ReNeuron

NEURAL STEM CELL DERIVED EVs AS NOVEL THERAPEUTICS AGENTS

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Vice President of Research

ISCT Scientific Signature Series – Therapeutic Advances with native and engineered human EVs

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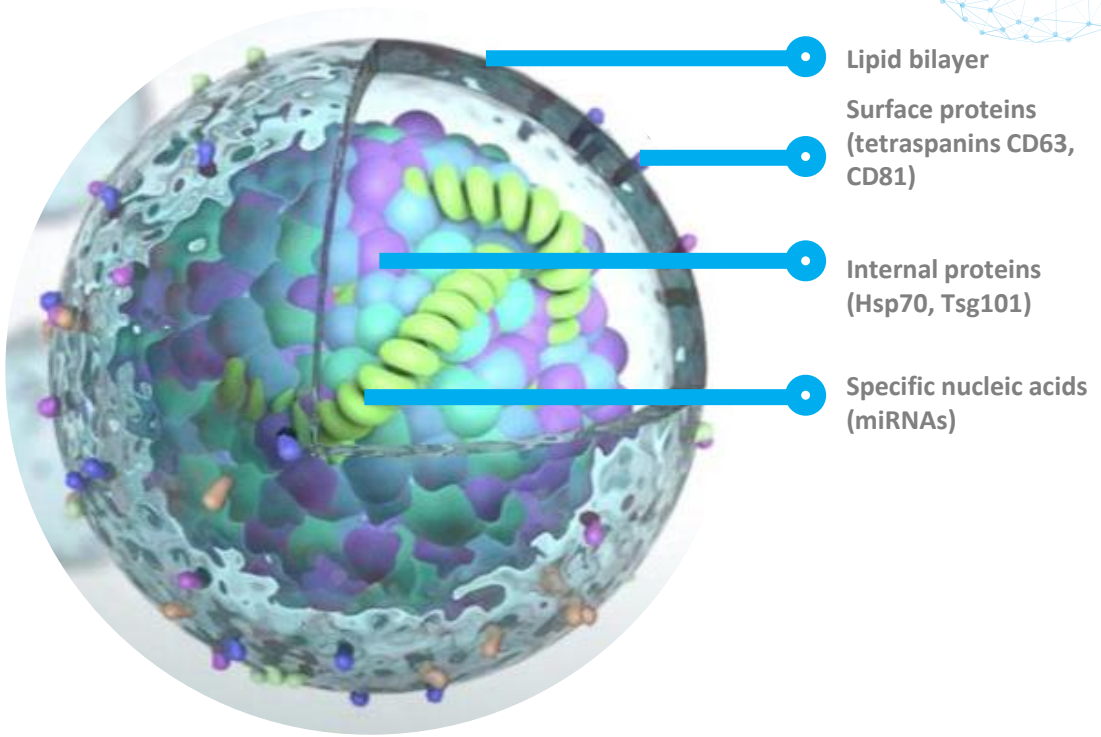
Extracellular Vesicles: A Targeted Delivery platform

Naturally occurring, nanoparticles released by all cell types in a functionally relevant manner as a means of intercellular communication

Proven ability to carry a range of biologically active cargos including nucleic acids and proteins

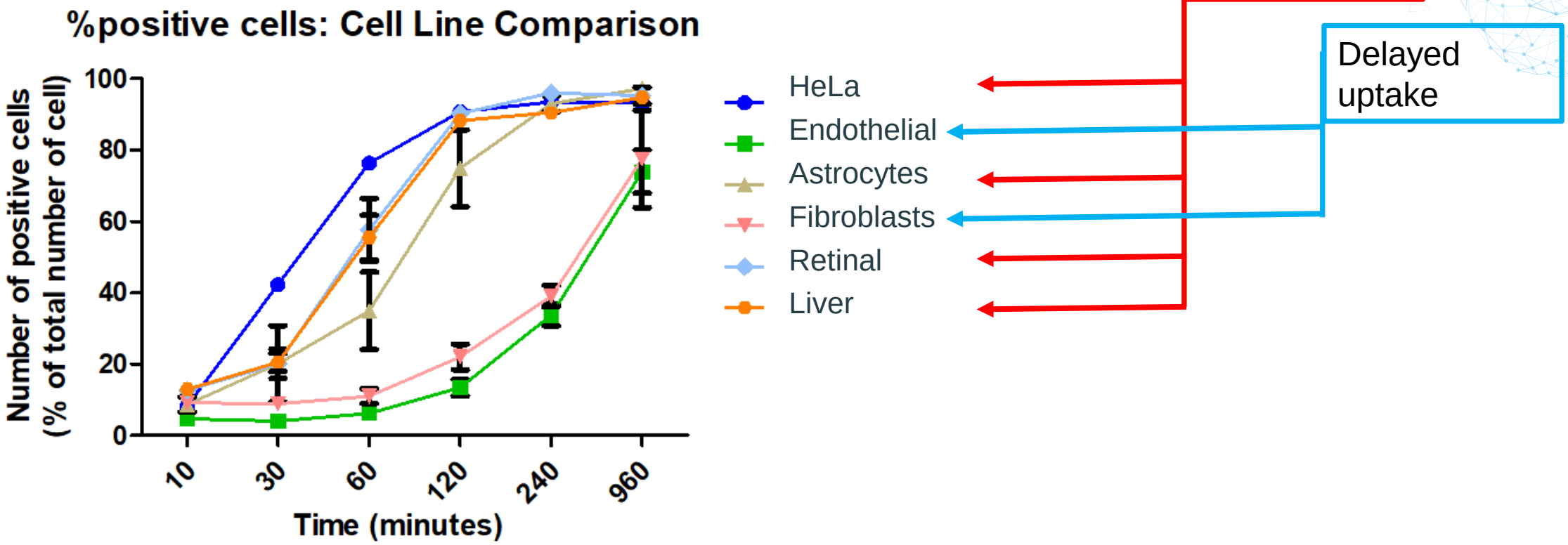
Target recipient cells via specific surface proteins that are determined by their cell of origin

Increasing interest across the industry in extracellular vesicles as biomarkers, standalone therapeutics and as delivery vectors for complex drug modalities



