Circular RNA expression systems for enhanced gene and cell therapies

Dr Erik D Wiklund, CEO

RNA Leaders Congress
Basel, 5 March 2025

1

circRNA introduction

2. circVec platform
3. circVec therapeutic development

Human circRNA was first described by Circio scientists

Dr Thomas B Hansen

Dr Erik D Wiklund

nature — 7,400 citations

Published: 27 February 2013

Natural RNA circles function as efficient microRNA sponges

Thomas B. Hansen, Trine I. Jensen, Bettina H. Clausen, Jesper B. Bramsen, Bente Finsen, Christian K. Damgaard & Jørgen Kjems

THE EMBO JOURNAL — EMBOpress — 30 September 2011 — 1,000 citations

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miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA

Thomas B Hansen, Erik D Wiklund, Jesper B Bramsen, Sune B Villadsen, Aaron L Statham, Susan J Clark, Jørgen Kjems

nature reviews genetics — January 2025

Review Article | Published: 09 January 2025

The therapeutic potential of circular RNAs

Eoghan O'Leary, Yanyi Jiang, Lasse S. Kristensen, Thomas B. Hansen & Jørgen Kjems

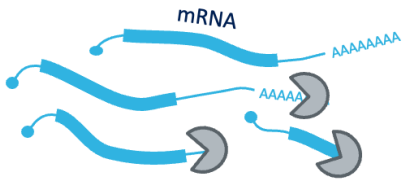
Nature Reviews Genetics (2025) | Cite this article

circio

circRNA increases durability and expression level to enhance the potency of nucleic acid medicines

