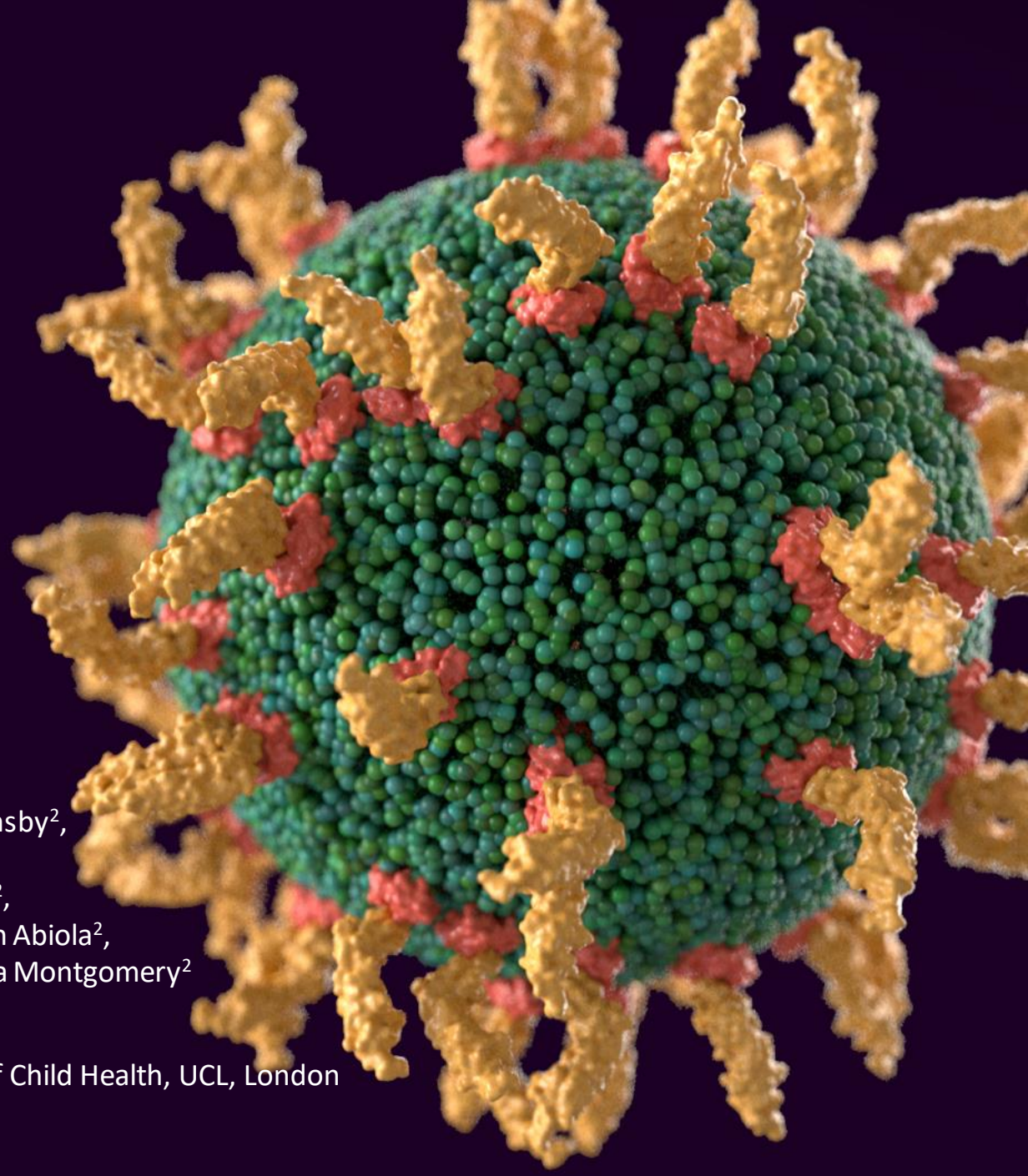
# Using Novel Intein Technology to Deliver Exosome-Based Therapeutics