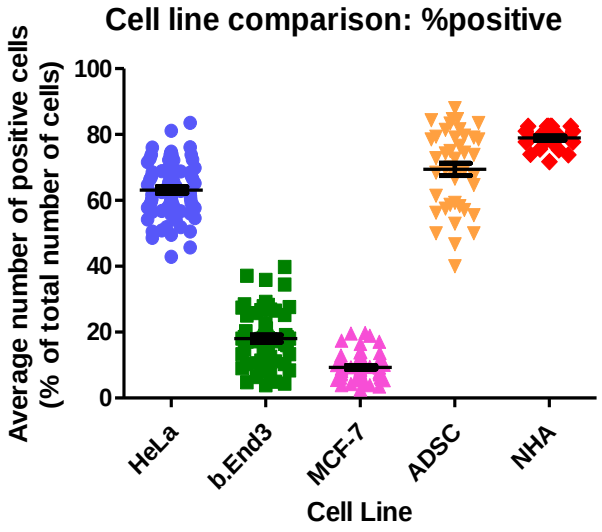
ReNeuron

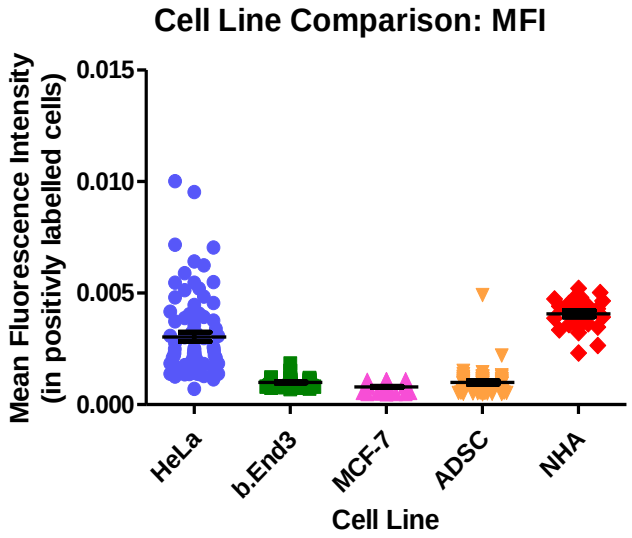
hNSC-derived EVs are Differentially Taken Up by Specific Cell Types

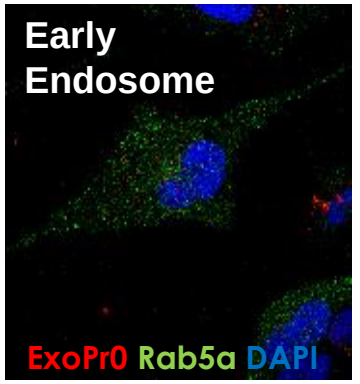


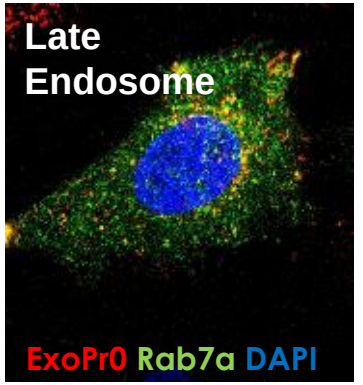
- By assessing the number of positive cells over time, 2 distinct profiles emerge
- HeLa, astrocytes, retinal and liver progenitors rapidly take up hNSC exosomes while fibroblasts and endothelial cells show delayed uptake

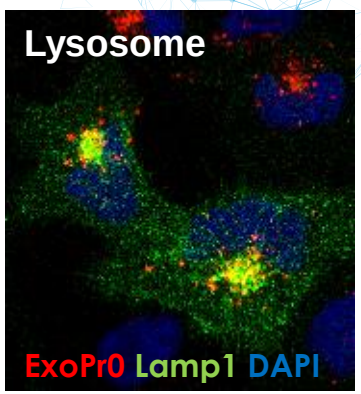
CTX-derived EVs are predominantly taken up by Clathrin mediated Endocytosis by specific cell types



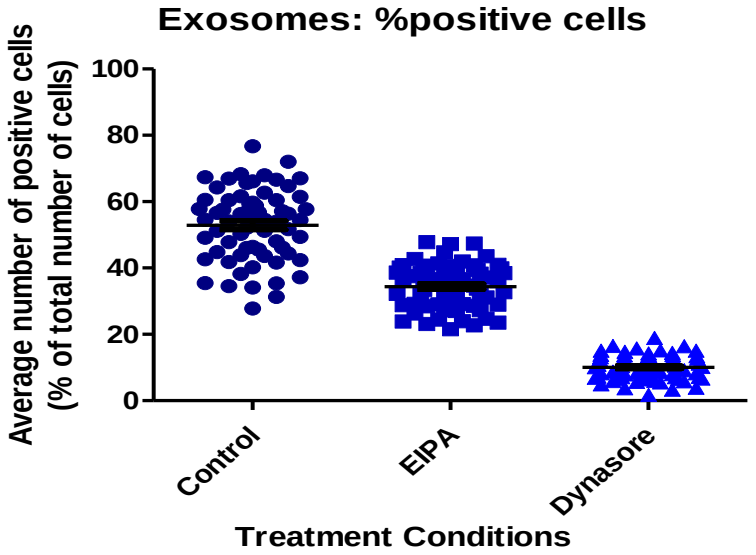






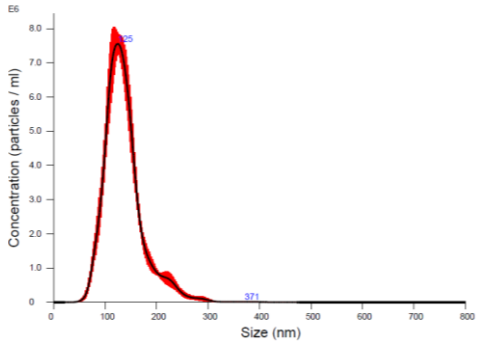


From a panel of 5 different cell types, CTX-derived EVs are taken up by the cells in the following order; Normal human astrocytes (NHA; ~85%), Adipose-derived stem cells (ADSC; ~75%), HeLa (~65%), b.End3 (endothelial cells; 18%) and MCF-7 (breast cancer; ~10%).

