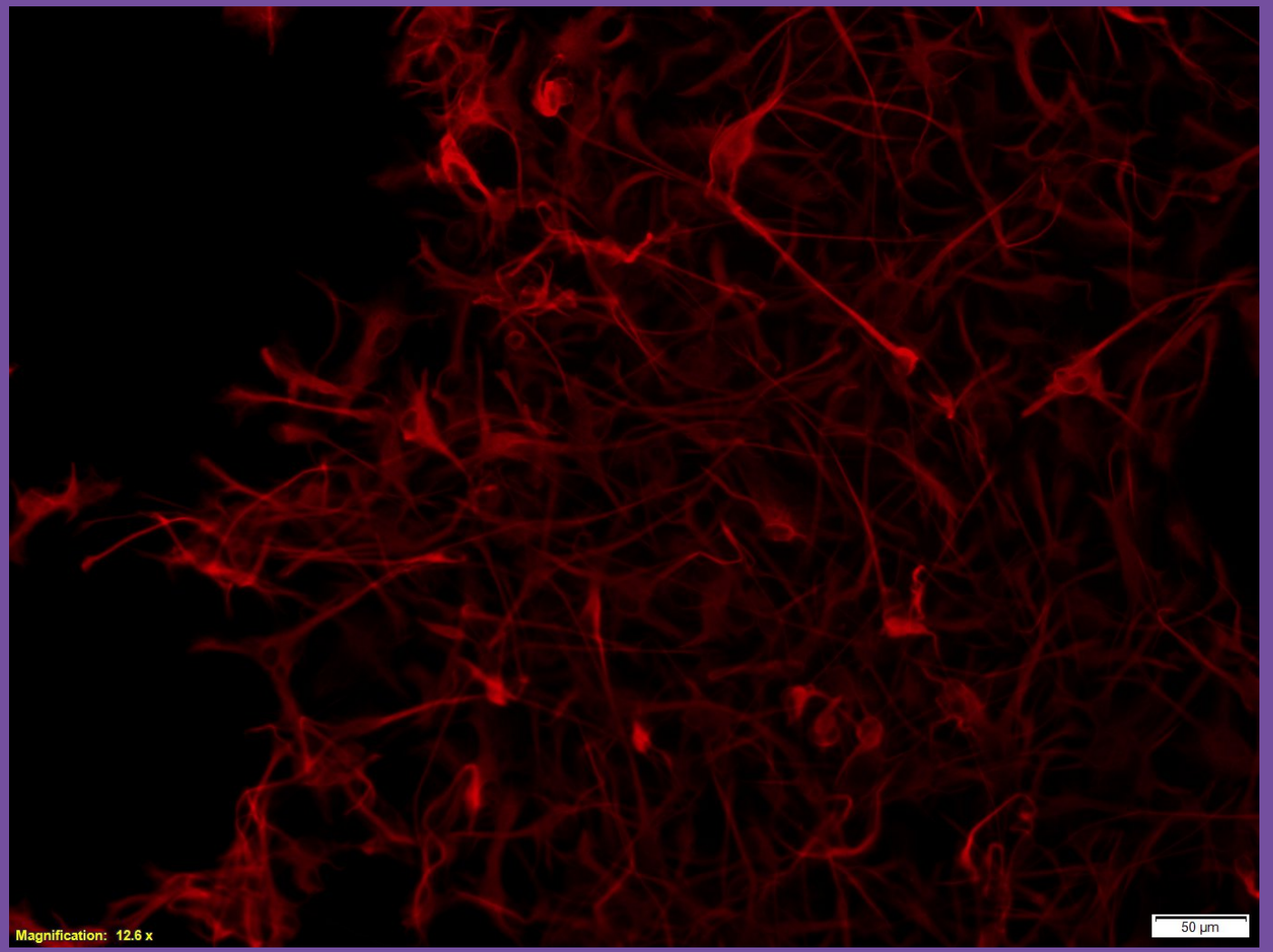
- DAPI is stains adenine–thymine-rich regions in DNA, staining all nuclei
- Slides can have hundreds of cells.
- Counting is tedious and time consuming



DAPI Stained Cells

The Cell Counting Problem

- Human Neural Progenitor Cells have many processes
- They originate from a few specific nuclei

