



Targeted Delivery of Therapeutic Payloads Using Engineered Stem Cell Exosomes

Randolph Corteling, PhD
Chief Scientific Officer

EV-based Therapeutics – Exploring Latest Advancements of EV-Based Cargo Delivery

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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



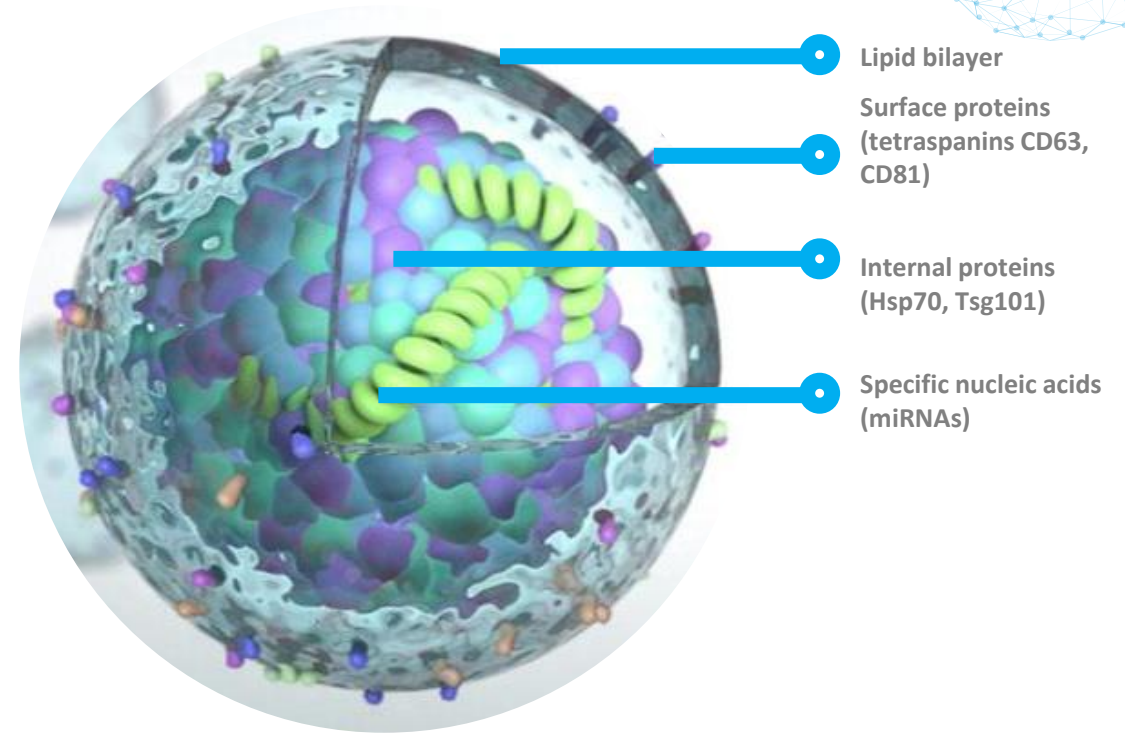
Target recipient cells via specific surface proteins that are determined by their cell of origin



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



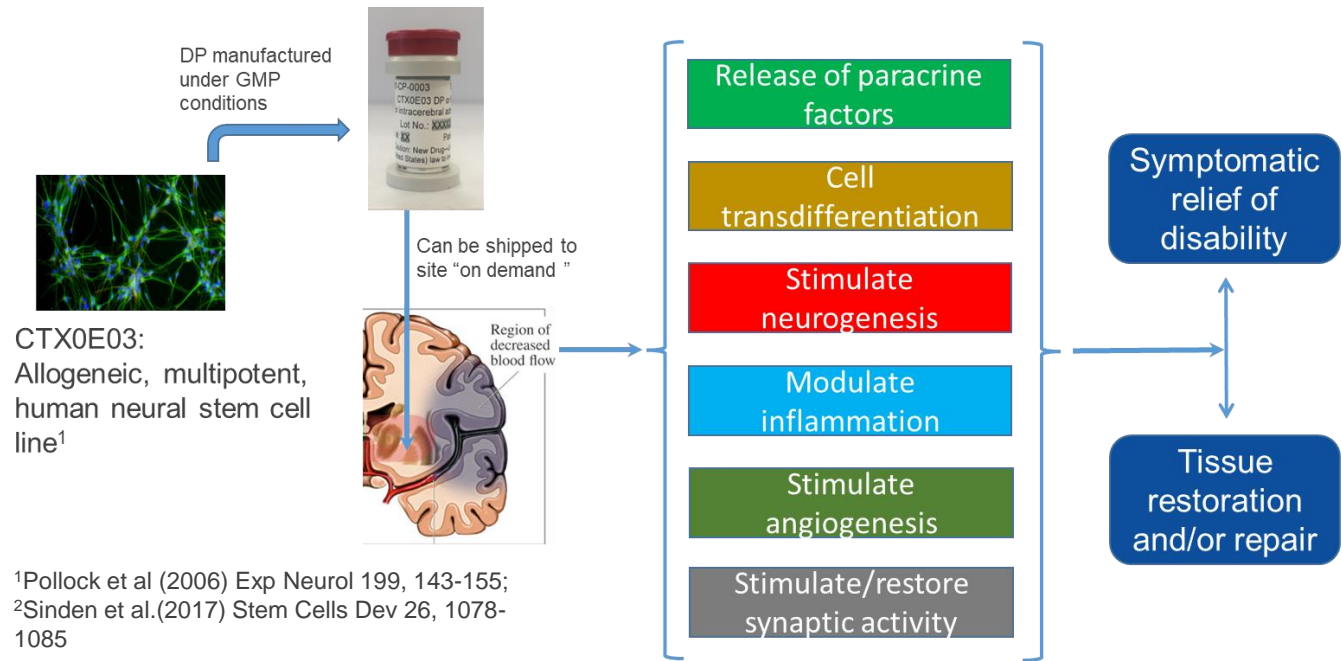
Increasing interest across the industry in extracellular vesicles as biomarkers, standalone therapeutics and as delivery vectors for complex drug modalities



CTX-derived EVs only partially recapitulate function of cells



Potential mechanism(s) of action²:

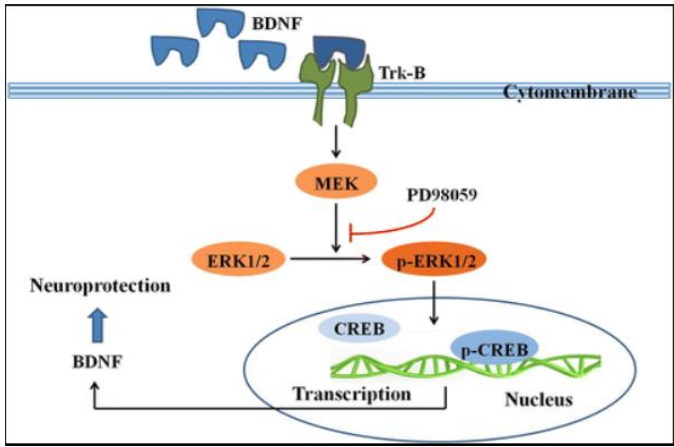
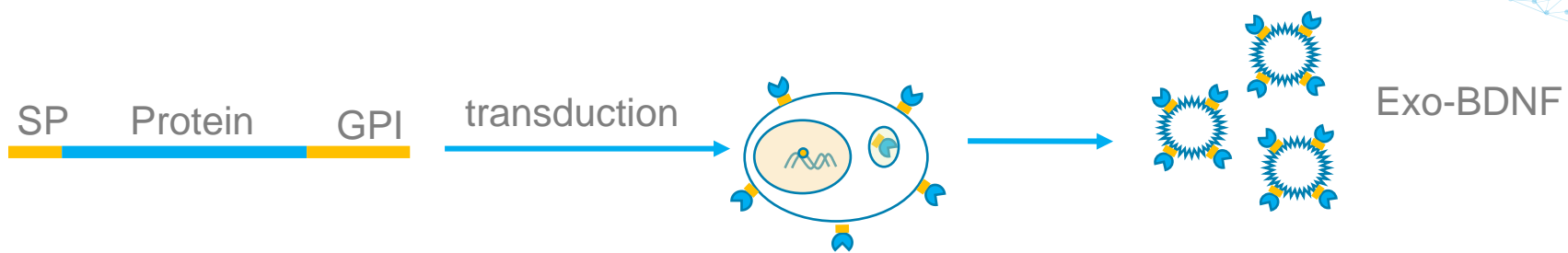


¹Pollock et al (2006) Exp Neurol 199, 143-155;
²Sinden et al.(2017) Stem Cells Dev 26, 1078-1085

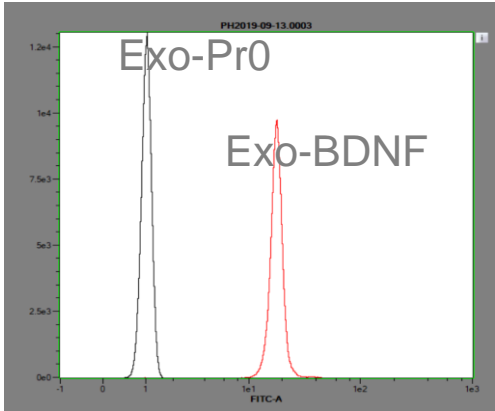
Host Response	Measure	CTX-derived sEVs
Angiogenesis	CD31	✓
Neurogenesis	DCX/Ki67	✓
Macrophage M1-M2	CD68/iNOS/CD206	X
Microglia	IBA1	X

- Stereotaxic implantation of CTX cells in MCAo model of stroke induced a number of repair mechanisms within the host tissue
- Potential mechanism of action for CTX cells *in vivo* include the release of extracellular vesicles.
- CTX-derived EVs alone however only partially recapitulate the function of the cells

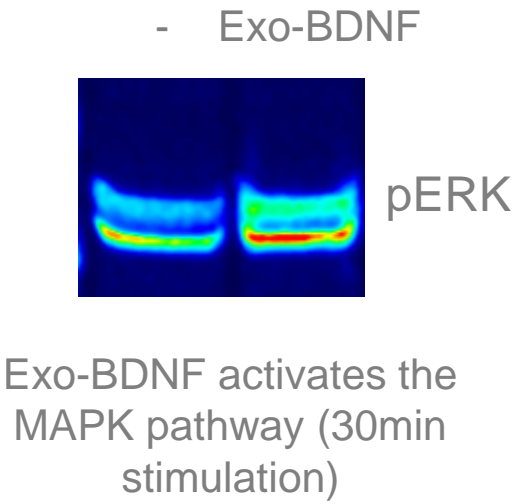
Directed Loading of Functional Protein via Surface Modification



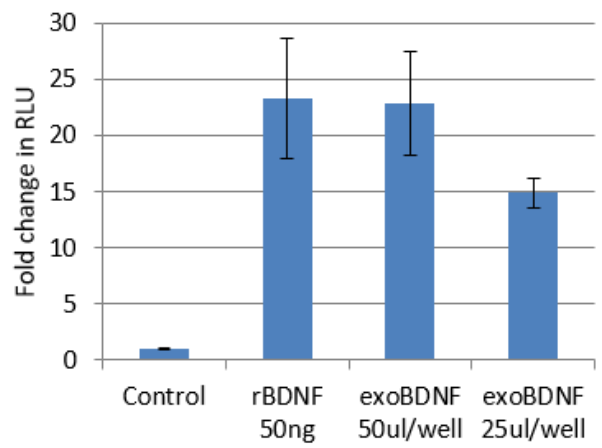
BDNF promotes gene transcription through the TrkB/MAPK/CREB pathway.