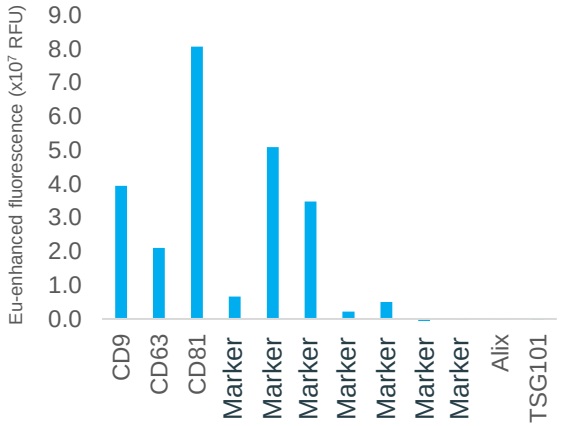


Distinct Surface Marker Profile

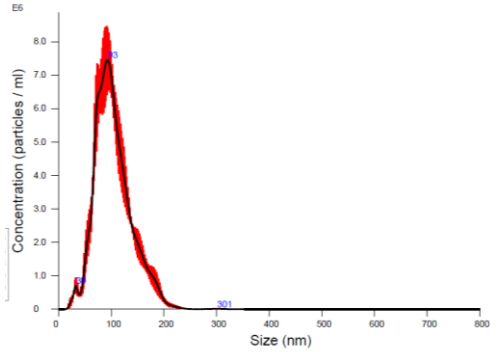
Nanotracking Analysis



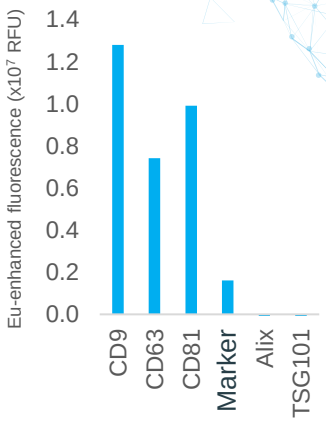
ELISA surface marker profile

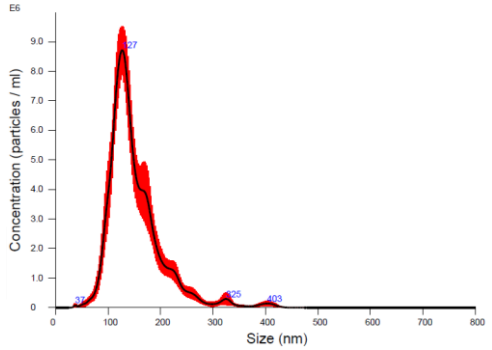


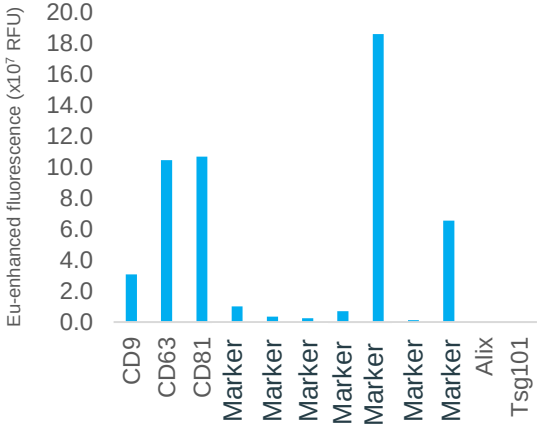
Nanotracking Analysis

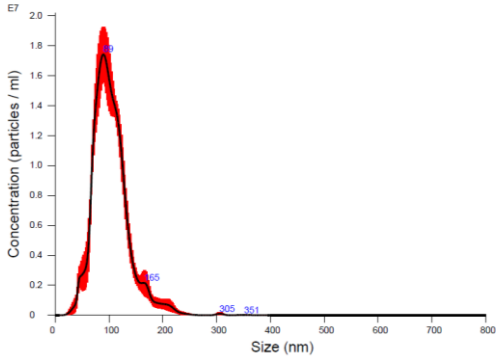


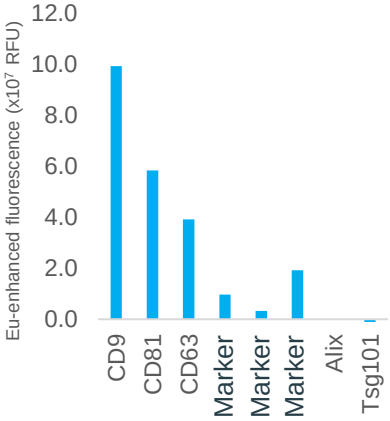
ELISA surface marker profile





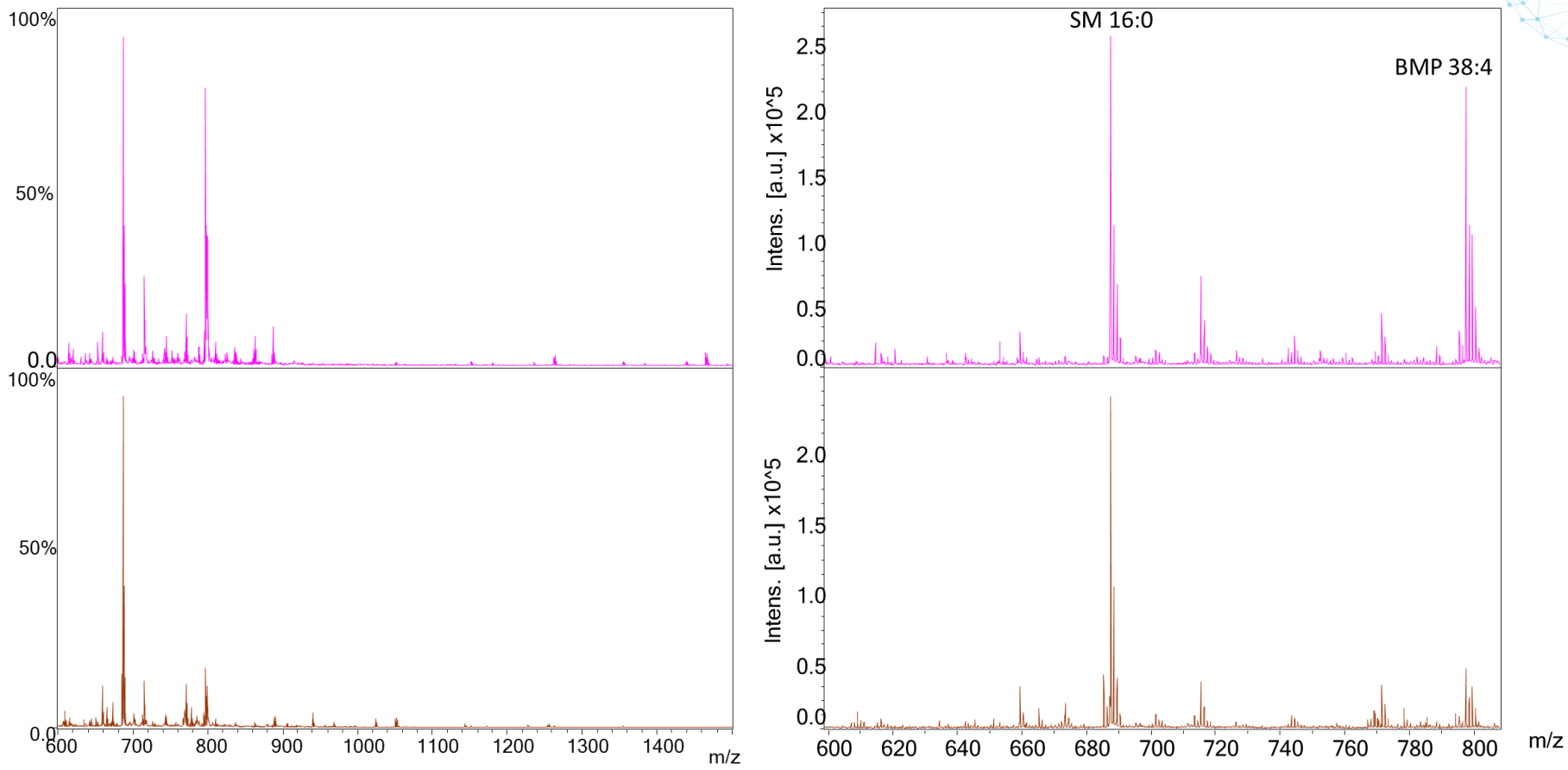






4 different producer cell lines, 4 different surface marker profiles

Distinct Lipid Membrane Composition

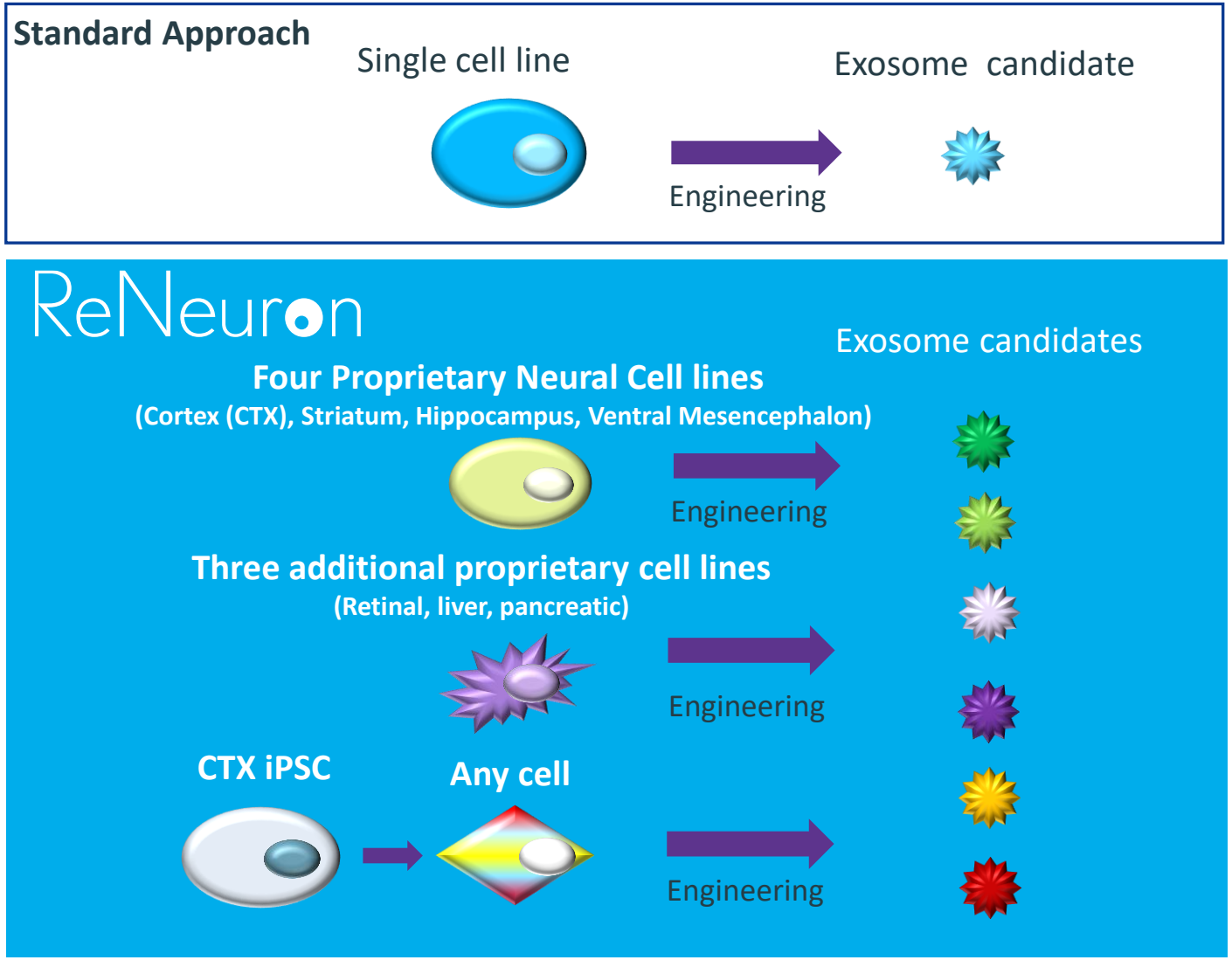


NSC-derived EVs

HEK 293-derived EVs

- MALDI-ToF - Qualitative technique, to identify species
- Sphingomyelin (**SM**) is a fundamental building block of membranes.
- Bis(monoacylglycero)phosphate (**BMP**) is enriched in endosomal membranes
- More complex and heavy gangliosides (glycosphingolipids) present in NSC derived EVs associated with CNS

Customisable, EV delivery platform optimised for specific drug delivery needs



'one-size fits all'

- Single cell line, single outcome