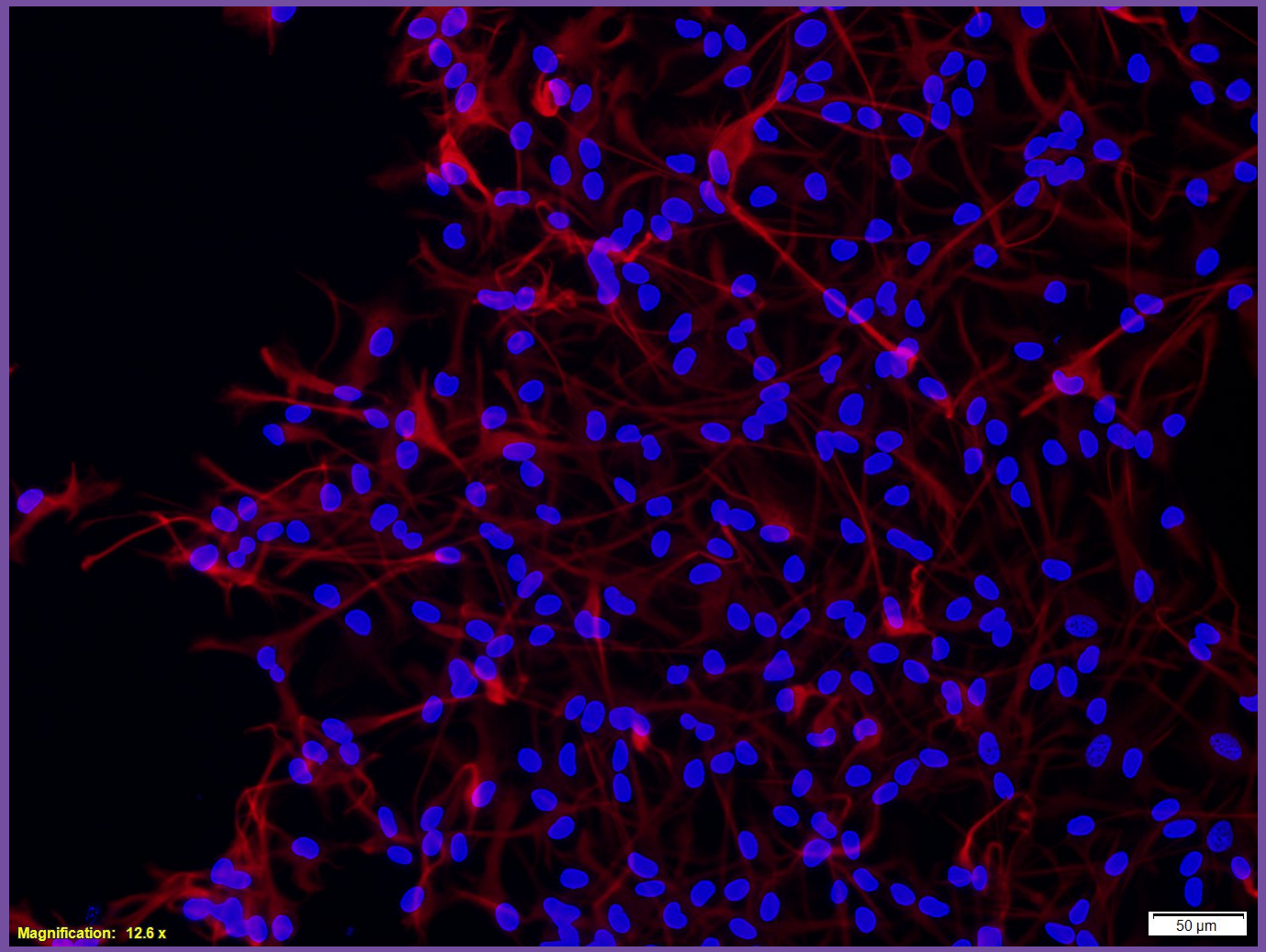


DAPI Stained Cells

The Cell Counting Problem

Merged image of DAPI and GFAP Stained Cells

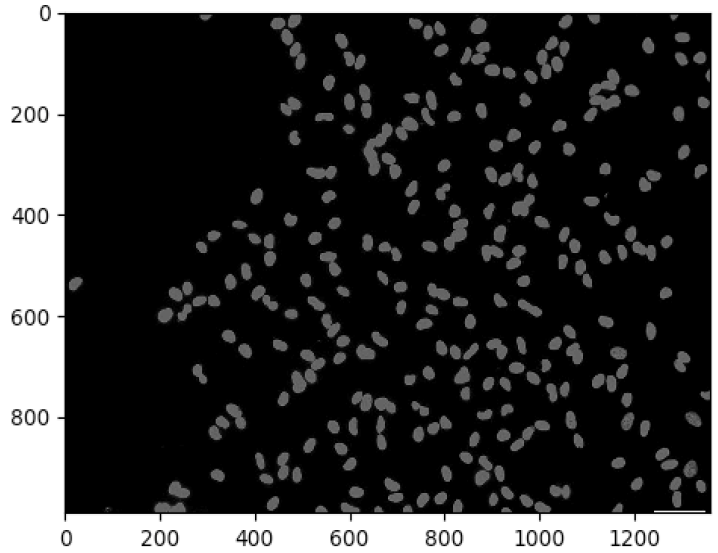
Is there a better way to count?