Exo-BDNF binds to the receptor TrkB

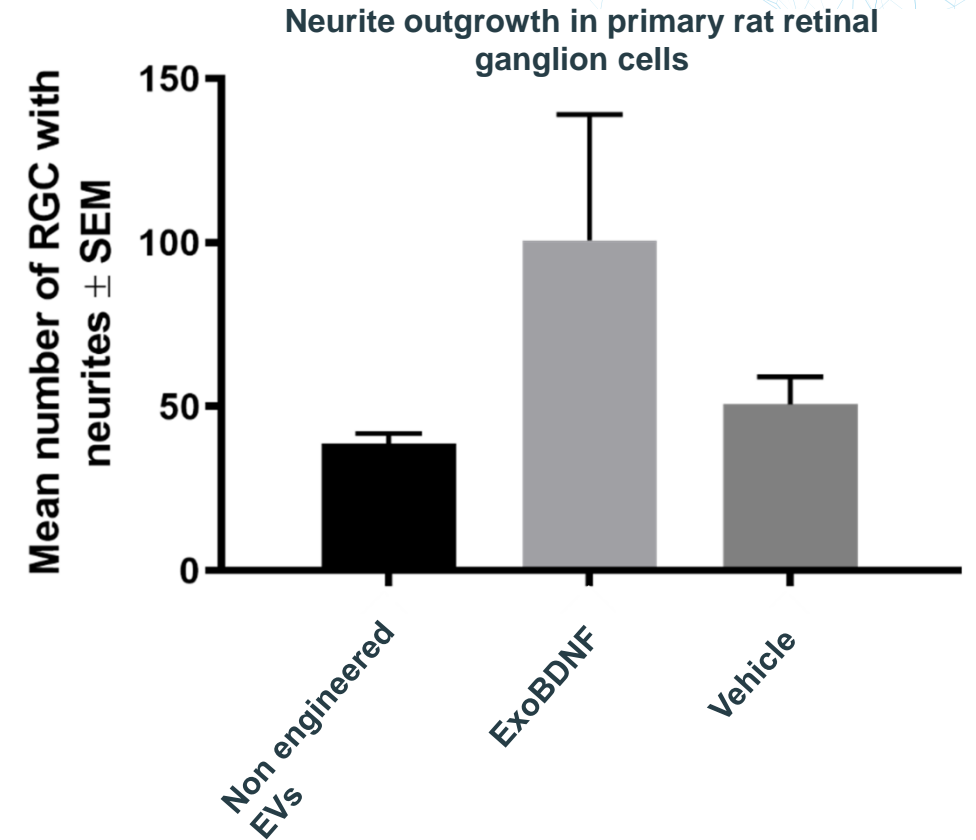
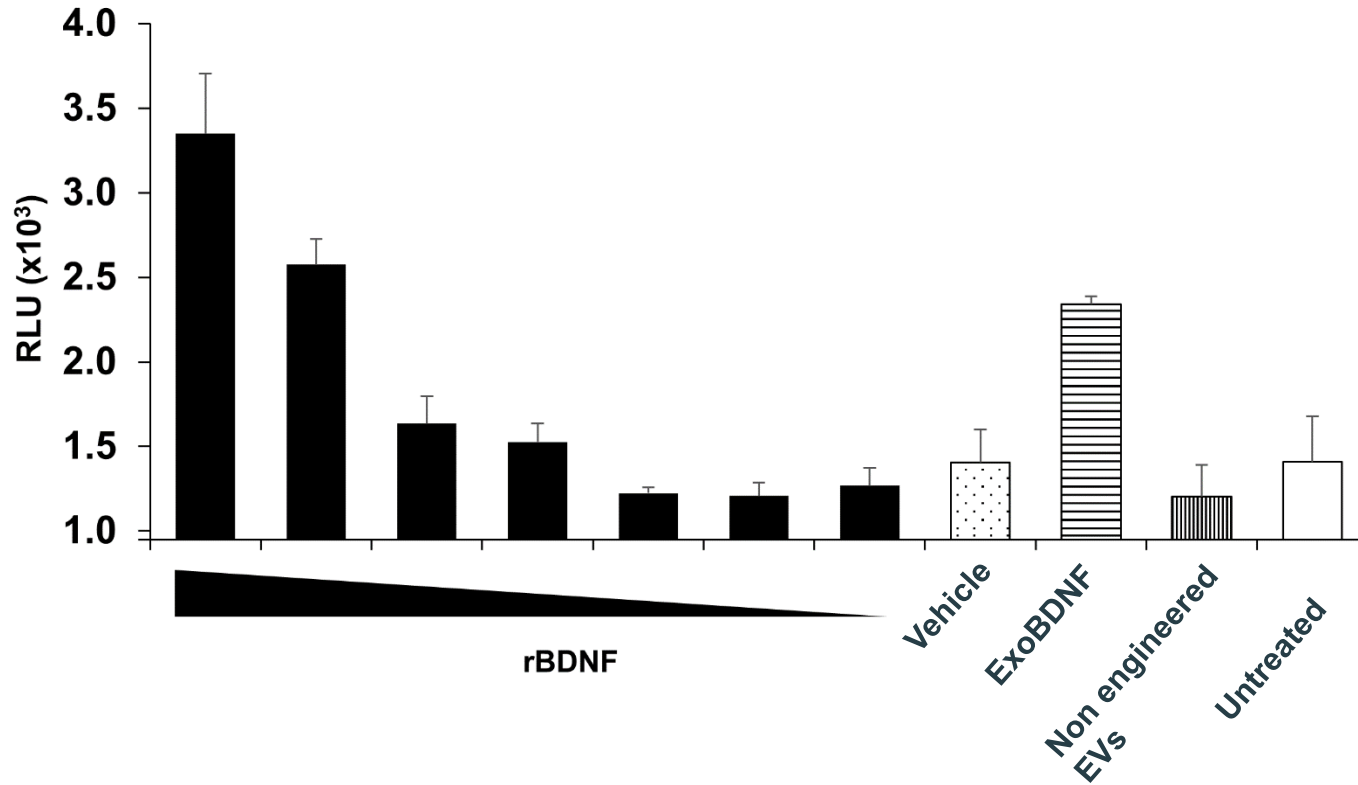


Exo-BDNF activates the MAPK pathway (30min stimulation)



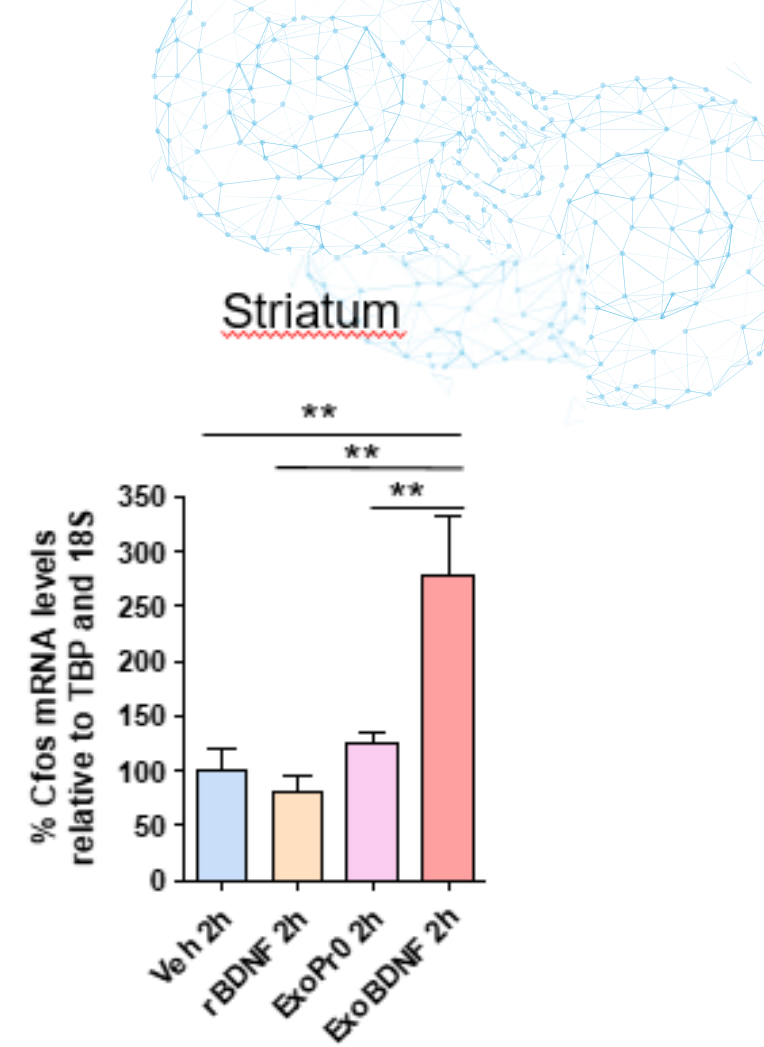
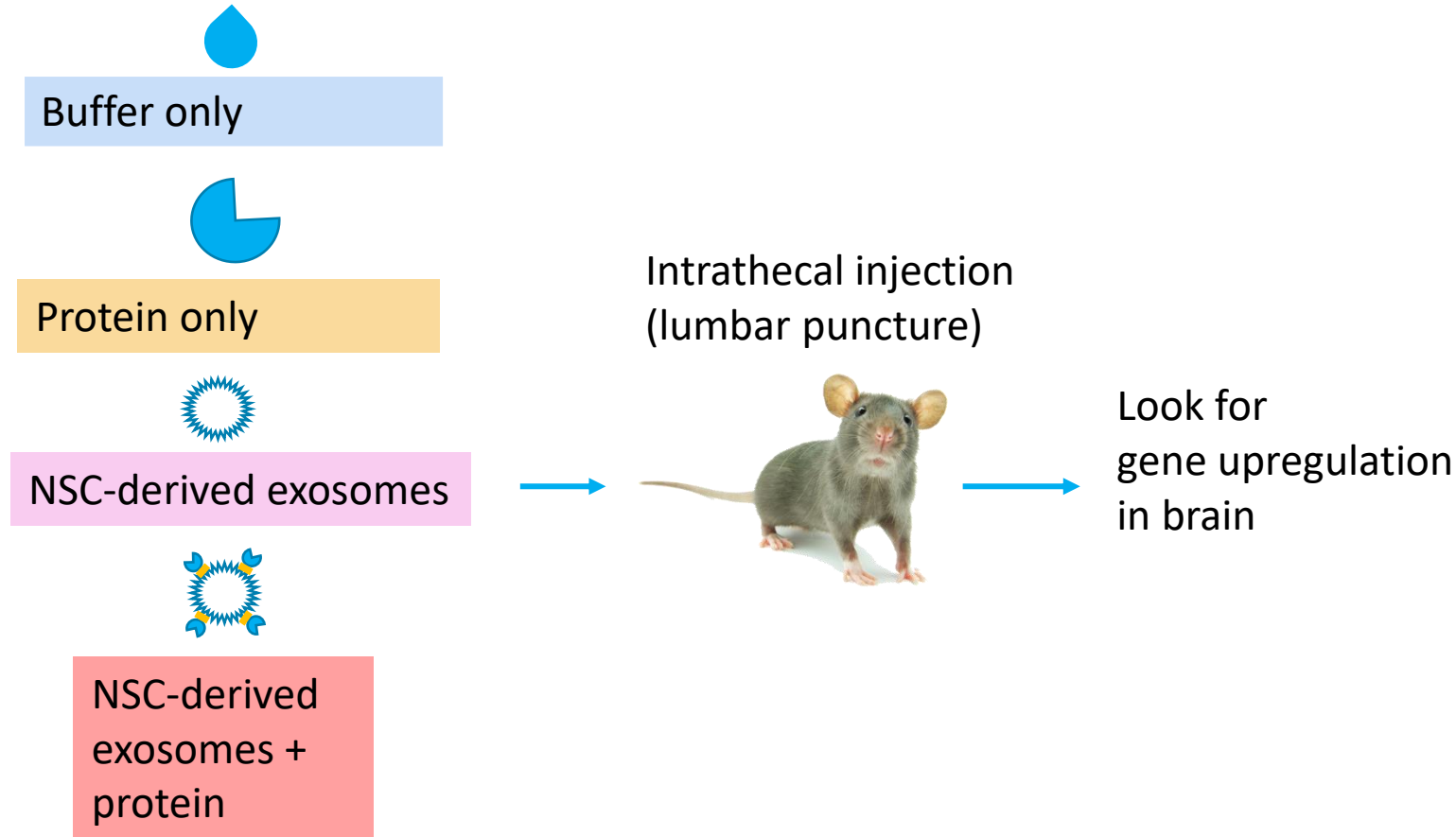
Exo-BDNF triggers CREB dependent gene transcription