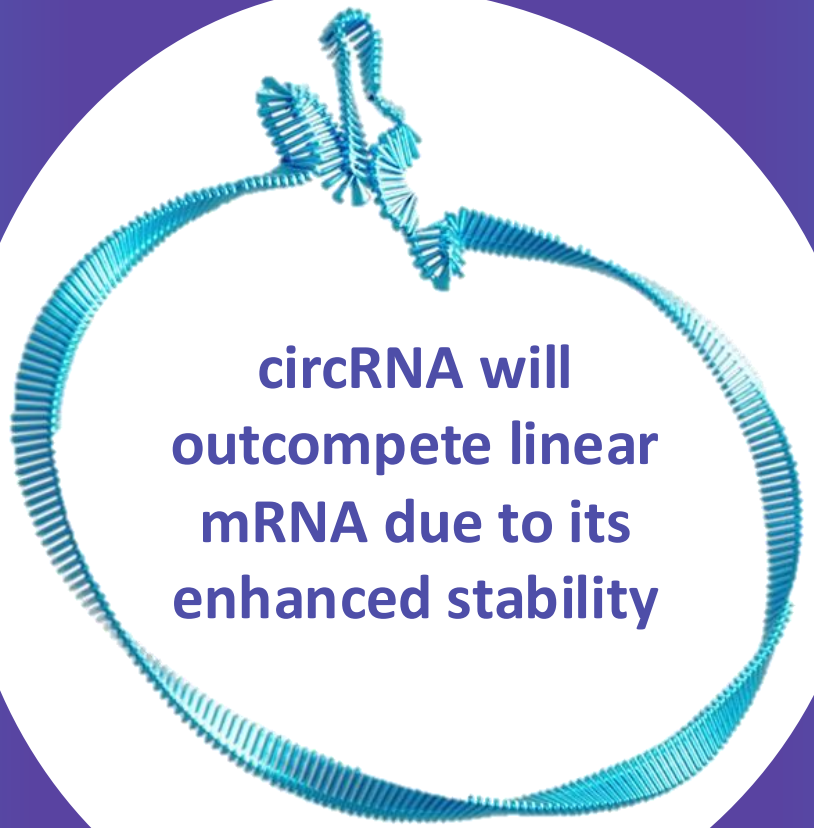
Extended RNA durability

70x half-life vs. mRNA in vivo



Higher protein expression

15x protein expression vs. mRNA in vivo



circRNA will outcompete linear mRNA due to its enhanced stability

Reduced toxicity

Dose sparing for improved safety

Modular & multi-functional

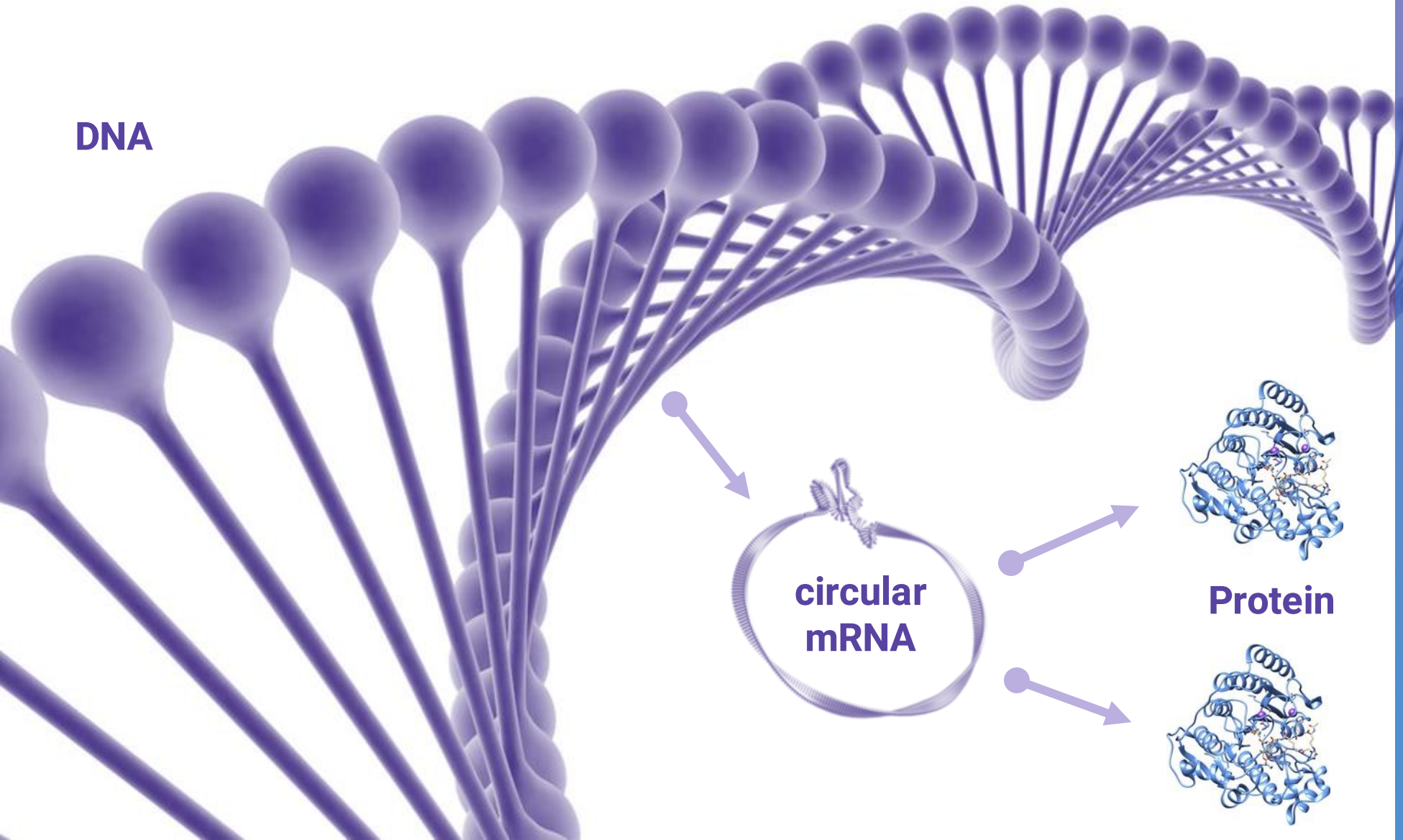
Addt´l ´remove & replace´ MoA

2

circVec platform

3. circVec therapeutic development

The unique circVec expression system:
Turning the patient´s cells into circRNA factories



circVec
DNA or viral
vector

Inject

circRNA
biogenesis

Potent and durable protein expression

circio

The circVec platform is technologically differentiated and creates novel opportunities for circRNA

		Expression durability	*Main opportunity in vaccines*	*Suitable for gene therapy*	*Delivery system*	*Existing CDMO manu-facturing*
circio	**circVec vector approach**	**months to years**	✓ VLP-format	✓	Viral or DNA-LNP	✓
ORNA, SAIL BIOMEDICINES	**Synthetic circRNA**	7-10 days	✓	✗	circRNA-LNP	✗
moderna, BIONTECH	**Synthetic mRNA**	2-3 days	✓	✗	mRNA-LNP	✓

Source: Circio analysis

circVec substantially outperforms the expression level and durability of mRNA-based systems in cells