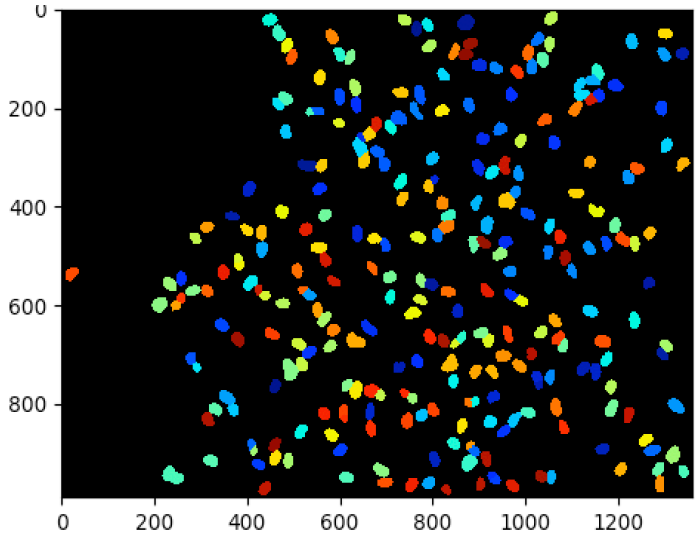


Merged image of DAPI Stained Cells

Data quantification using CellProfiler™ cell image analysis software

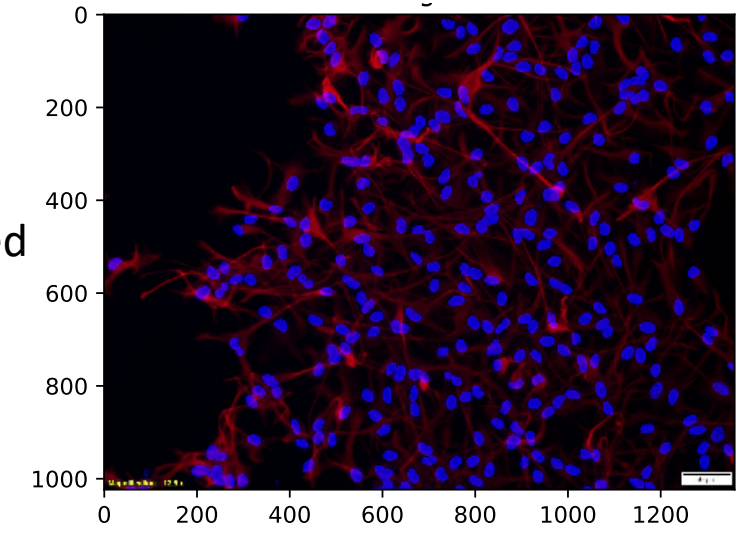
DAPI

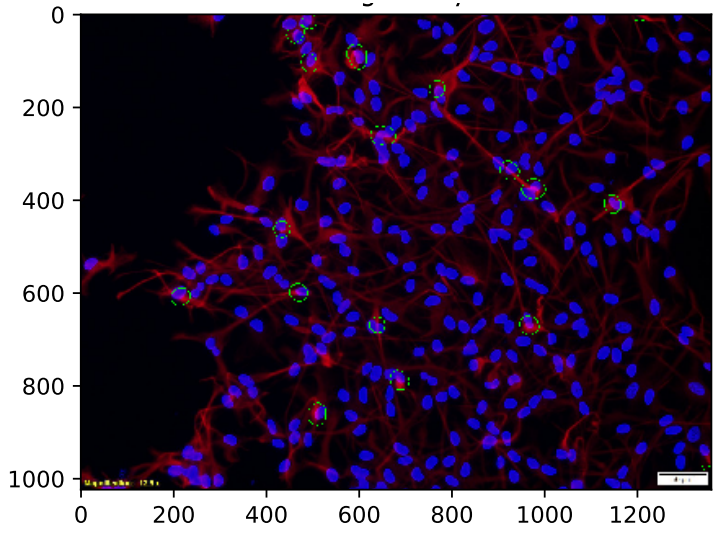




# of accepted objects	277
10th pctile diameter	22.5 pixels
Median diameter	27.3 pixels
90th pctile diameter	29.9 pixels
Area covered by objects	11.9 %
Thresholding filter size	1.0
Threshold	0.0833
Declumping smoothing filter size	6.7
Maxima suppression size	6.7

Merged





# of accepted objects	18
10th pctile diameter	14.0 pixels
Median diameter	38.3 pixels
90th pctile diameter	47.0 pixels
Area covered by objects	1.5 %
Thresholding filter size	0.0
Threshold	0.00301
Declumping smoothing filter size	10.0
Maxima suppression size	0.7

Does Enzyme Replacement with TPP-1 Induce Cell Death in human cortical neural progenitor cells?

rhTPP-1 Standard Curve

ApoTag(TUNEL) Assay

rhTPP-1 Group / Date	0.1µg	1.0µg	10µg	100µg
	0µg rhTPP-1 (control)			