Target engagement and functional effect of cargo *In vitro*



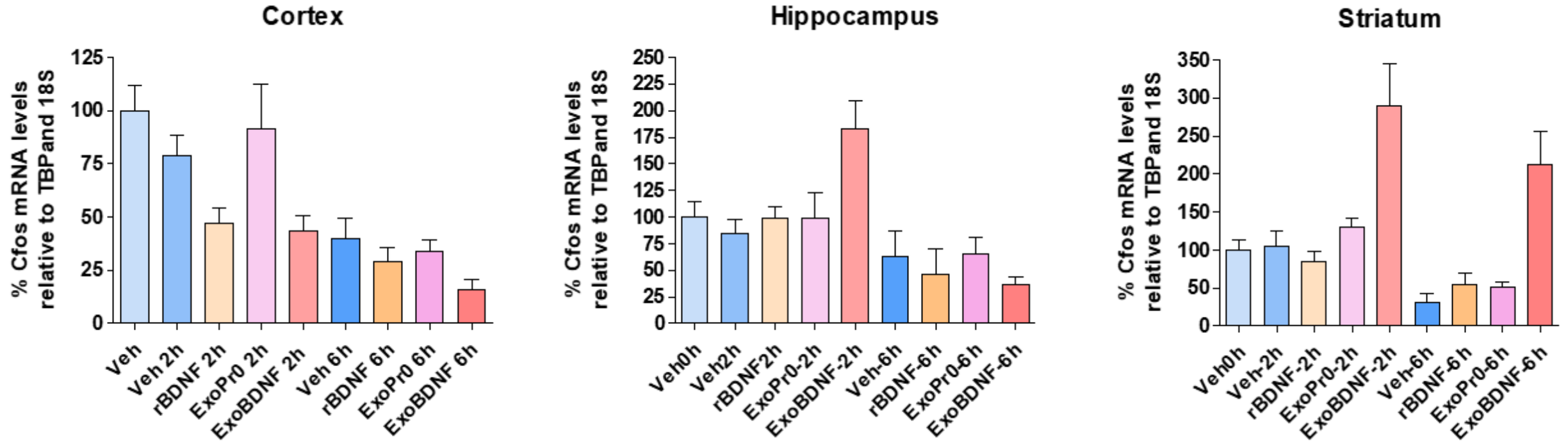
- Confirmation of BDNF specific activity using a commercially available TrkB reporter assay
- Demonstration of *in vitro* functional recover by Exo-BDNF in a primary RGC neurite outgrowth assay

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



- 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

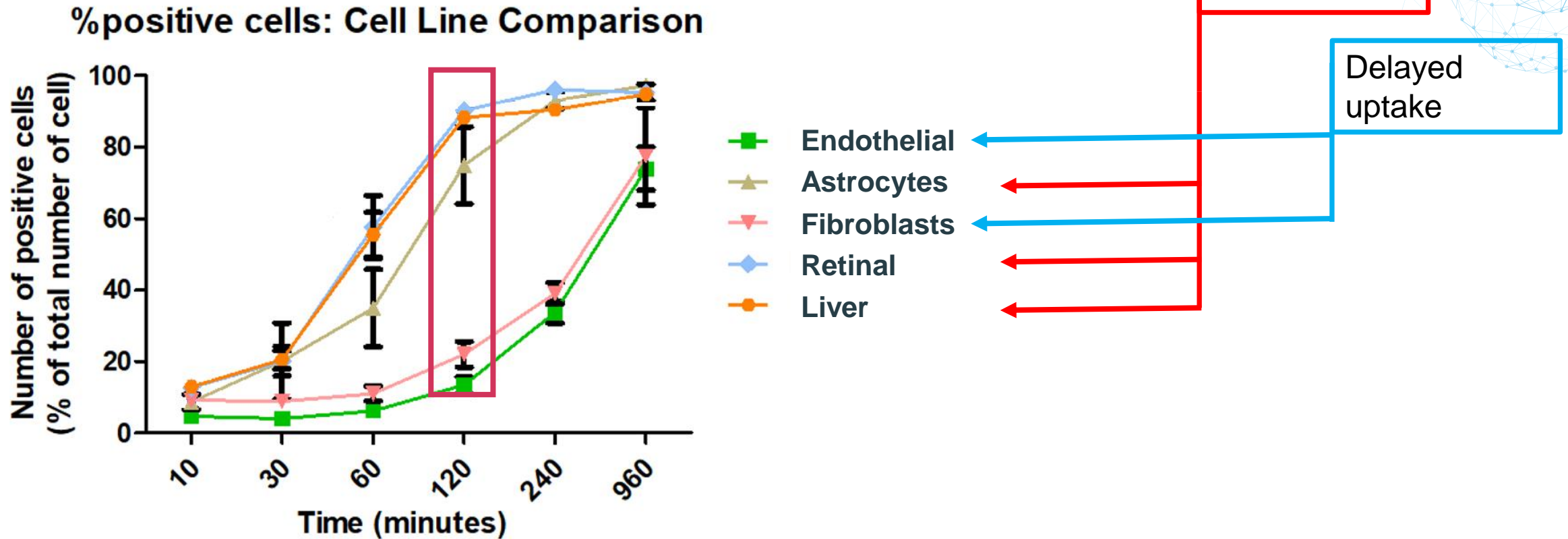
Summary

- CTX-derived EVs can be engineered to express functional proteins on their surface
- Protein cargo can bind to its specific receptor and activate appropriate second messenger pathway
- Exo-BDNF can induce pro-survival signals in primary RGC *in vitro*
- Following intrathecal administration into naïve mice, Exo-BDNF can reach deep structures within the brain (striatum and hippocampus) and induce a sustained activation of pro-survival signals associated with BDNF for the potential treatment of neurodegenerative disease



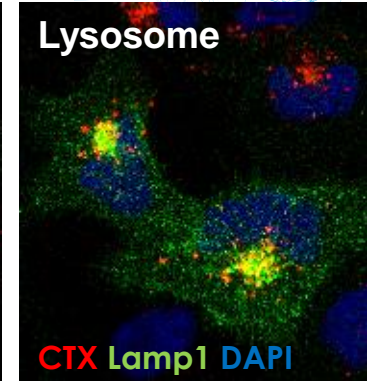
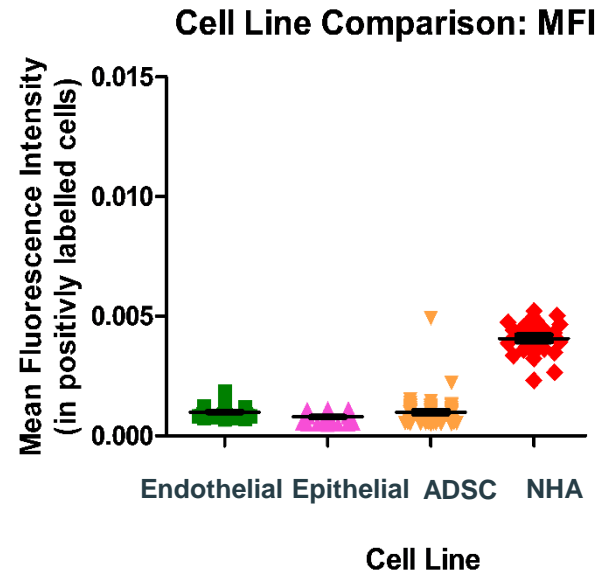
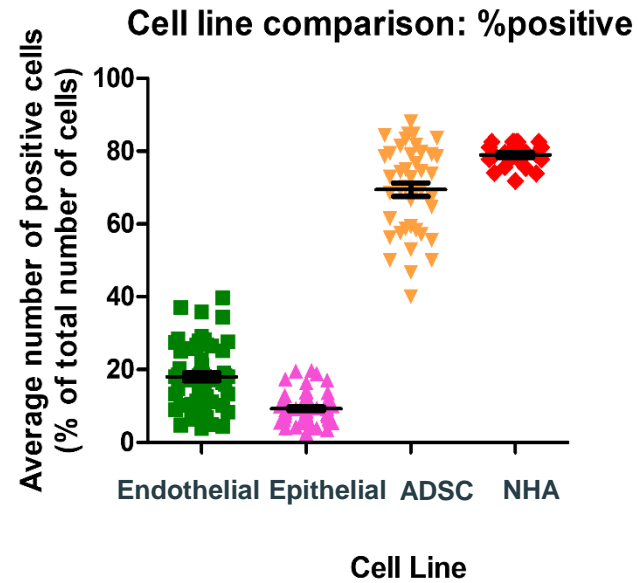
EV-based Delivery Platform Beyond the CNS