Portfolio of EVs

- EVs have fundamental characteristics based on their parental cell
- Multiple conditionally immortalized cell lines allows EVs to be customised and optimised for a specific payload and target

EV Producer cell line optimized for:

- Engineering efficiency
- Tissue targeting (on and off-target effects)
- Delivery (cytoplasmic / nuclear)

Proprietary Assets

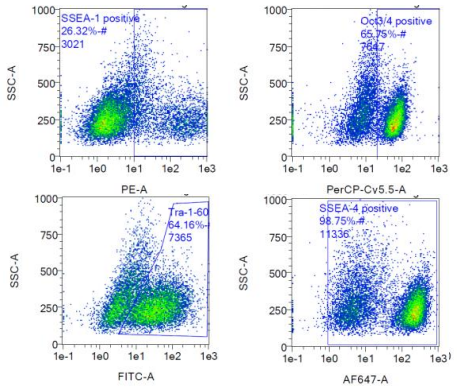
Human neural stem cell EVs (hNSC)	EVs from human liver (LIV), retinal (RET) and pancreatic progenitors (PIC)	Inducible pluripotent stem cell-derived EVs (CTX-iPSC)
Producer stem cell lines from 4 distinct brain areas	Further proprietary immortalized stem cell lines	CTX-derived induced pluripotent stem cell platform
Cortex (CTX), Striatal (STR), Hippocampal (HPP) and Ventral mesencephalon (VM)	EVs with distinct characteristics	EV production from parental CTX-iPSC and MSC lineage confirmed
Conditionally immortalized for stable and scalable production	Stable cell lines generated through immortalization or via CTX-iPSCs	Potential for new EV producer cell lines from any cell type
GMP-compliant source stocks		