rhTPP-1 Group / Date	0.1µg	1.0µg	10µg	100µg
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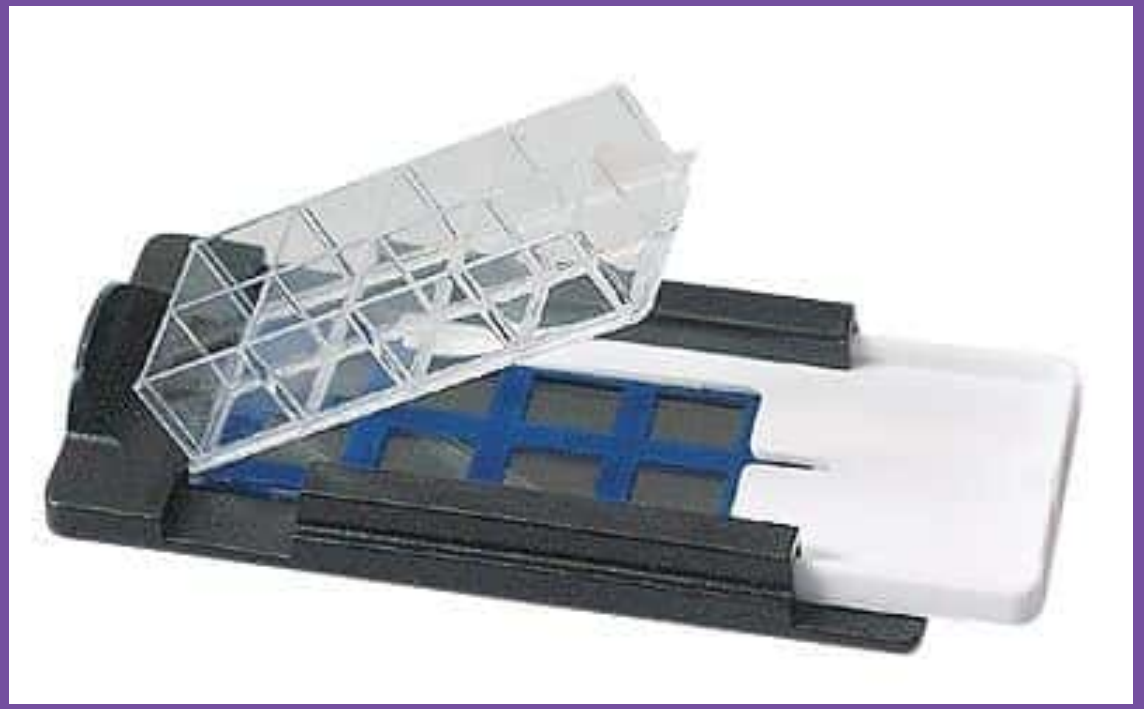
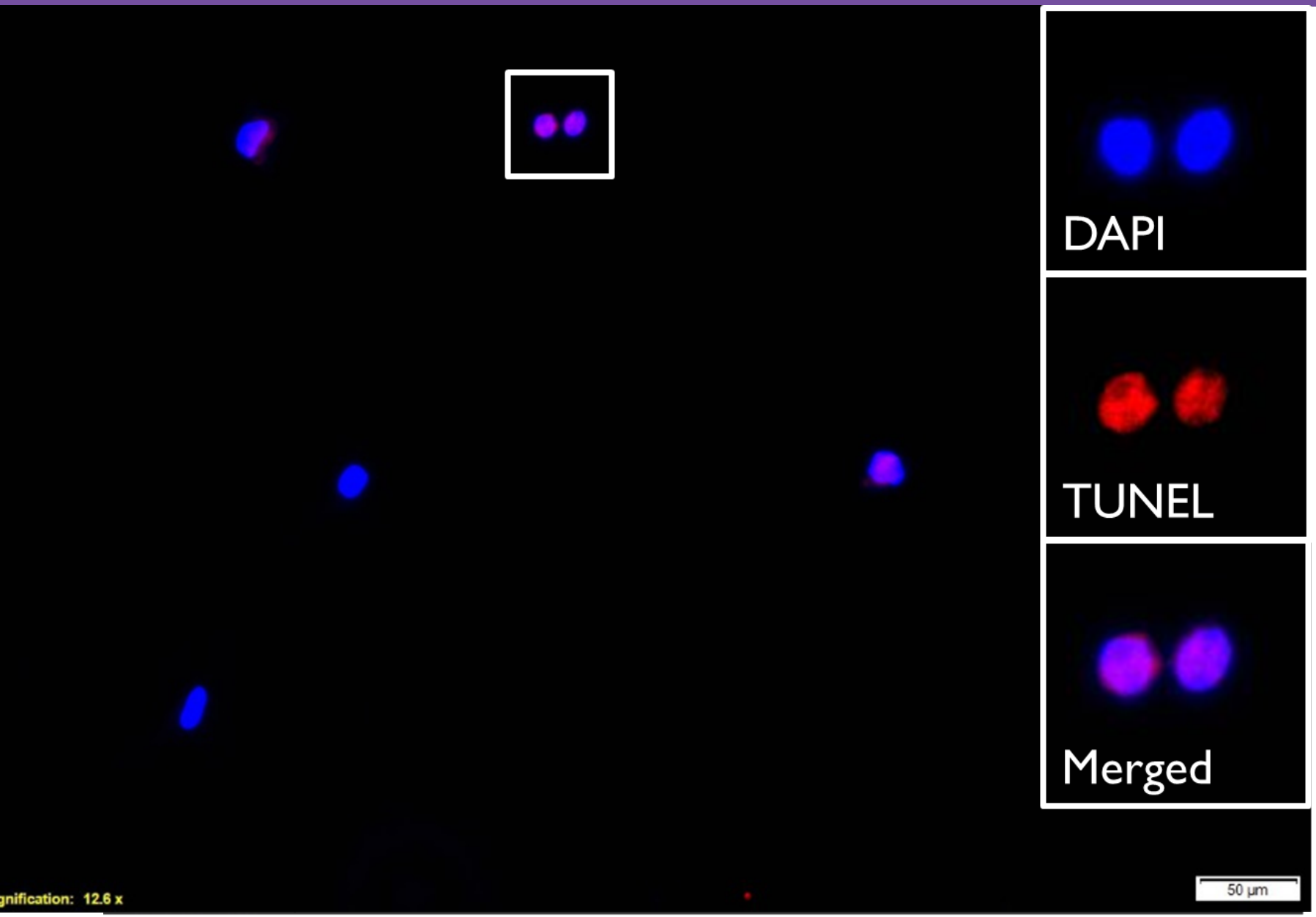
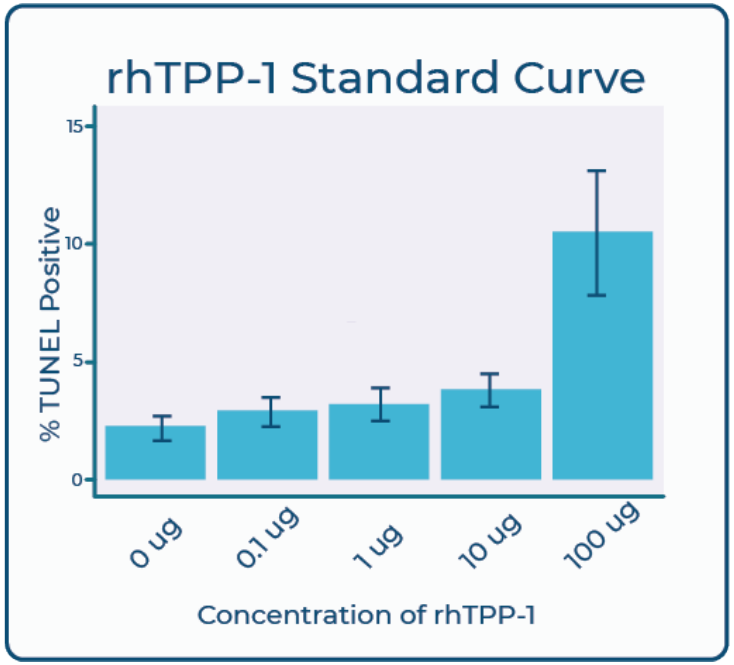