Justin Hean[2], Claire Pearce[2], Pijus Brazauskas[2], Charlie de Havilland[2], Charlotte Stainsby[2], Maria Jacintha Victoria[2], Thaine Mayes[2], Zoe Rees[2], Xiuna Yang[2], Jorge Salazar[2], Trang Mai[2], Vrinda Sreekumar[2], Gianmaria Vincenzi[2], Luke Haslett[2], Stefania Fedele[2], Yi Yang[2], James Veale[2], Daria Farcas[2], Andrew Christie[2], Jacqueline Vieira[2], Olusegun Abiola[2], Tania Selas-Castineiras[2], Dany Perocheau[1], Sonam Gurung[1], Julien Baruteau[1], Sonya Montgomery[2]

**Affiliations:**

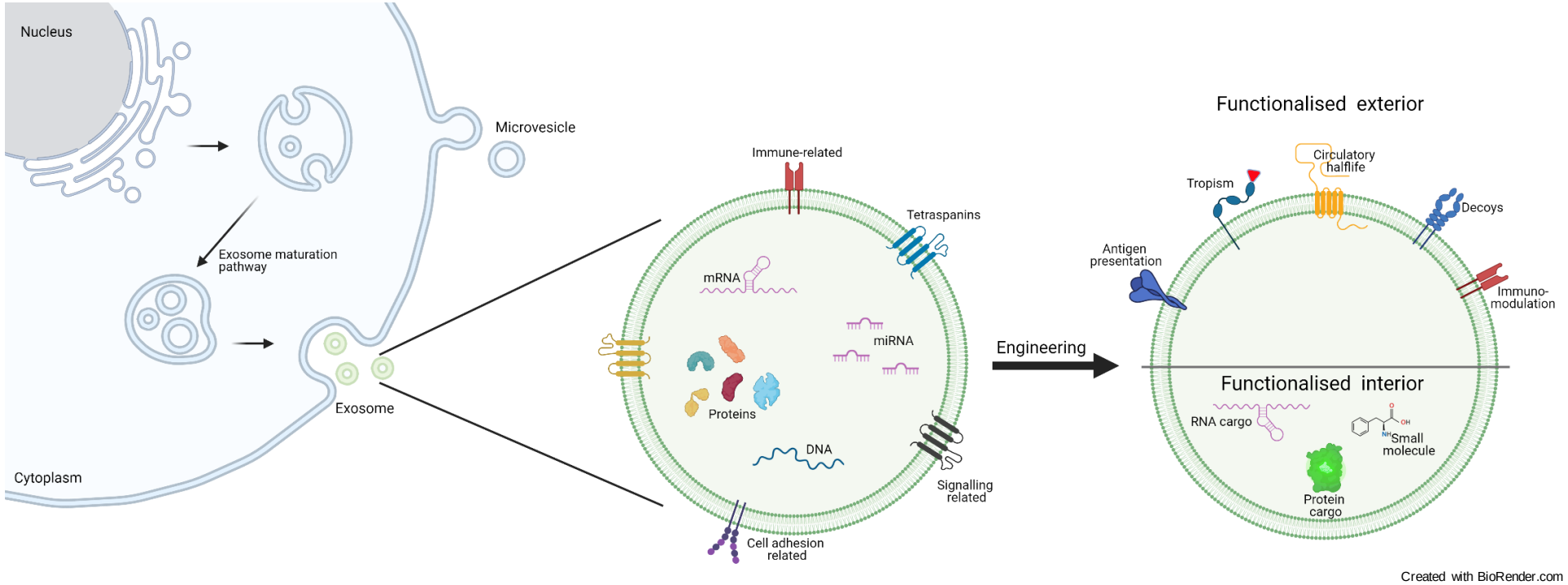
1. Genetics and Genomic Medicine Programme, Great Ormond Street Institute of Child Health, UCL, London
2. Evox Therapeutics, Oxford



This work was supported by a grant from Innovate UK

# Exosomes – a new therapeutic modality

## Introduction





### Summary

Exosomes are a novel class of promising biologics for the delivery of therapeutics