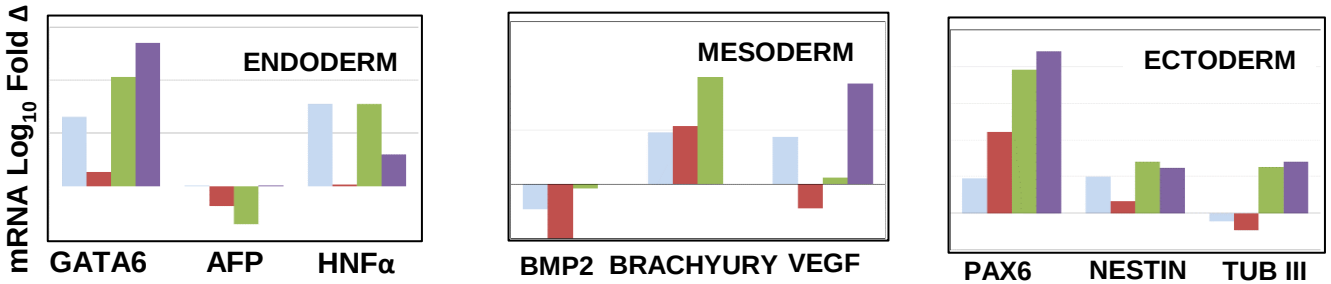
The Future: Range of Bespoke Exosomes from iPSCs

Pluripotency

Expression of pluripotency markers

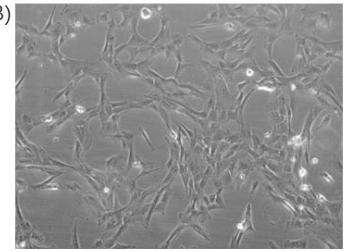
Line	Marker	%+ve
CTX-iPSC-1.1	SSEA-1	2.31
	OCT4	87.02
	SSEA-4	98.2
	TRA-1-60	90.9
CTX-iPSC-1.3	SSEA-1	11.24
	OCT4	68.86
	SSEA-4	96.92
	TRA-1-60	61.87
CTX-iPSC-1.4	SSEA-1	1.11
	OCT4	77.24
	SSEA-4	99.6
	TRA-1-60	92.4
CTX-iPSC-1.5	SSEA-1	26.16
	OCT4	65.47
	SSEA-4	98.19
	TRA-1-60	41.63
CTX-iPSC-1.6	SSEA-1	1.7
	OCT4	88.79
	SSEA-4	99.46
	TRA-1-60	55.13
CTX-iPSC-vx1	SSEA-1	47.37
	OCT4	16.44
	SSEA-4	65.24
	TRA-1-60	12.26



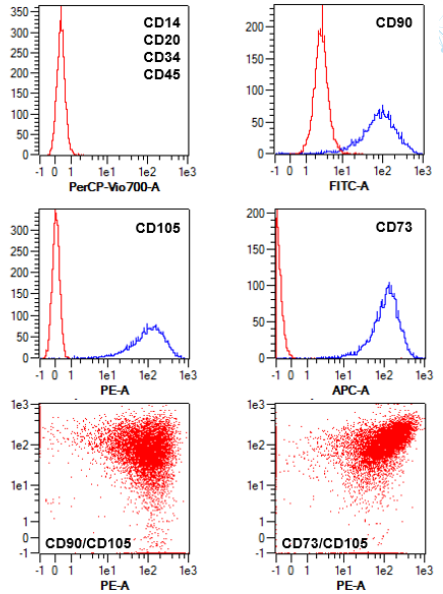


Therapeutic derivatives (MSCs) from CTX-iPSCs

(B)



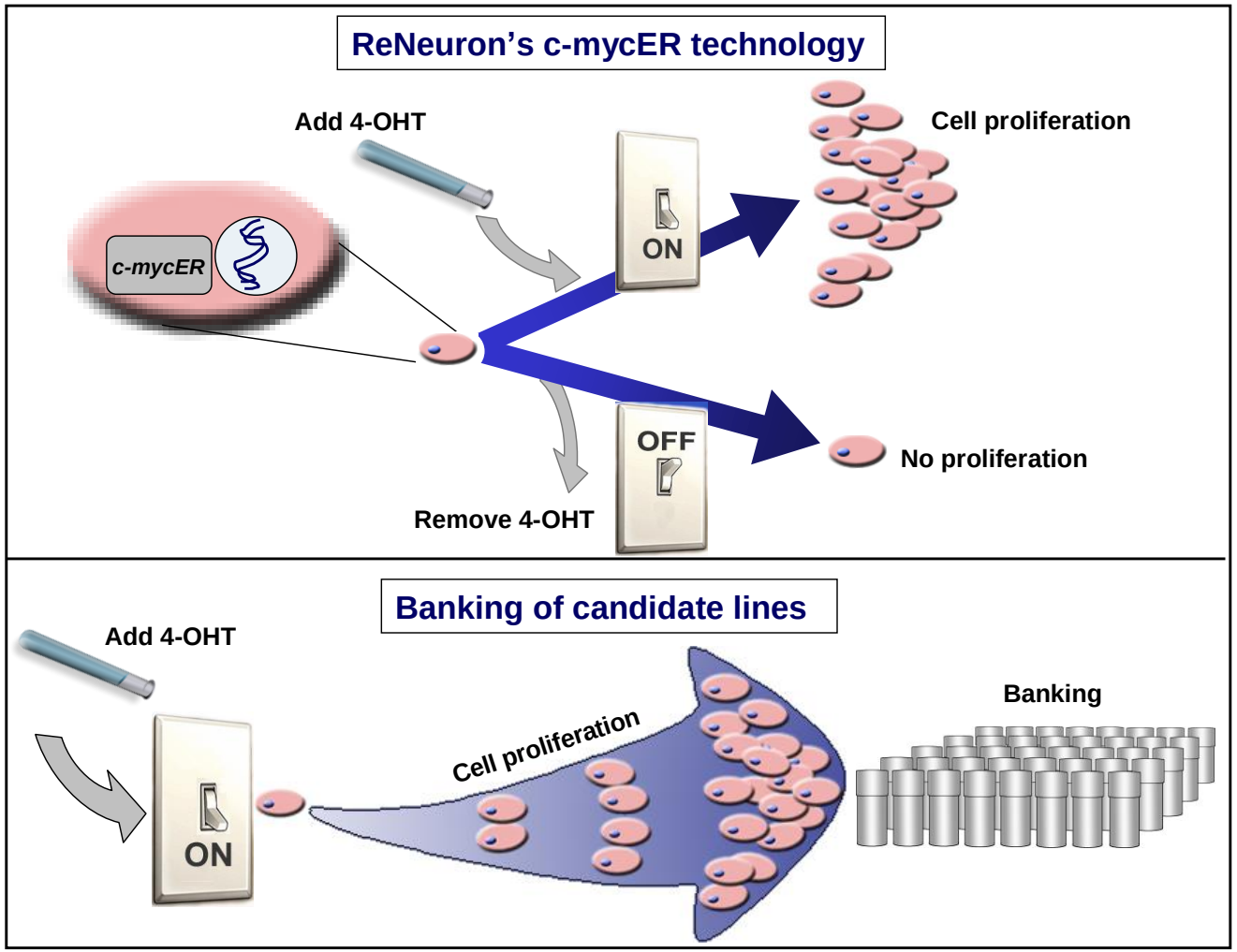
(C)