ReNeuron's CTX-derived EVs are Differentially Taken Up by Specific Cell Types

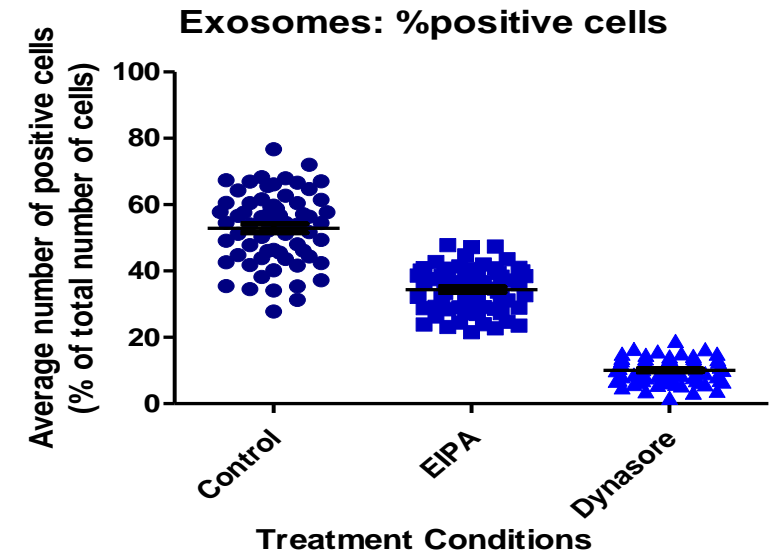


- By assessing the number of positive cells over time, 2 distinct profiles emerge
- Astrocytes, retinal and liver progenitors rapidly take up CTX-derived EVs while fibroblasts and endothelial cells show delayed uptake

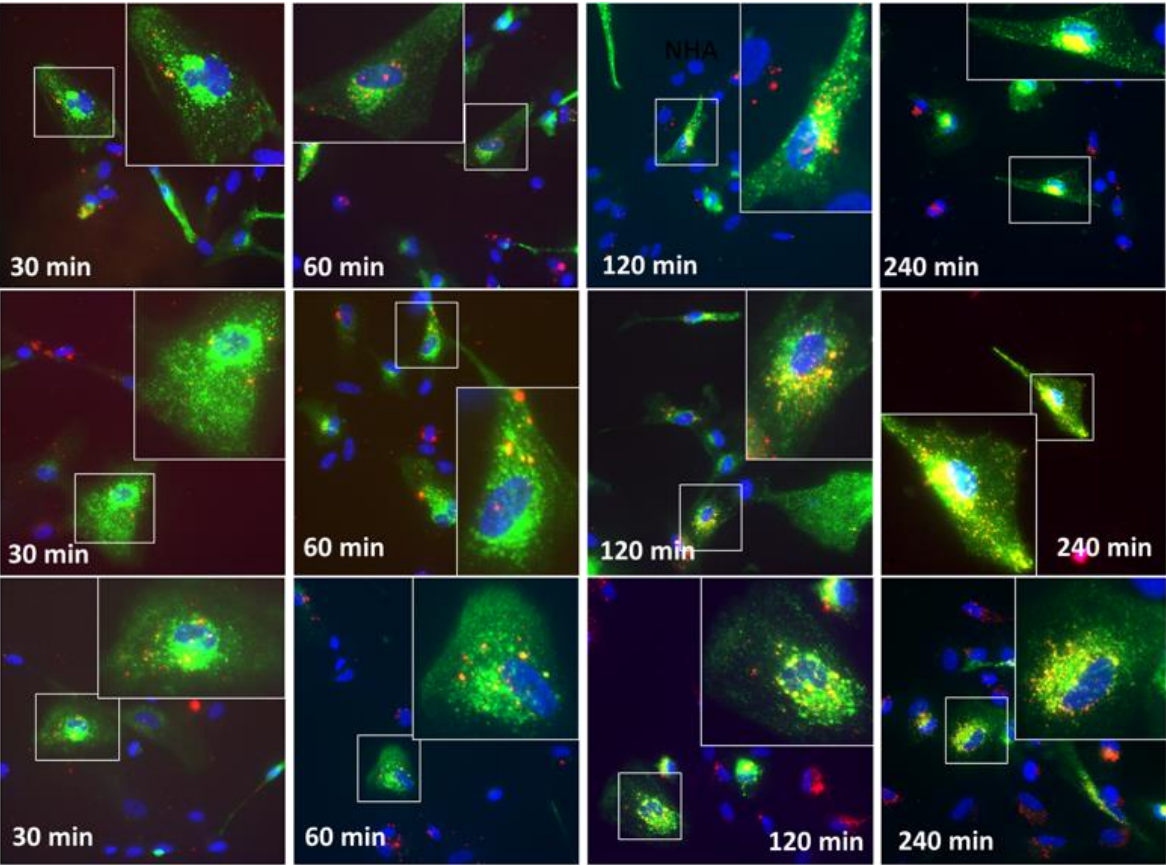
ReNeuron's CTX-derived EVs are Predominantly taken up by Clathrin mediated Endocytosis by Specific cell types



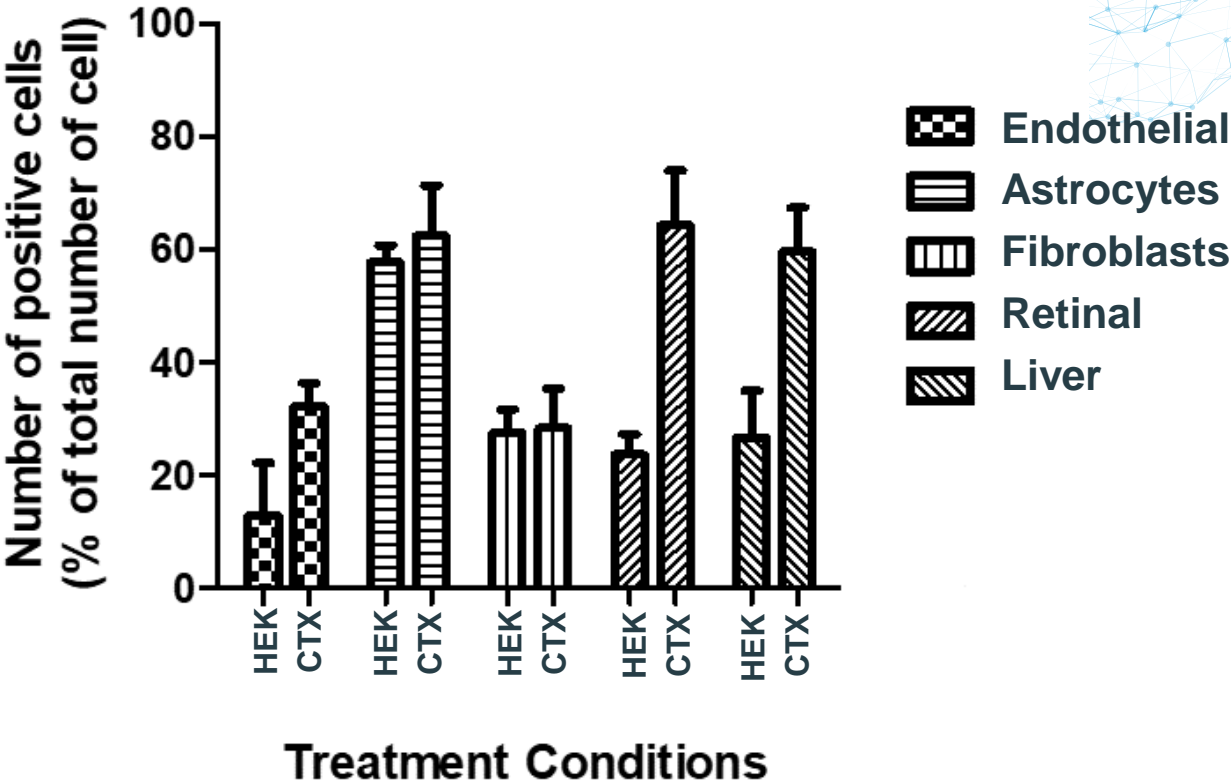
From a panel of 4 different cell types, CTX-derived EVs are taken up by the cells in the following order; Normal human astrocytes; ~85%, Adipose-derived stem cells; ~75%, Endothelial; ~18% and Epithelial; ~10%.



Efficient EV Uptake is Dependent Upon Cell Line of Origin



Uptake of EVs from various cell sources into Normal Human Astrocytes (NHA)



Uptake of HEK293 and CTX-derived EVs when applied at the **same time** to different recipient cell types. CTX-derived EVs were shown to be rapidly and efficiently taken up in 3/5 cell types versus 1/5 for HEK293-derived EVs.