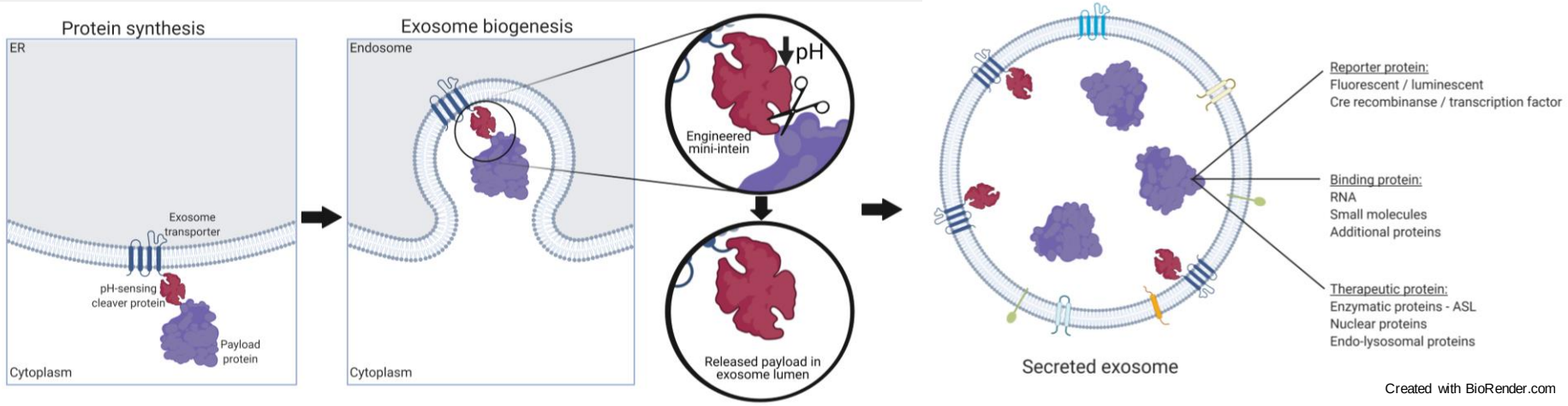
Naturally secreted by nearly all cells, can contain a variety of cargos, including proteins and nucleic acids

Exosome therapeutics are non-immunogenic

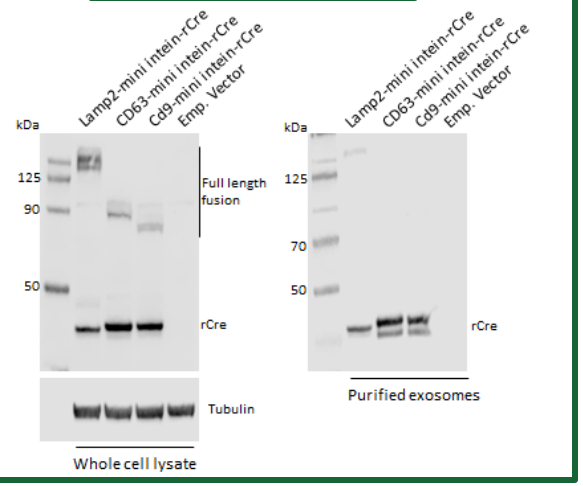
Platform for combinatorial biologic engineering

# Exosome engineering strategy

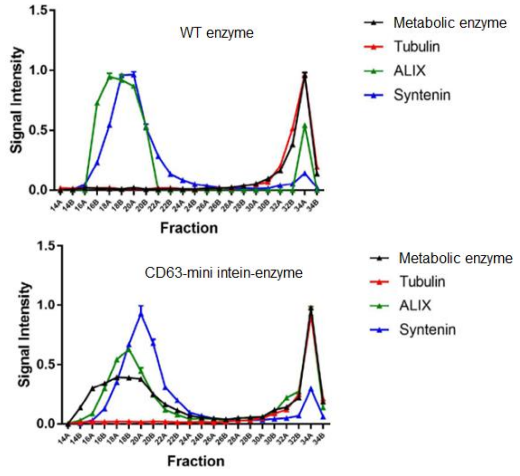
## Proof of concept: Loading of soluble enzymes into exosome lumen