Increased expression level

Prolonged durability

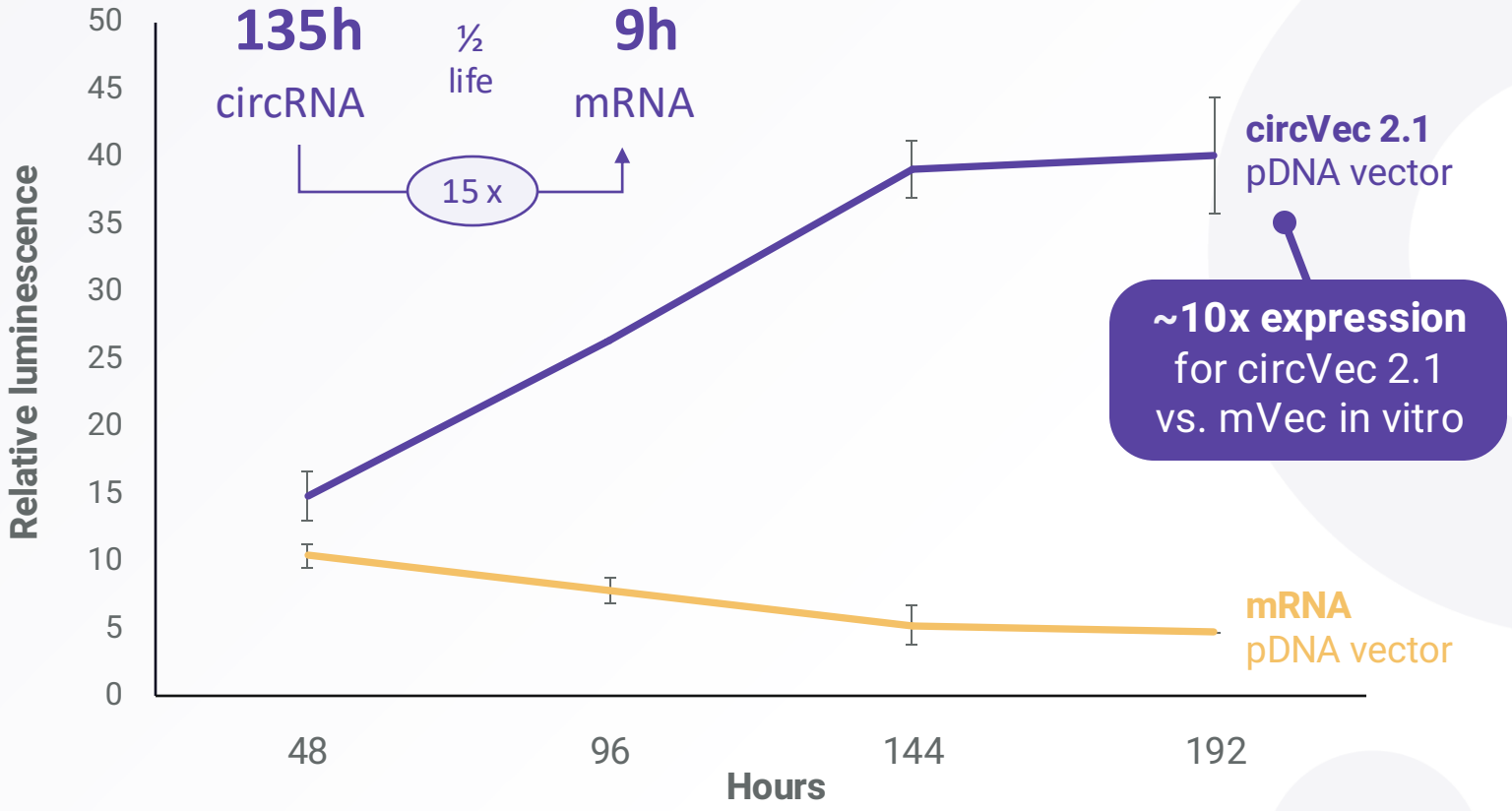
→ Enhanced therapeutics

"Due to its significant advantages, circRNA systems can be expected to replace mRNA-based expression for DNA format therapeutics in the future – just as synthetic circRNA can be expected to replace current mRNA formats"