CustomEx™ - A Customisable, EV delivery platform Optimised for Specific Drug Delivery needs

Standard Approach

Single cell line



Engineering

Exosome candidate



'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:

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

CustomEx™

Exosome candidates

Four Proprietary Neural Cell lines

(Cortex (CTX), Striatum, Hippocampus, Ventral Mesencephalon)



Engineering



Three additional proprietary cell lines

(Retinal, liver, pancreatic)

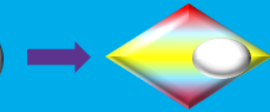


Engineering



CTX iPSC

Any cell

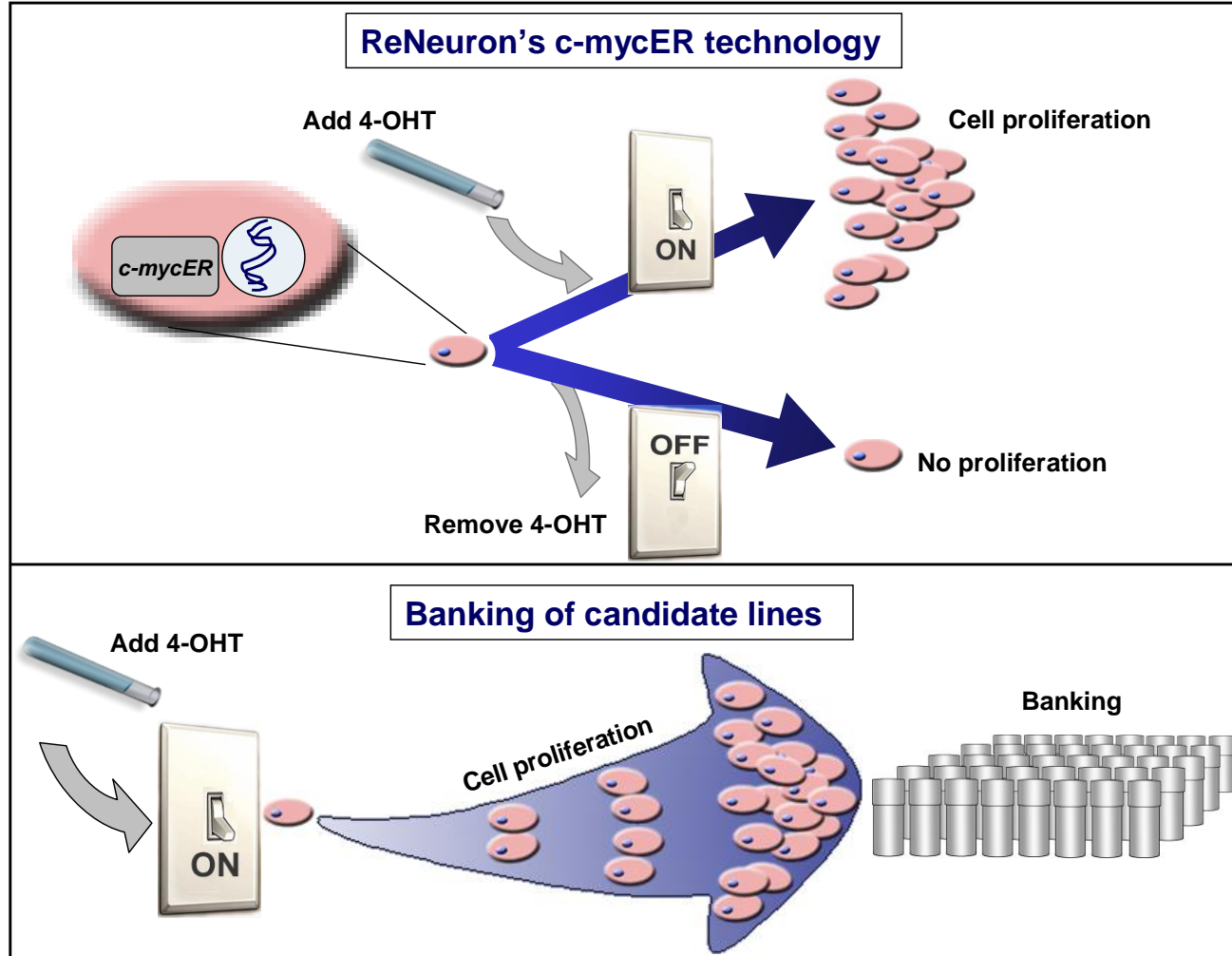


Engineering

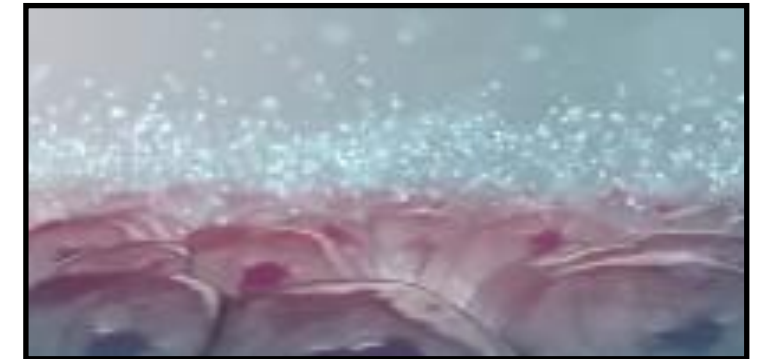


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Consistent and Scalable EV Production through Conditional Immortalisation



- 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



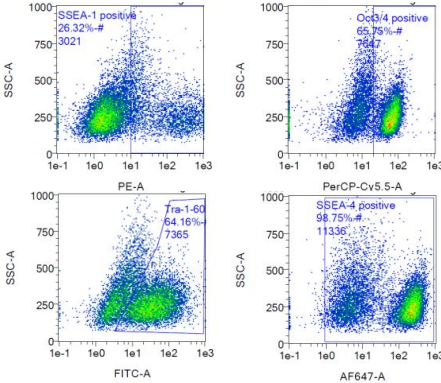
EVs harvested from CTX producer cells

A Range of Bespoke EVs from iPSCs

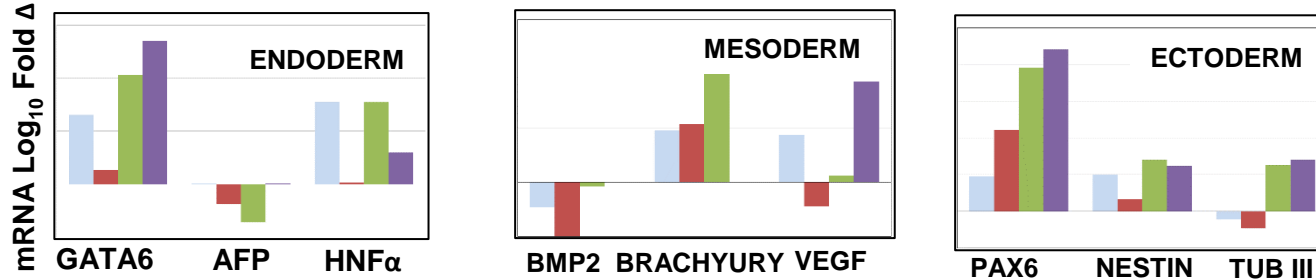
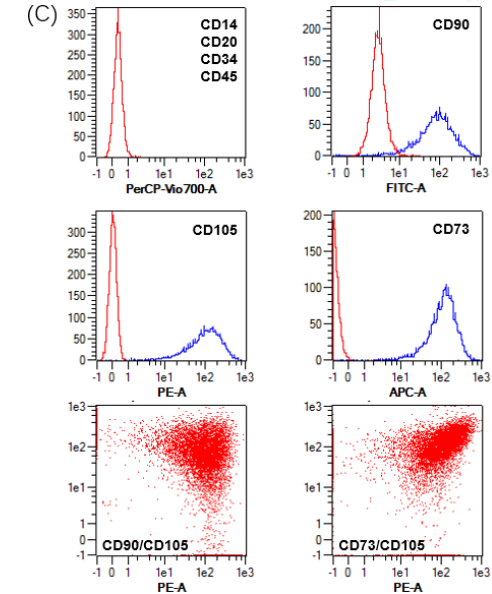
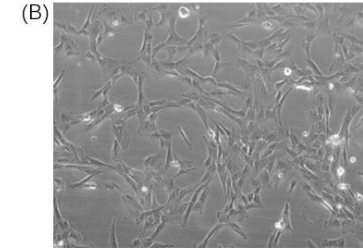
Pluripotency

Expression of pluripotency markers

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



Therapeutic derivatives (MSCs) from CTX-iPSCs



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

Proprietary Assets



Human neural stem cell EVs (hNSC)

Producer stem cell lines from 4 distinct brain areas

Cortex (CTX), Striatal (STR), Hippocampal (HPP) and Ventral mesencephalon (VM)

Conditionally immortalized for stable and scalable production

GMP-compliant source stocks



EVs from human liver (LIV), retinal (RET) and pancreatic progenitors (PIC)

Further proprietary immortalized stem cell lines

EVs with distinct characteristics

Stable cell lines generated through immortalization or via CTX-iPSCs



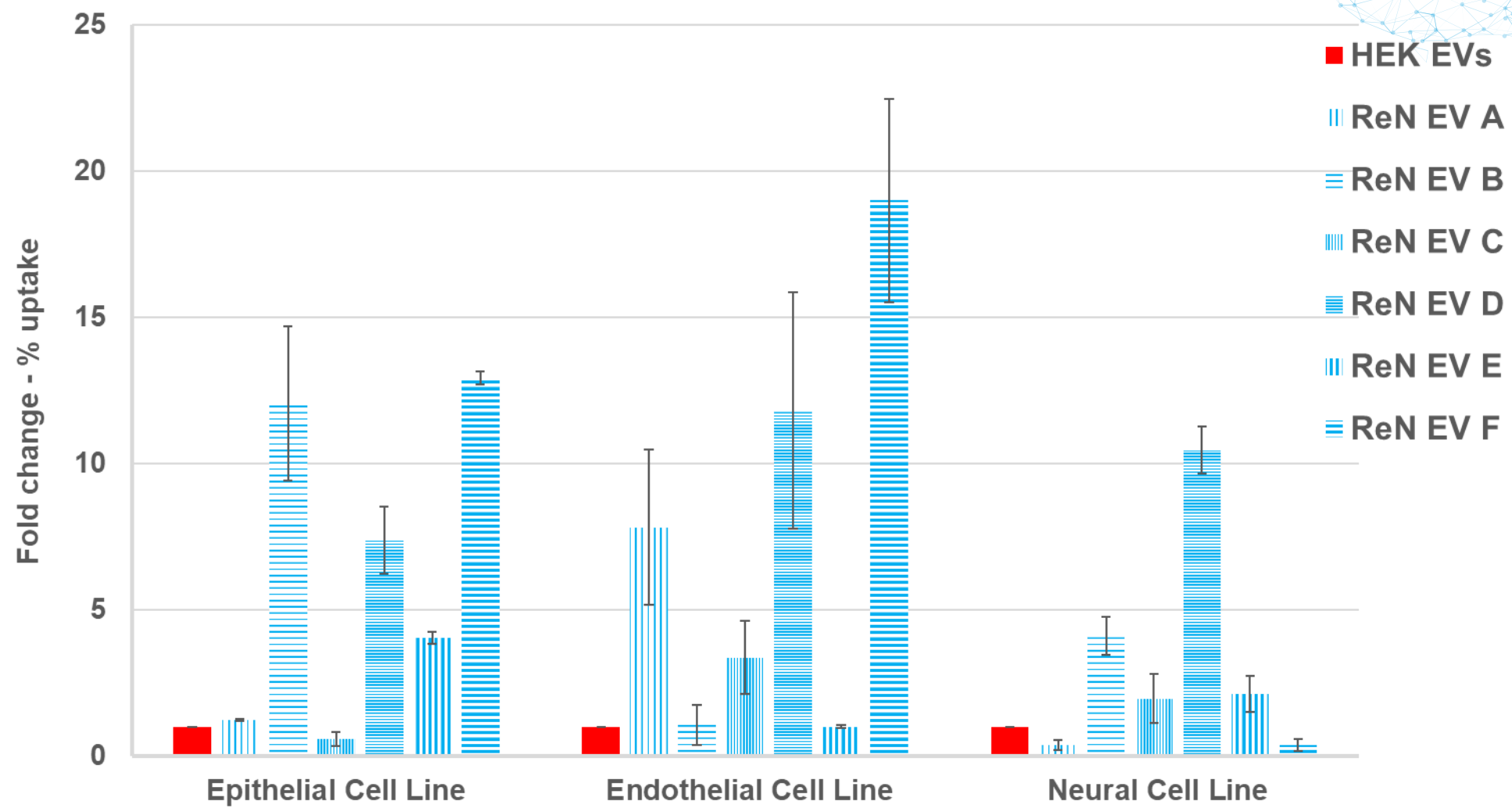
Inducible pluripotent stem cell-derived EVs (CTX-iPSC)