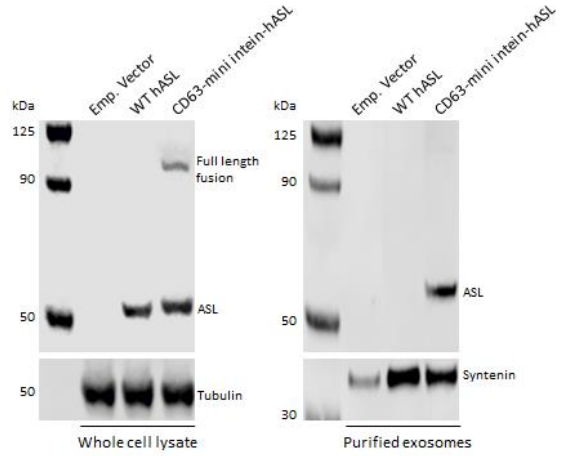

### Concept 1: rCre




### Concept 2: Metabolic enzyme




### Concept 3: ASL




### Summary

By including a pH-sensing mini intein module, the active payload is released from its exosome transporter following loading

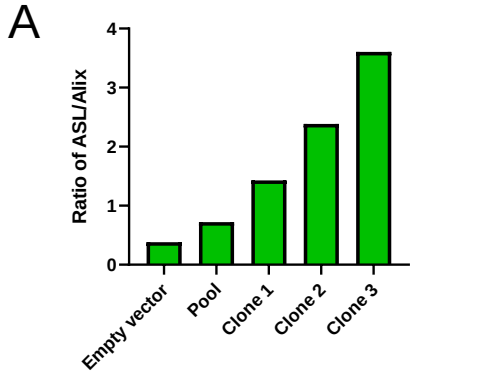
A variety of payload proteins have been successfully loaded, including:
1) The DNA modifying enzyme, Cre recombinase
2) An undisclosed metabolic enzyme
3) Cytoplasmic urea cycle enzyme argininosuccinate lyase