Dr. Alex Wesselhoeft

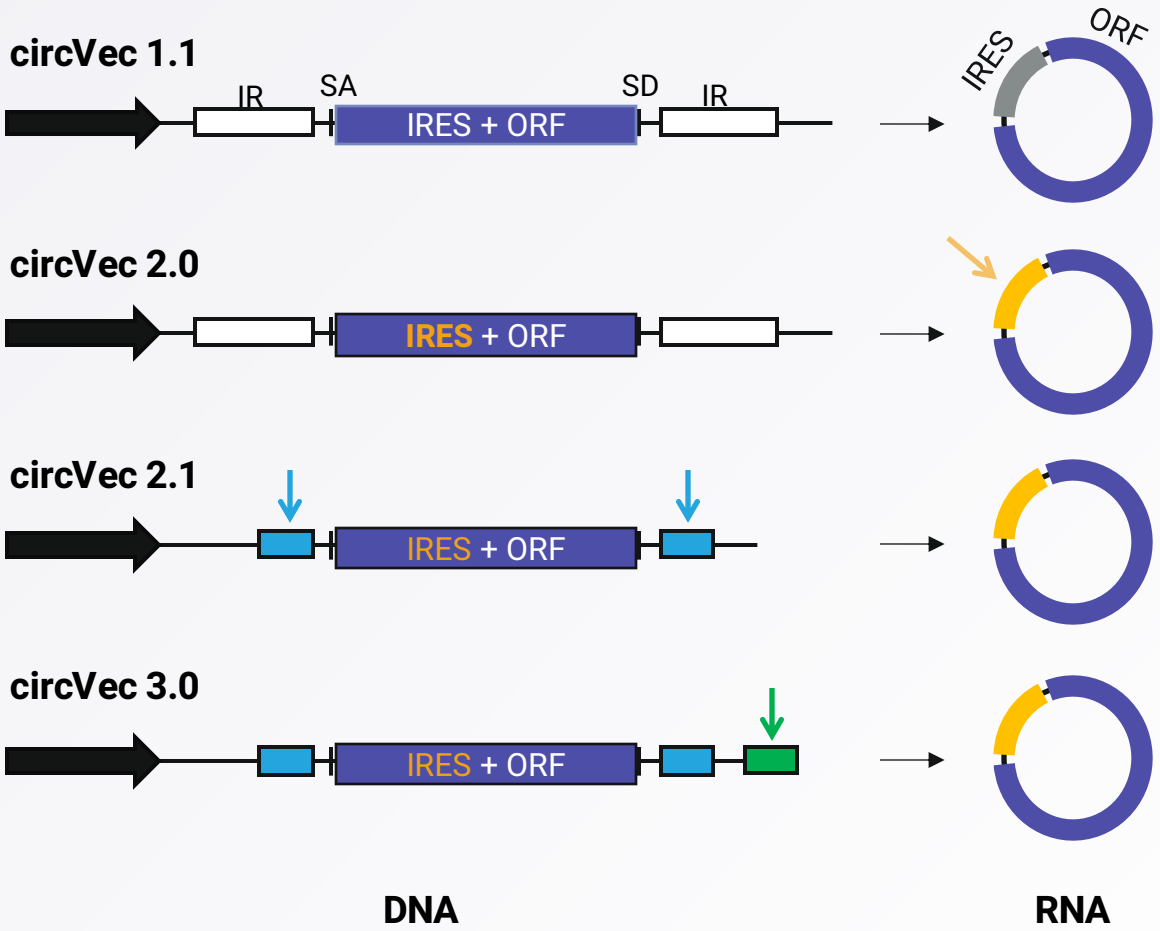
Scientific founder
oRNA Therapeutics

circVec 2.1 vs. mVec (mRNA) luciferase reporter expression; in vitro

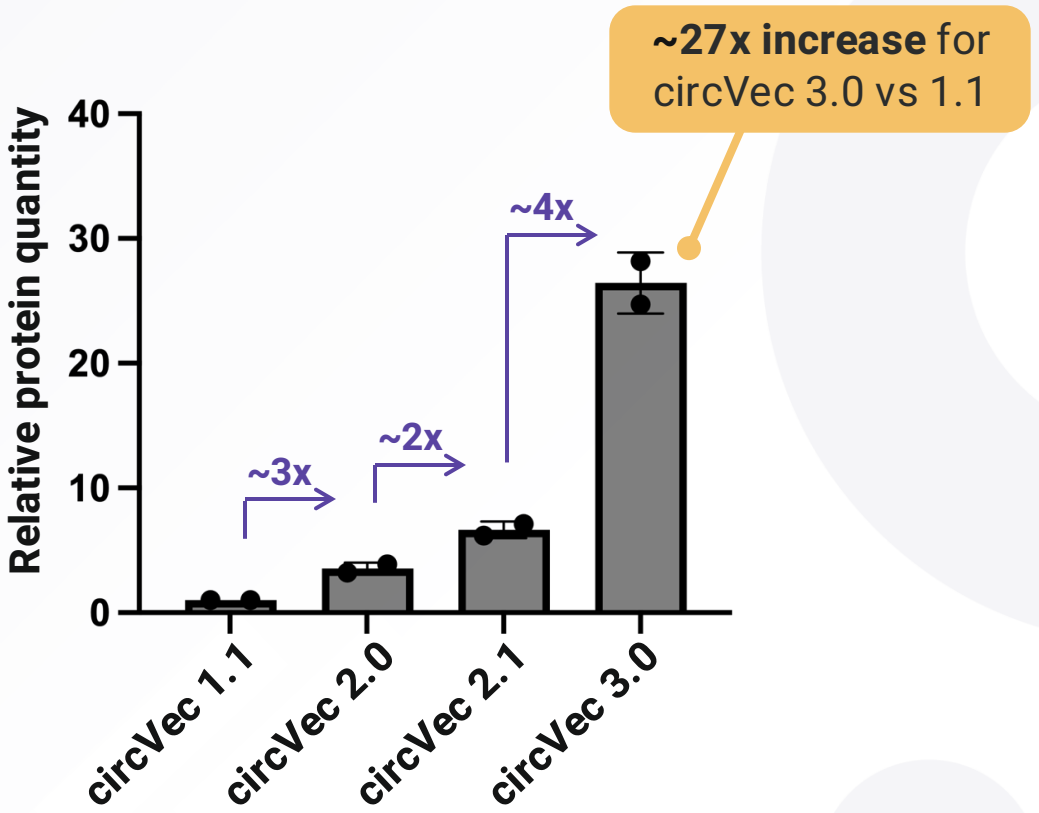


New and enhanced circVec 3.0 in current testing

circVec generation 1.X – 3.X, design schematics



circVec protein quantification, Western blot



circVec 2.1 achieves > 6 month expression durability on one single injection in immuno-competent mouse muscle