CTX-derived induced pluripotent stem cell platform

EV production from parental CTX-iPSC and MSC lineage confirmed

Potential for new EV producer cell lines from any cell type

Producer Cell line Selection Important Consideration when Developing EV-based Drug Delivery System



EV biology influences tropism and delivery of cargo

Distinct Surface Marker Profile

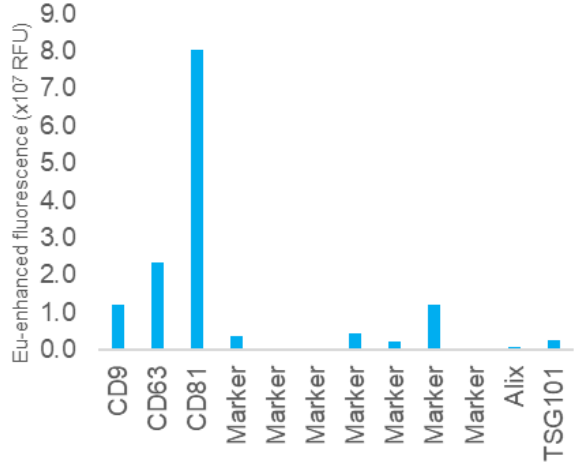
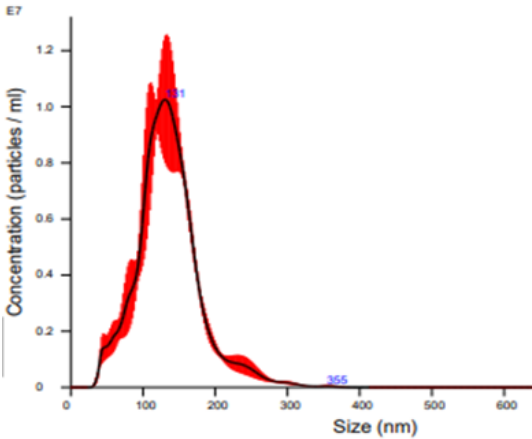
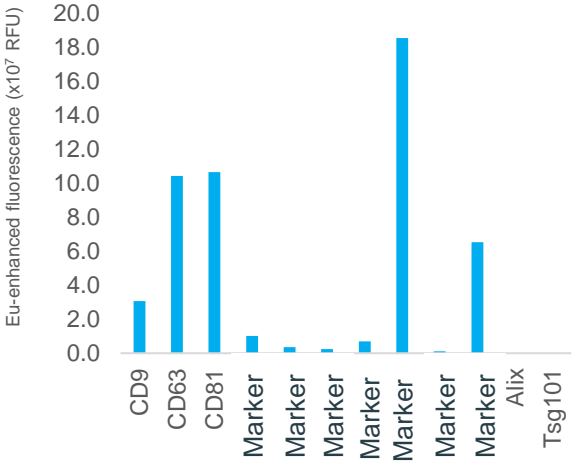
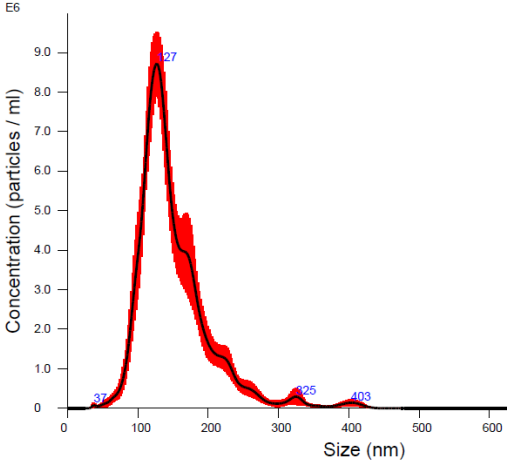
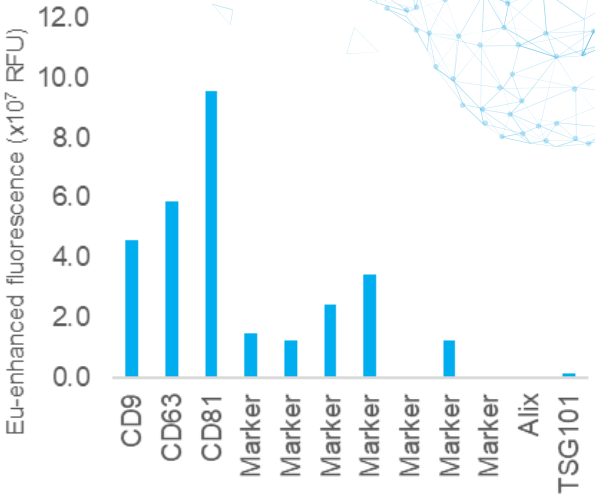
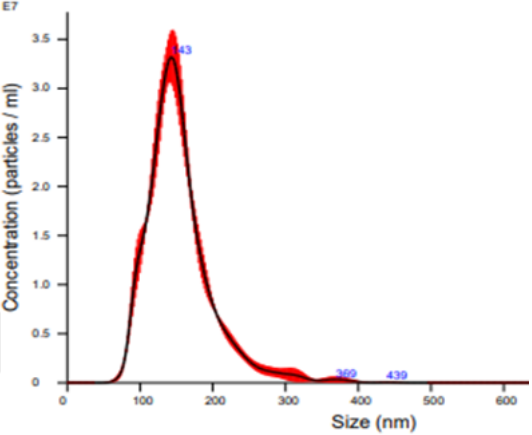
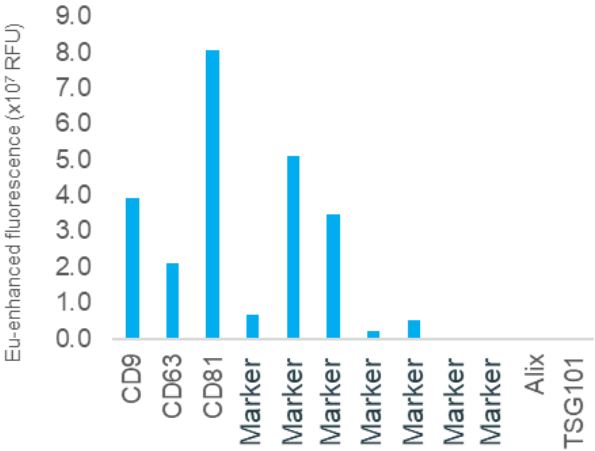
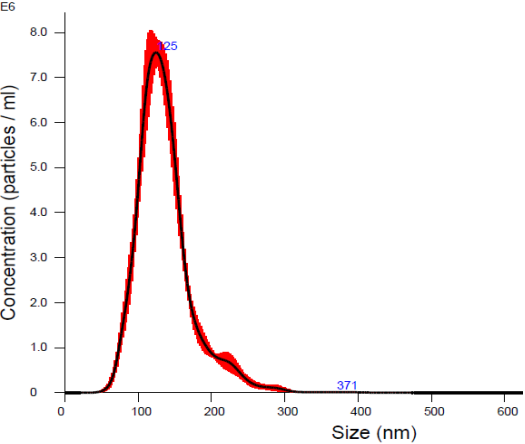