High Concentrations of TPP-1 Enzyme Induce Cell Death





*F(4,34)= 6.87, p < 0.05***

Experimental Design

hNPCs in proliferation media

NSC P1 Prolif. Group				
Date	mNestin + rNestin	mHuCD + rNeuN	mNestin + rDCX	rNeuN
	mOligo + rDC	mHuCD +	mHuCD + rGFAP	rGFAP

hNPCs in differentiation media

NSC P1 Diff. Group				
Date	mPCNA + rDCX	mHuCD + rGFAP	mPCNA + rDCX	rGFAP
	mPCNA+ rNestin	mPCNA + rDCX	mHuCD + rGFAP	rNeuN

ApoTag (TUNEL) Assay

NSC P1 Prolif. Group				
Date	Con	eGFP	OE	KD
	Con	eGFP	OE	KD

ApoTag (TUNEL) Assay

NSC P1 Diff. Group				
Date	Con	eGFP	OE	KD
	Con	eGFP	OE	KD

EdU (Proliferation) Assay

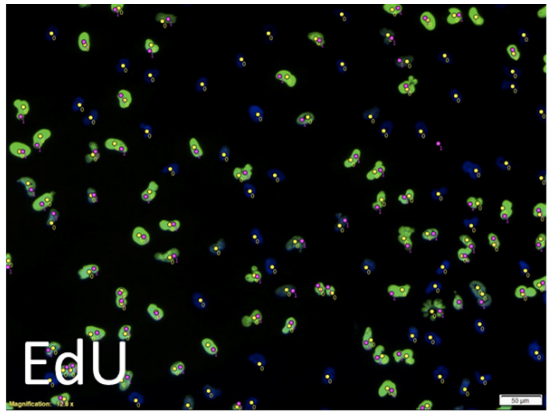
NSC P1 Prolif. Group				
Date	Con	eGFP	OE	KD
	Con	eGFP	OE	KD