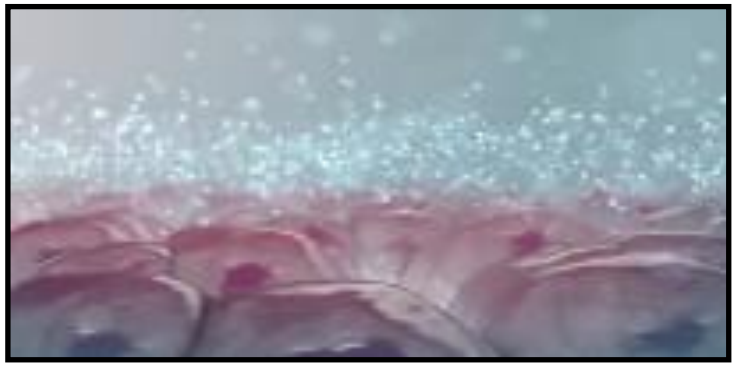
MSC differentiation accompanies expression of MSC-specific markers

- Pluripotent cell lines from the hNSC line retain conditional immortality
- A pluripotent stem cell line opens a range of opportunities to create *any* desired cell line and their EVs

Consistent and Scalable EV Production: Conditional Immortalisation



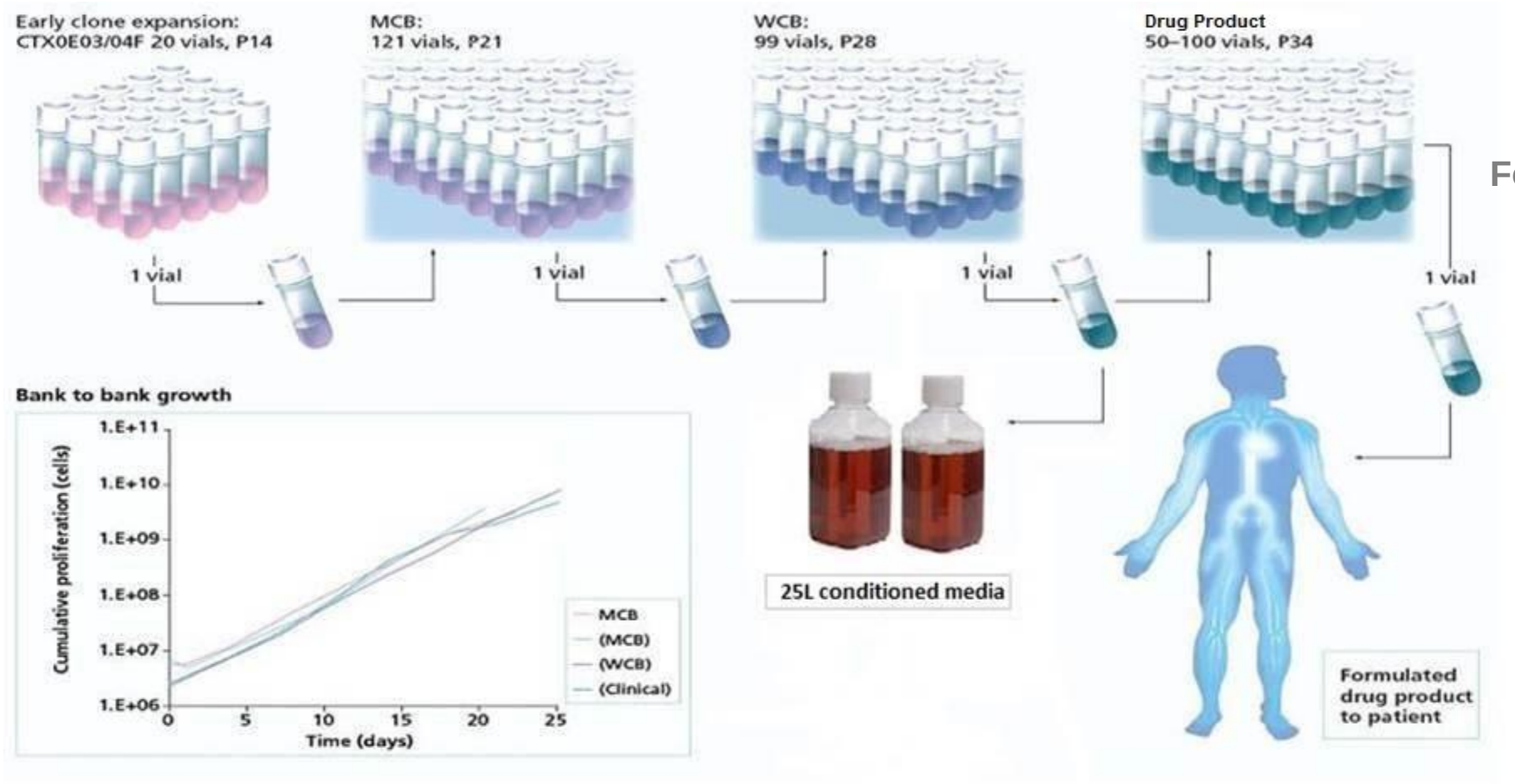
- Stable producer cell line - Consistent phenotype maintained over multiple passages
- Fully qualified xeno-free GMP process – tightly controlled USP with strict release criteria
- Scalability - produced to a commercially relevant scale in multi-tier tissue culture flasks or bioreactors
- Stable exosome product at 4'C, -80'C
- Safe: No c-MycERTAM within exosomes



EVs harvested from CTX producer cells

4-OHT = 4-hydroxy tamoxifen

Xeno-free, Scalable GMP Process

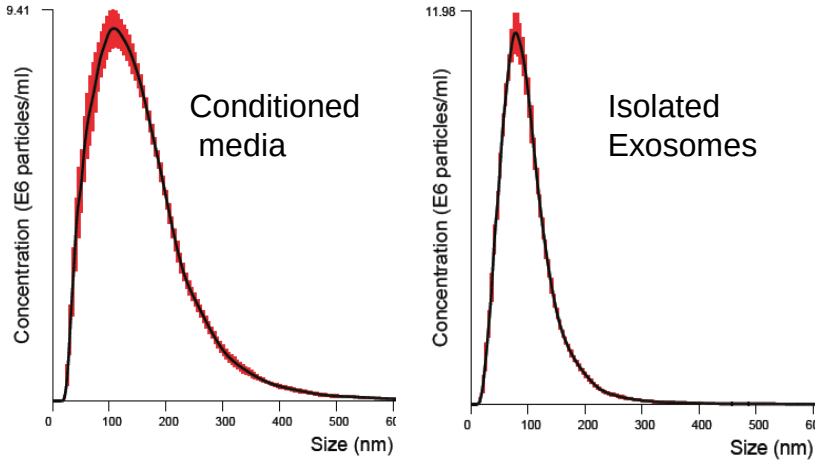


Formulation:

- Very simple: PBS at 2-8°C
- Estimated stability 6-12 months
- Possibility for enhanced formulation: frozen (-20°C), lyophilisation for long-term stability.