Nanotracking Analysis

ELISA surface marker profile

Nanotracking Analysis

ELISA surface marker profile



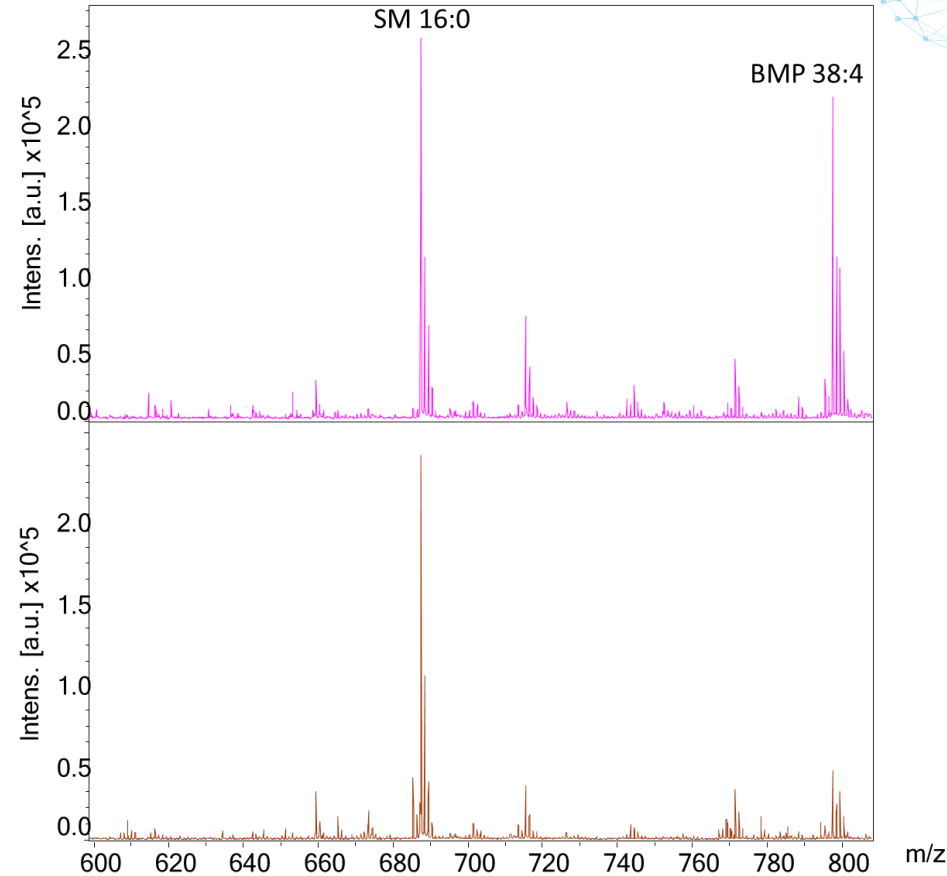
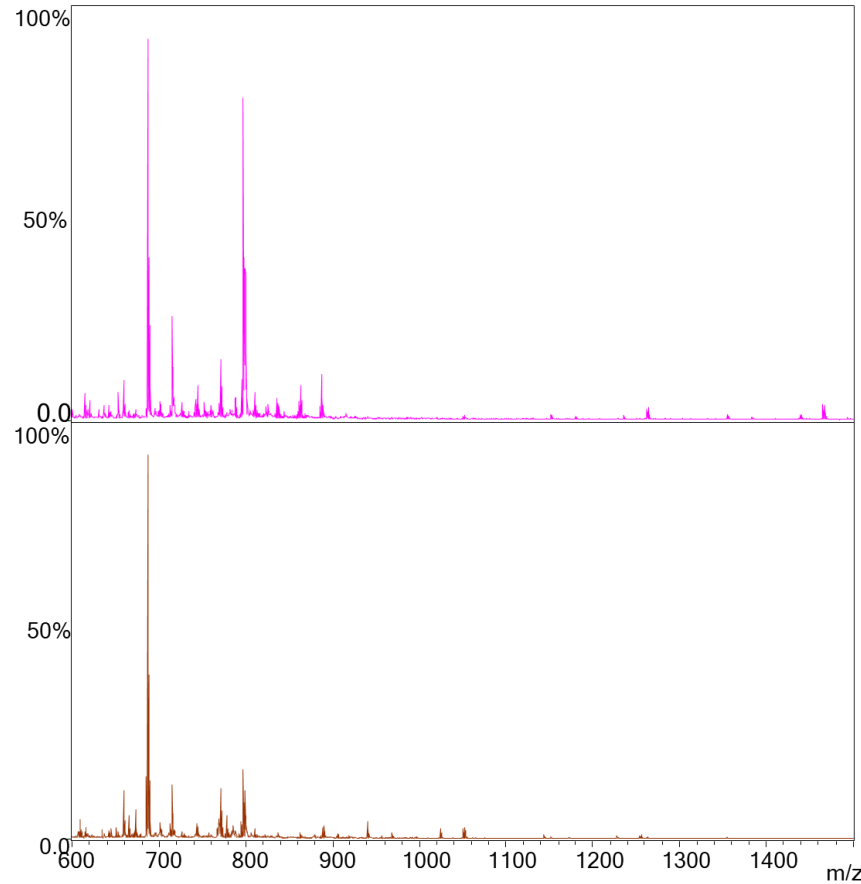
3 different neuronal producer cell lines, 3 different surface marker profiles