# From Proof-of-Concept to Functional Product

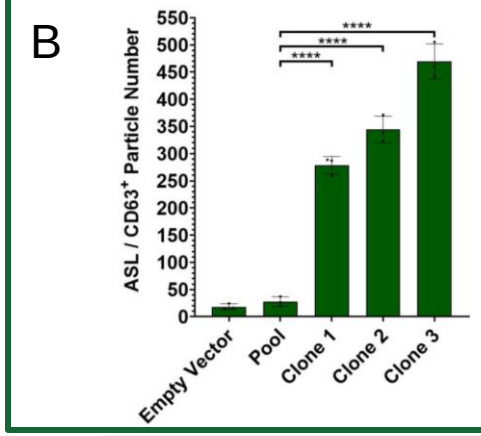
## Cell Line Development and Analytical

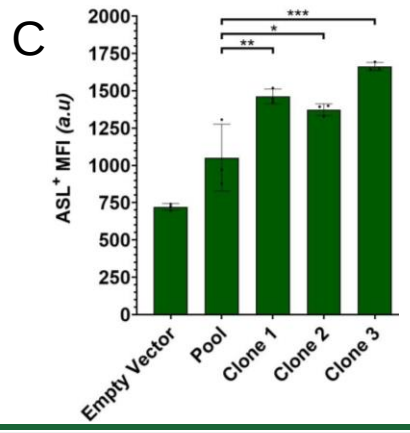
### ASL EV loading

CD63 enriched EV lysate western blot



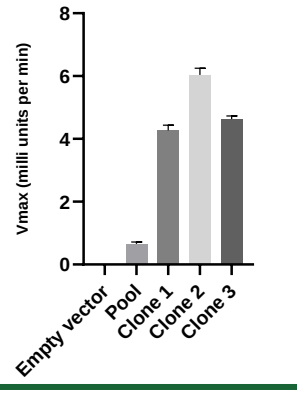

Intraluminal ExoView – ASL+CD63+ EV number and intraluminal ASL MFI



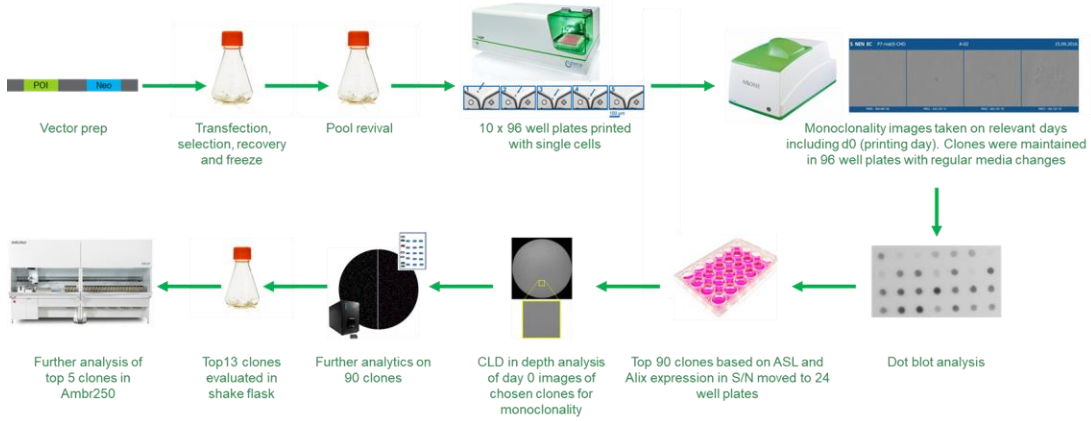




### ASL activity

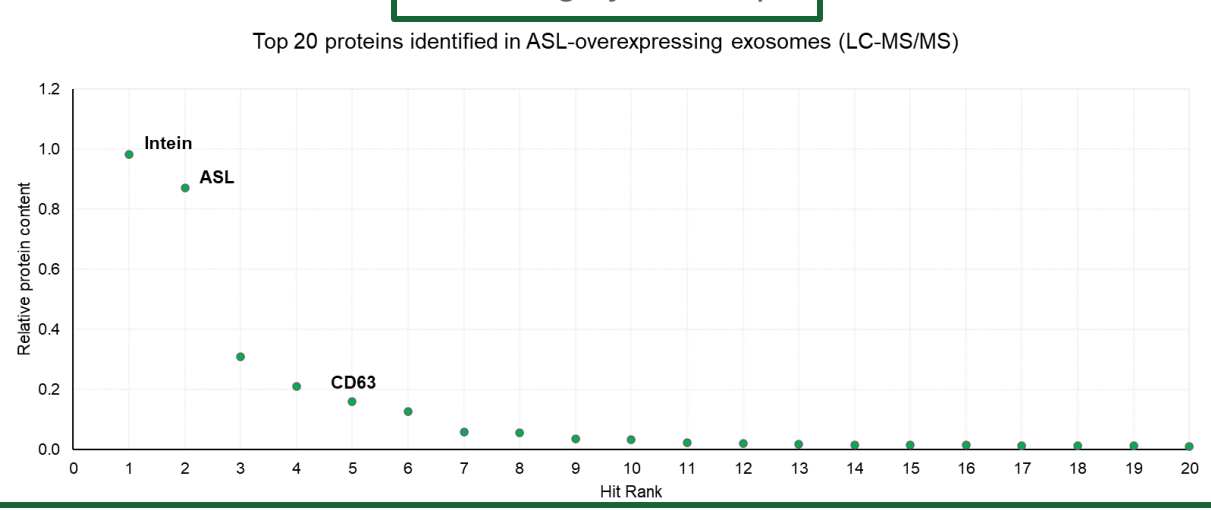
A240 assay




### CLD Workflow




### EV loading by mass spec




### Summary

CLD campaign yielded clones enriched for ASL enzyme loading into EVs, subsequently shown to be functionally active