Stable and Consistent Product

Nanoparticle Tracking Analysis (NTA):

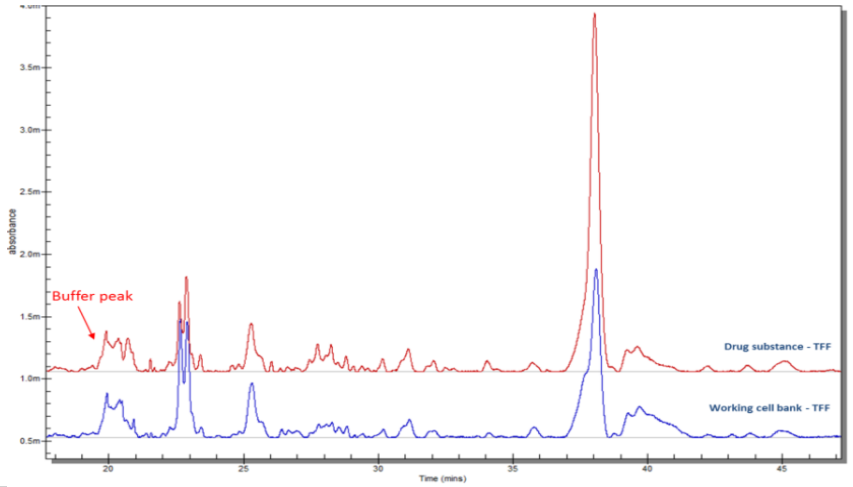


Next-Generation miRNA Sequencing (NGS):

	Batch 1	Batch 2	Batch 3	Batch 4
hsa-miR-A	1	2	1	1
hsa-miR-B	2	1	3	3
hsa-miR-C	3	3	4	4
hsa-miR-D	4	5	2	2
hsa-miR-E	5	7	6	7
hsa-miR-F	6	6	5	5
hsa-miR-G	7	12	9	10
hsa-miR-H	8	8	8	8
hsa-miR-I	9	11	12	15

Characteristic	Assay	Test	Specification
Purity			
Vesicle no. and Size distribution	**Established**	NTA (30-200nm)	Mode particle size 100±25nm
Protein content	**Established**	A280	10^8 vesicles/µg protein
Identity			
Surface markers	**Established**	ELISA (CD63, 81, 9)	CD81>CD9>CD63 Other specific surface markers
miRNA profile	NGS (**Established**) QPCR modification (in development)	PCR	Presence of specific miRNA
Proteomic fingerprint	**Established**	Capillary Electrophoresis (AUC)	Peak 1 – 10 ± 2% Peak 2 – 10 ± 2% Peak 5 - 19 ± 4%
FIO			
Visualisation	**Established**	TEM	Particle size 20-250nm

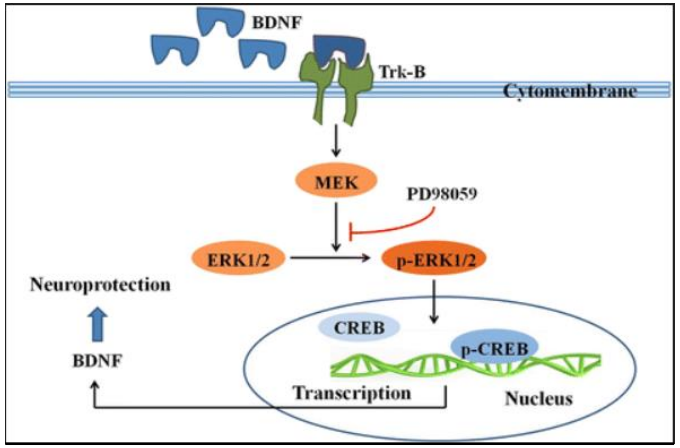
Proteomic Fingerprint - Capillary Electrophoresis (CE):



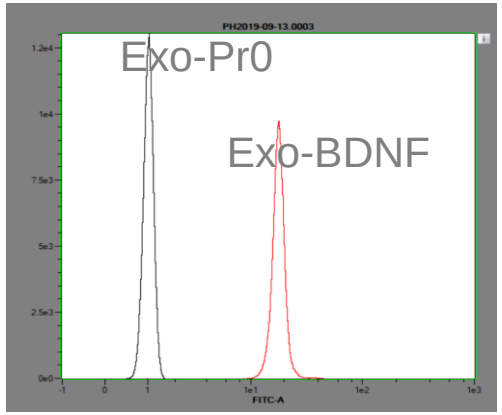
ReNeuron

Delivering a Functional Protein to the Brain

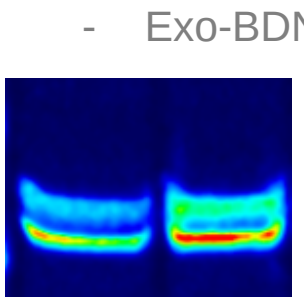
Directed Loading of Functional Protein via Surface Modification



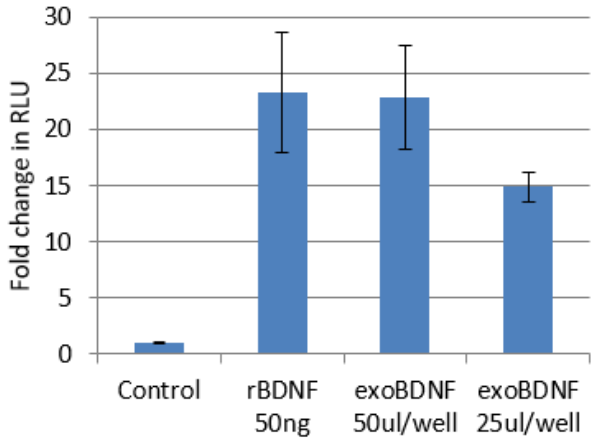
BDNF promotes gene transcription through the TrkB/MAPK/CREB pathway.