Distinct Lipid Membrane Composition



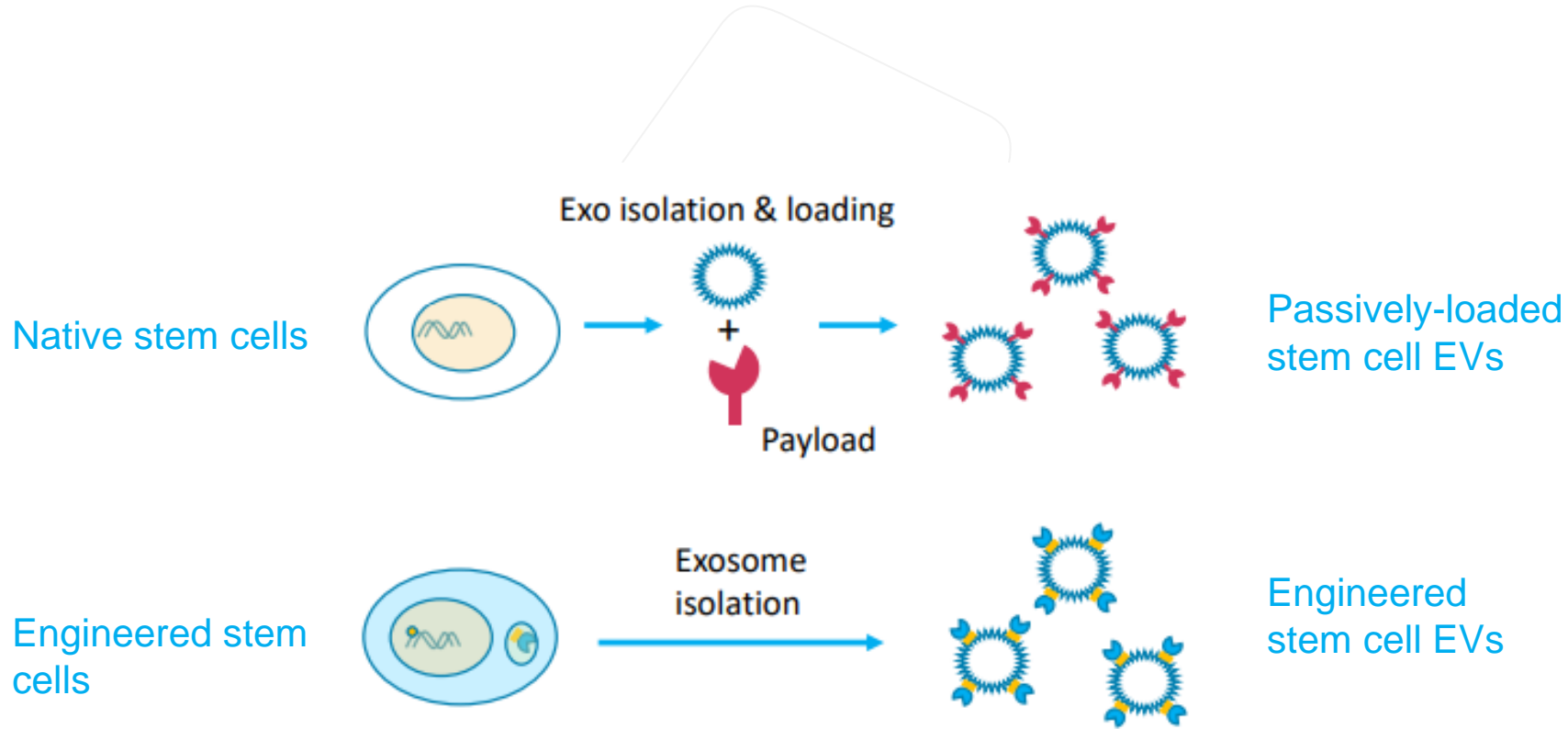
NSC-derived
EVs

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

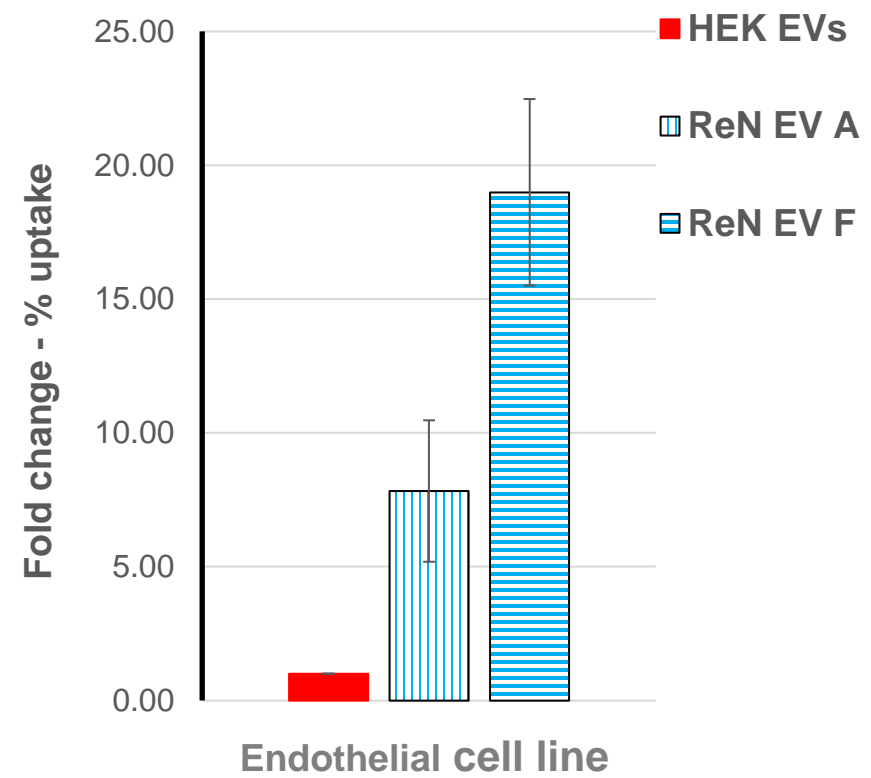
Ability to load and engineer EVs



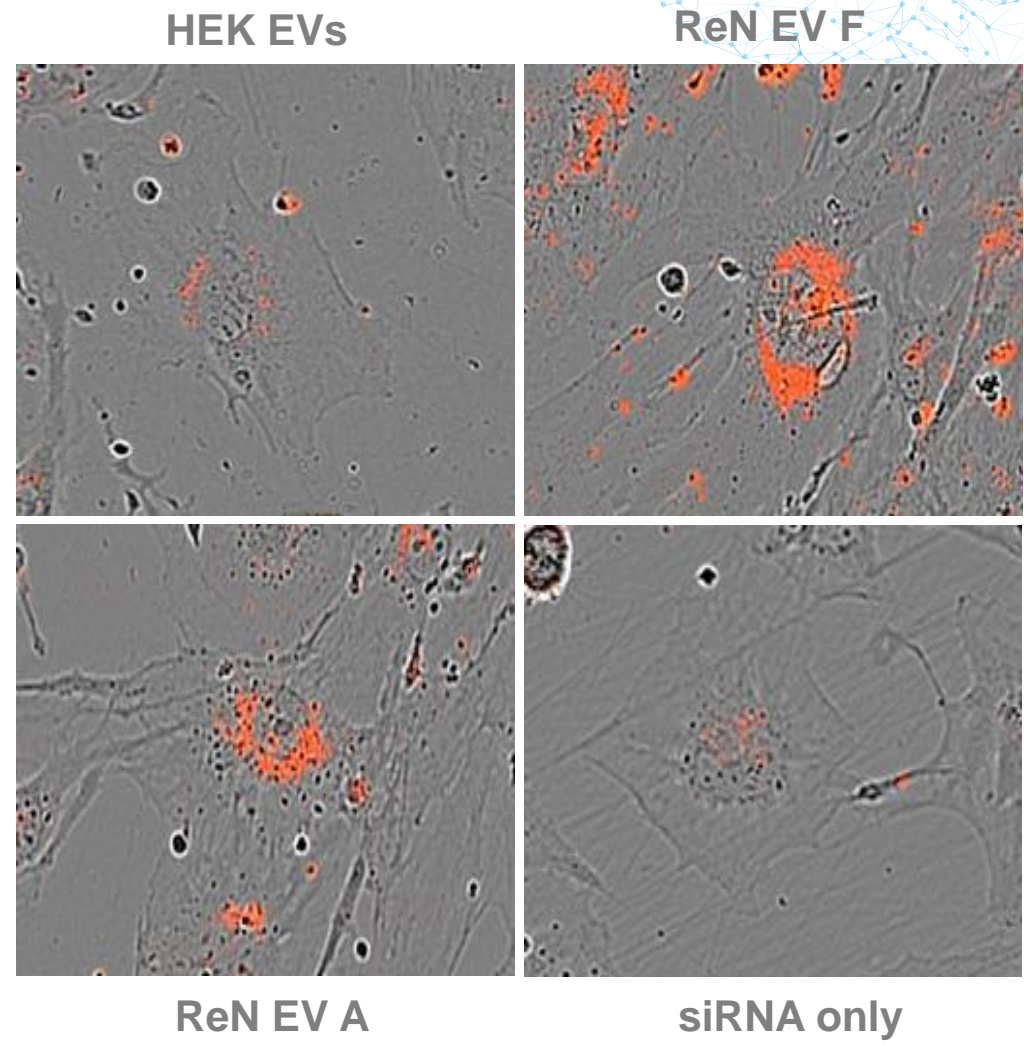
- Passive diffusion
- Forced evaporation
- Reverse permeabilization
- Electroporation

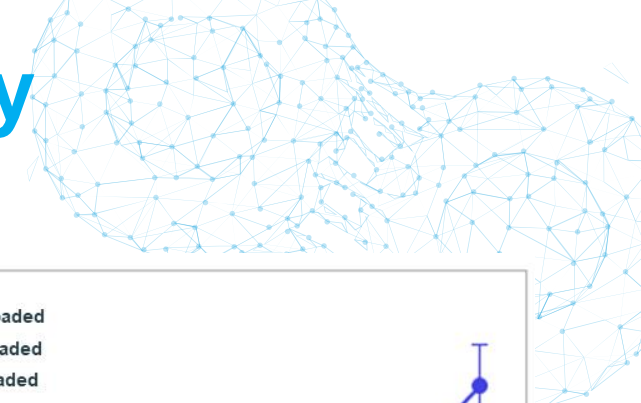
- Surface presentation
- Luminal loading
- combination

Greater the uptake, greater the delivery of cargo

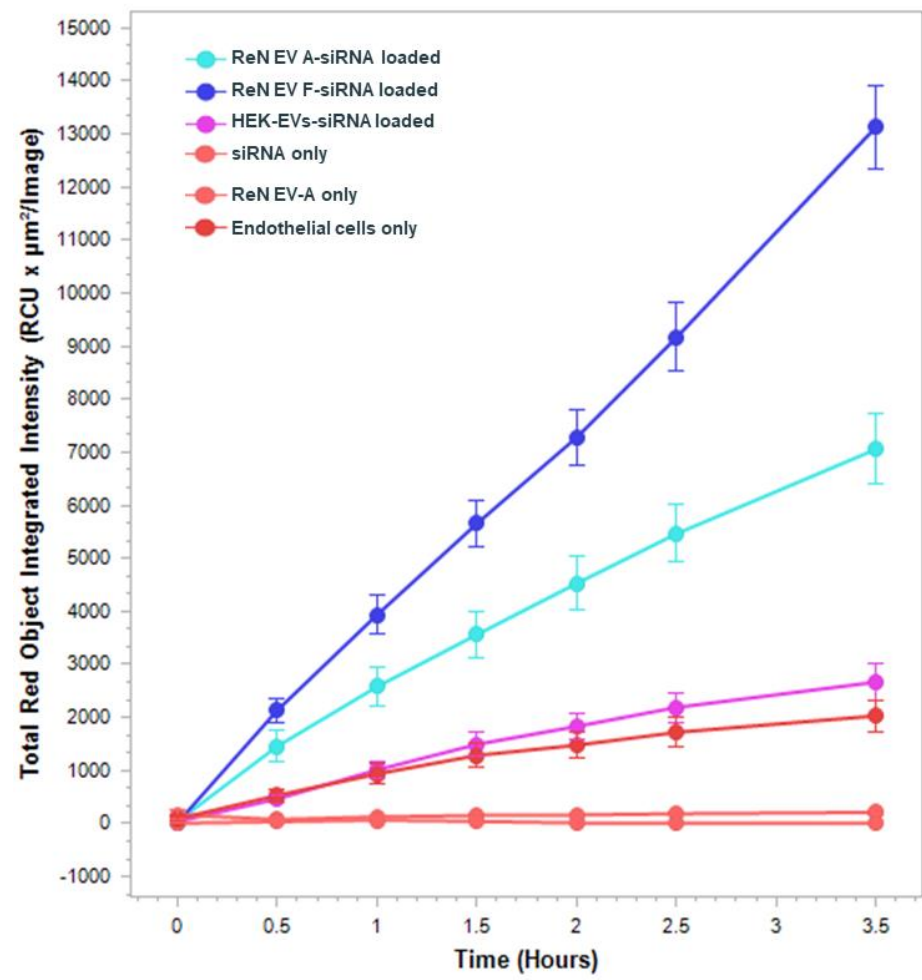
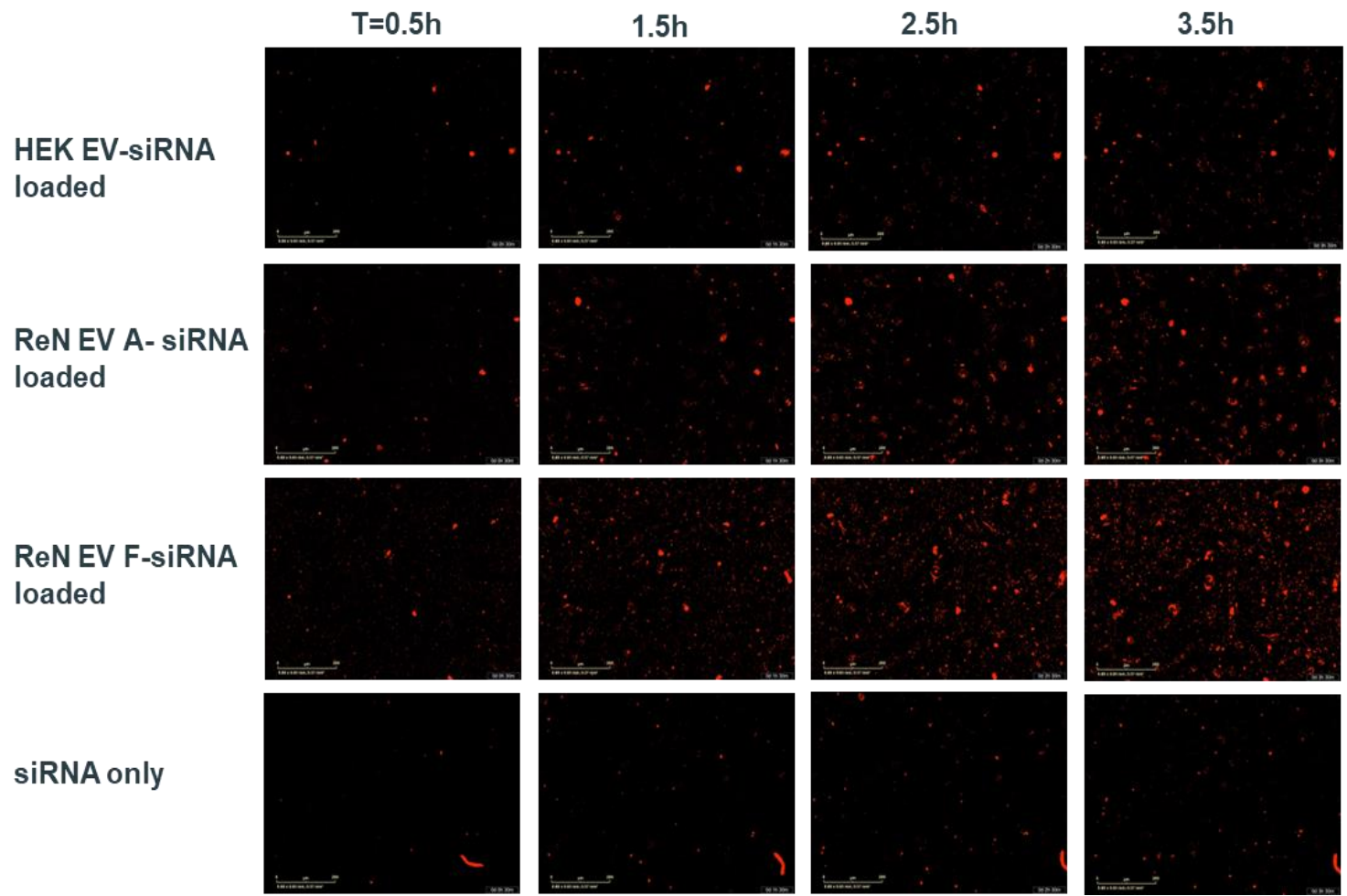


Delivery of fluorescently labelled siRNA loaded onto different EV types. The greater the level of EV uptake (left), the greater the delivery of siRNA visualised by pre-nuclear fluorescence.





Clear advantage over self delivering siRNA only



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 type of origin
 - Unique proteomic and lipidomic profiles
 - Not all EVs are the same
- CustomEx™ - a proprietary stem cell-based EV drug delivery platform that can be optimised for specific targets and payloads using different producer cell types and engineering tools
- EV producer cell line selection is an important consideration when developing a targeted DDS
- For a greater chance of success

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ReNeuron

Pencoed Business Park | Pencoed

Bridgend | CF35 5HY | UK

T +44 (0) 203 819 8400 | E info@reneuron.com

www.reneuron.com

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