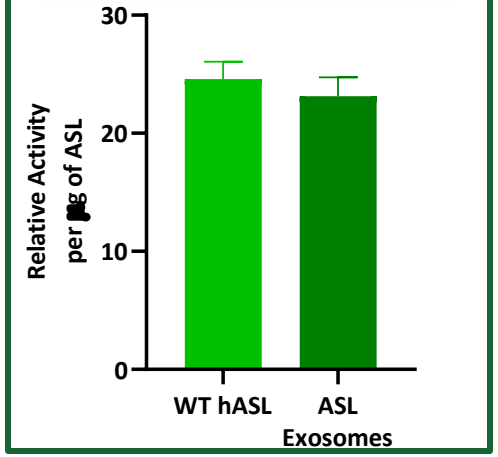
# Translation into a therapeutic

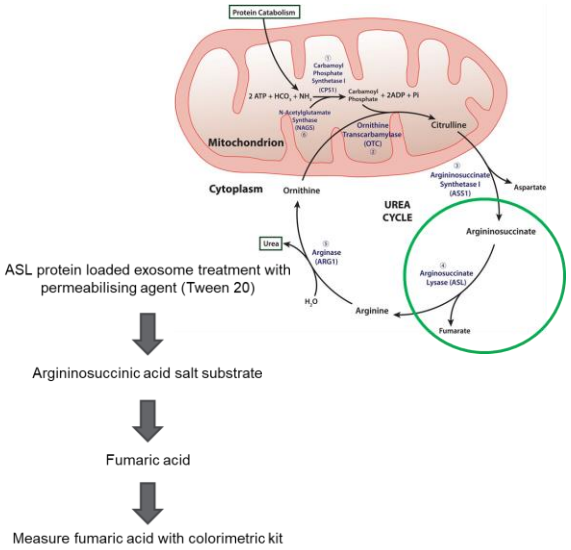
*In vitro* & *in vivo* proof of concept in the urea cycle disorder Argininosuccinic aciduria (ASA)

## ASA: an inborn error of metabolism

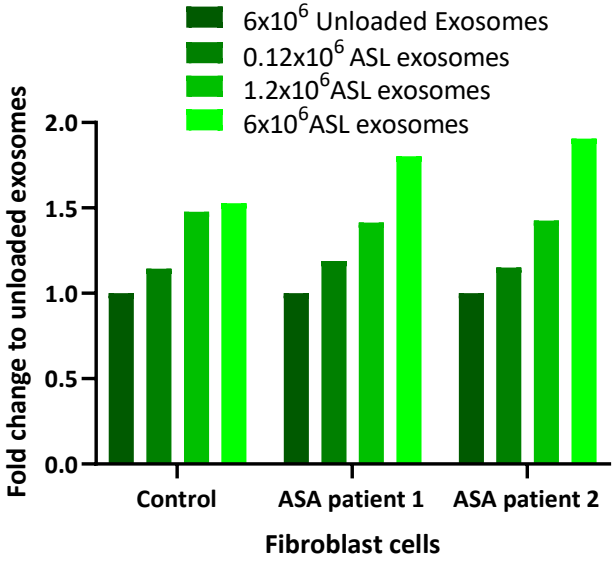
- A rare autosomal recessive life-threatening disorder caused by a mutation in the Argininosuccinate lyase (ASL) gene
- ASL enzyme deficiency results in elevated levels of ammonia & a defective nitric oxide pathway
- Long-term complications include liver dysfunction and neurocognitive deficits

## ASL Enzyme activity by fumarate production

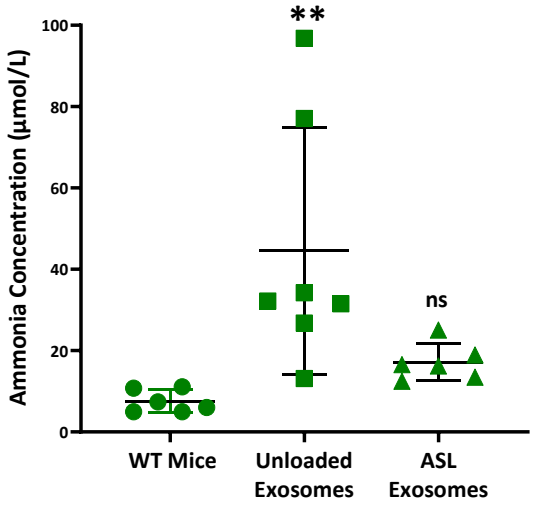



## *In vitro* PoC demonstrating hASL expression after 24 hours




## *In vivo* PoC in ASA hypomorphic mouse model




## Summary

- ASL loaded exosomes demonstrate dose responsive hASL expression in patient fibroblasts
- Functional activity of ASL enzyme post-intein cleavage is comparable to wild type human ASL
- ASL exosomes reduce ammonia in the ASA hypomorphic mouse model