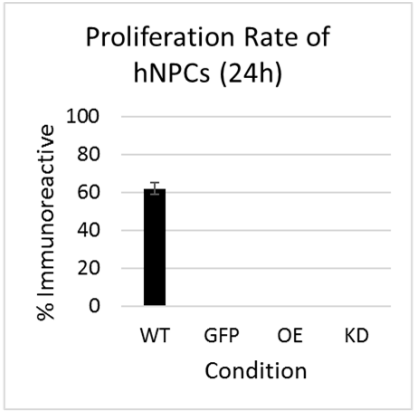
EdU (Proliferation) Assay

NSC P1 Diff. Group				
Date	Con	eGFP	OE	KD
	Con	eGFP	OE	KD

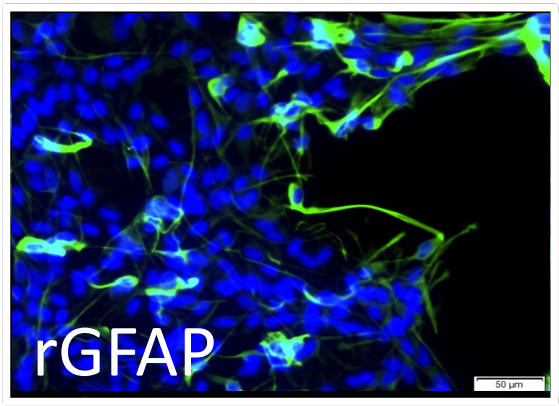
1 Set of above slides/group. Groups included (1) baseline controls, (2) eGFP controls, (3) TPP-1 OE, (4) TPP-1 KD

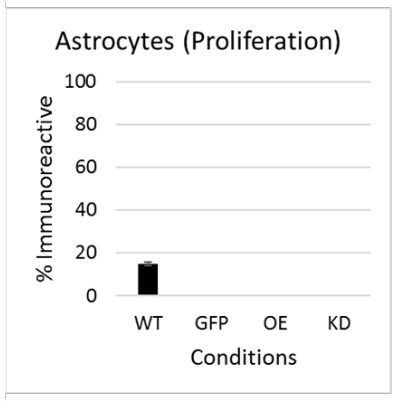
DOES ENZYME REPLACEMENT THERAPY WITH TPP-1 ALTER DIFFERENTIATION OF HUMAN CORTICAL NPCS?





WT = Wild Type
GFP = Control
OE = overexpression
KD = knock-down





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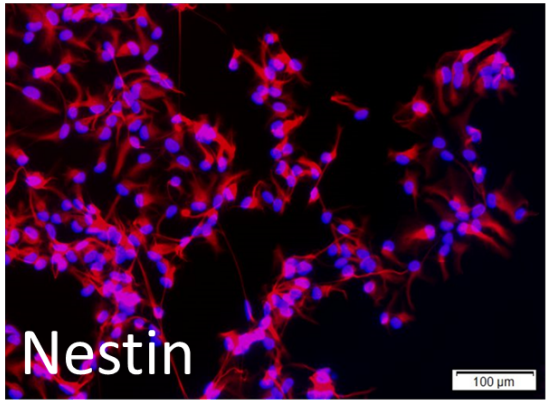

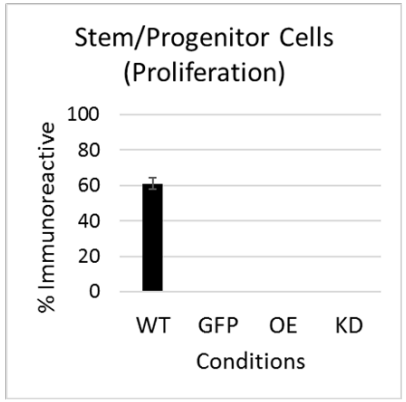

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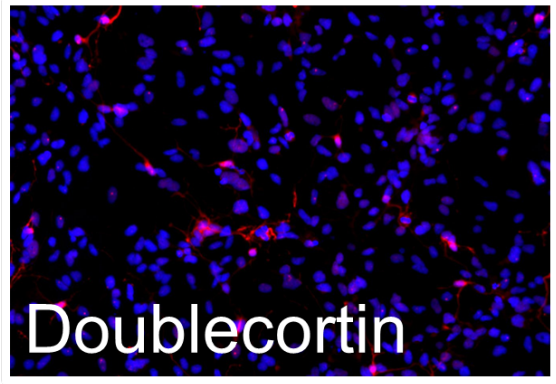

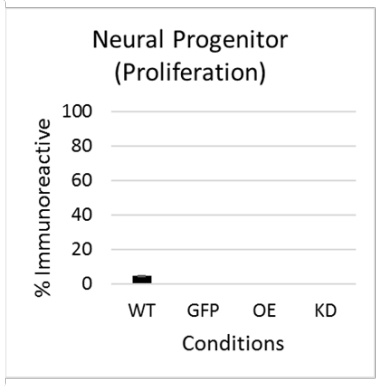

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Thank You!
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