Exo-BDNF binds to the receptor TrkB

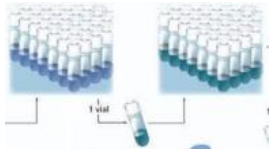


Exo-BDNF activates the MAPK pathway (30min stimulation)



Exo-BDNF triggers CREB dependent gene transcription

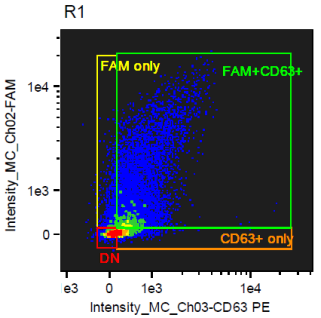
Scalable Manufacturing Process



MCB / WCB

Conditioned media

Tangential Flow Filtration (TFF)



UPSTREAM PROCESSING

DOWNSTREAM PROCESSING

CELL BANKING

- Native CTX / Engineered
- Cryopreservation & cryostorage
- Master Cell Bank (MCB)
- Working Cell Bank (WCB)

EXOSOME PRODUCTION

- 2D adherent
- Flask based
- Collection of conditioned media

EXOSOME PURIFICATION & CONCENTRATION

- Centrifugation
- Tangential flow filtration (TFF)
- Buffer exchange
- Size exclusion
- IEX
- Affinity

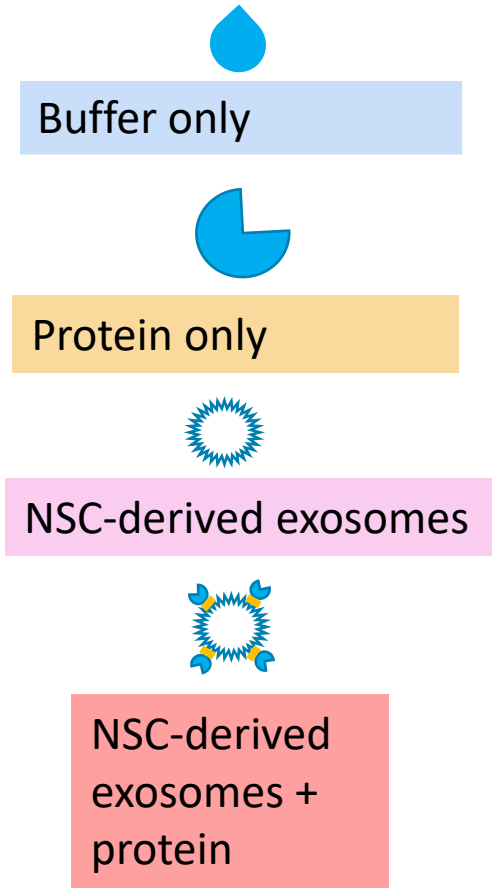
FILL/FINISH

- Sterile dispense

ANALYTICS

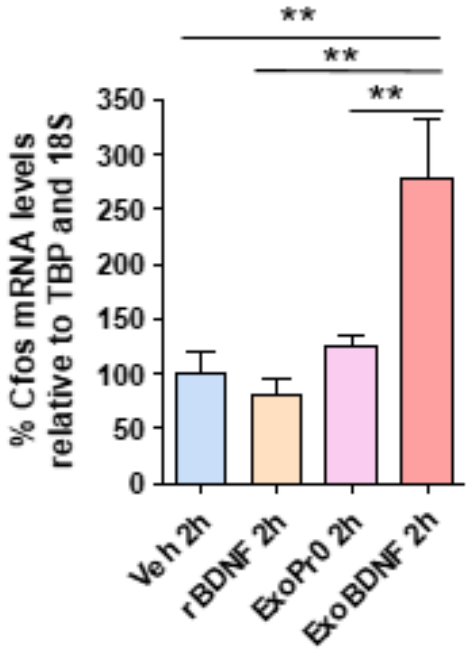
- Protein concentration
- Particle concentration
- Sterility
- Mycoplasma
- Endotoxin
- HCP
- **Advanced analytics**

Targeted Delivery of BDNF to the Striatum using engineered NSC-derived EVs



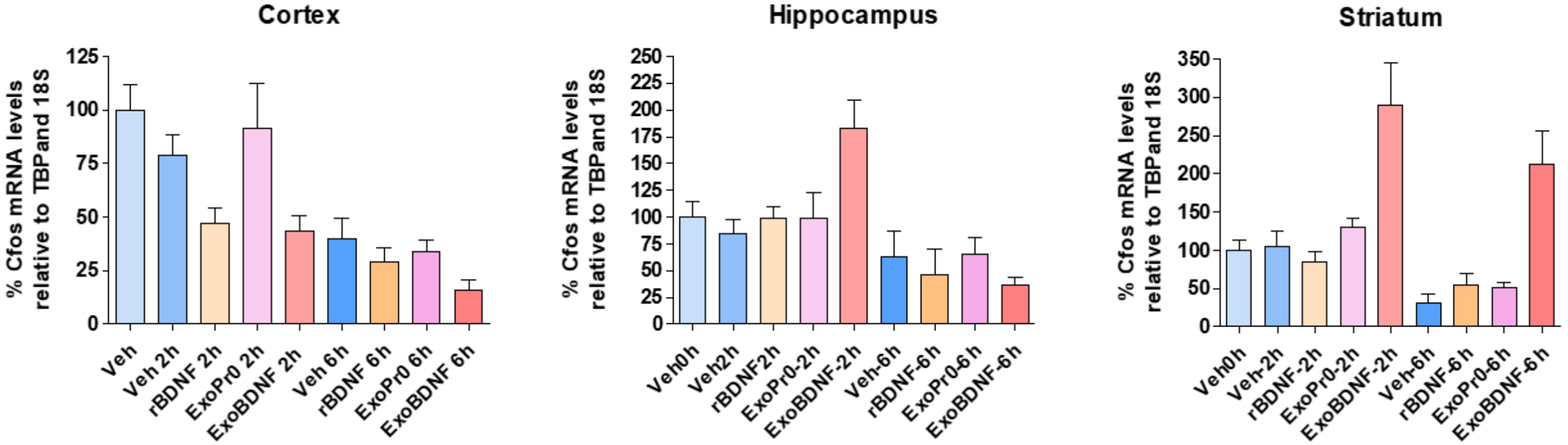
Intrathecal injection (lumbar puncture)

Look for gene upregulation in brain



- Pre-clinical proof of concept showing significantly improved delivery of functional protein to the brain
- EVs have the potential to transform effective drug delivery for key neurological diseases

Sustained Target Engagement in the Striatum



- C-fos mRNA measured in Cortex, Hippocampus, Striatum, Thalamic + Hypothalamic area, Midbrain, Brain Stem and Cerebellum
- Increase observed only in hippocampus (transiently) and striatum (sustained)
- Loss of function in the striatum associated with Parkinson's and Huntington's disease

Summary

- EVs have a proven ability to carry a range of biologically active cargos
- They target recipient cells via specific surface proteins that are determined by their cell of origin
- Proteomic and lipidomic profiling illustrate not all EVs are the same
- Multiple conditionally immortalised EV producer cell lines have been generated from different tissues
- Conditionally immortalised iPSC lines for rapid generation of new lines
- Producing a flexible EV platform that can be customised and optimised for specific payloads and targets for a greater chance of success
- The addition of highly efficient engineering techniques allow cargos to be loaded and delivered to specific tissues or cells

Acknowledgements

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- Anna Figueras
- Leila Barwani
- Zara Waheed
- Madeleine Miles
- Jade Hopkins

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- Aled Clayton
- Pete Watson
- Phil Stephens
- Rob Knight

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- Dan Bracewell
- Ben Barnes
- Derek Yellon
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