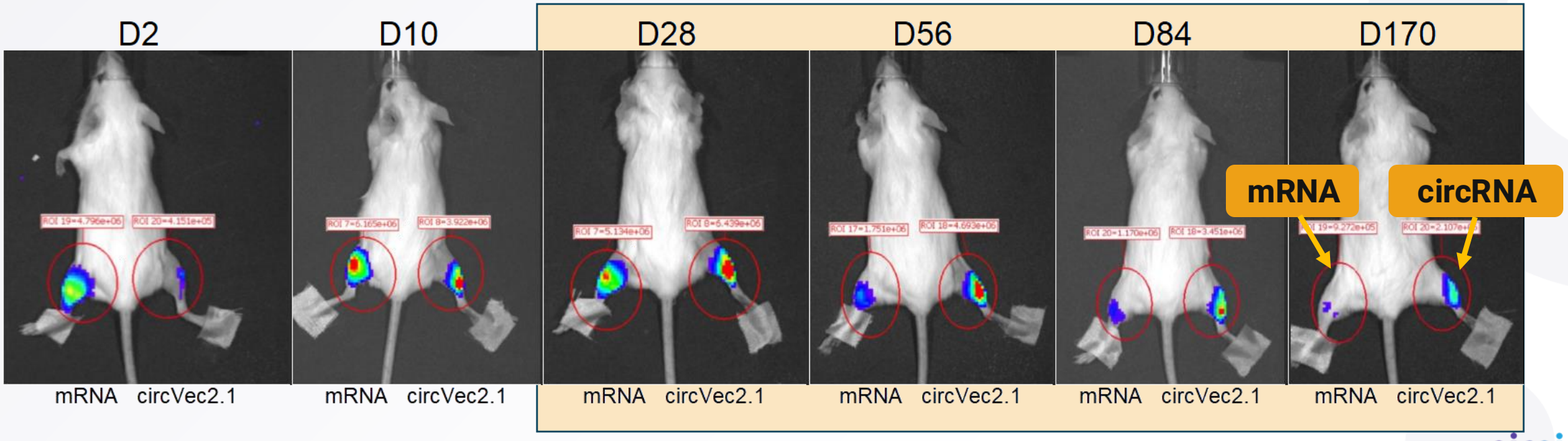
BALB/c
Immuno-competent mice

mRNA-vector-*Luciferase*
Left hindleg

circVec 2.1-*Luciferase*
Right hindleg

Intra-muscular injection
3 dose levels

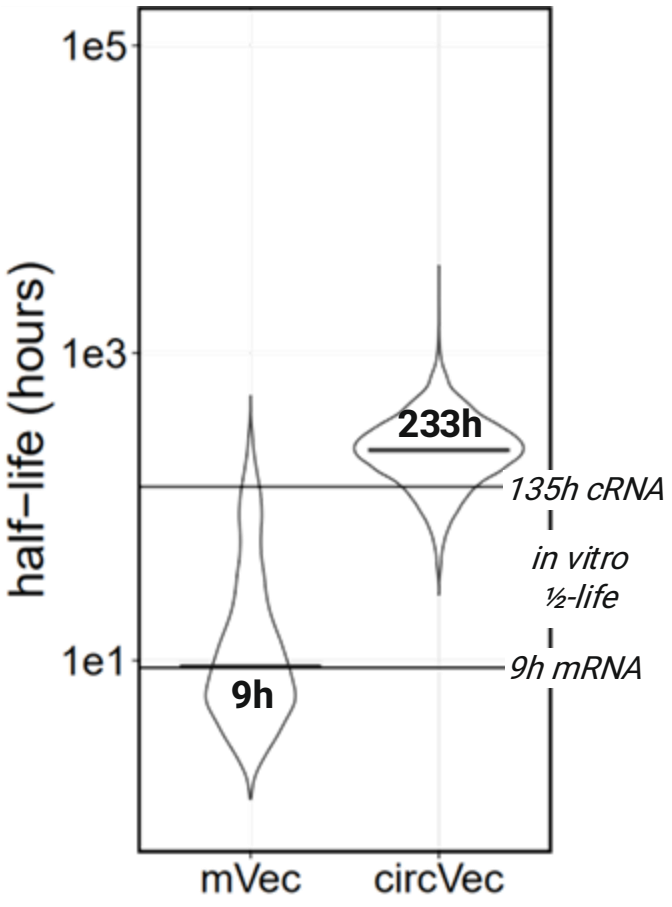
Monitor expression over time



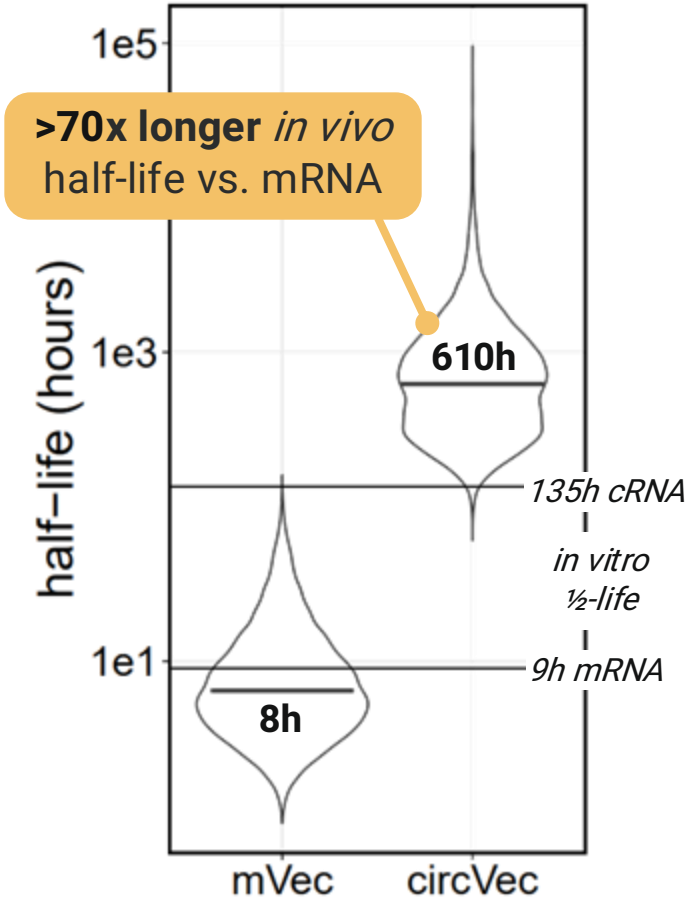
Low dose example animal

Bioinformatic analysis of circVec 2.1 in vivo data indicates over 70 times increased half-life of circRNA vs. mRNA

Inferred in vivo RNA half-life (hours), bioinformatic modelling

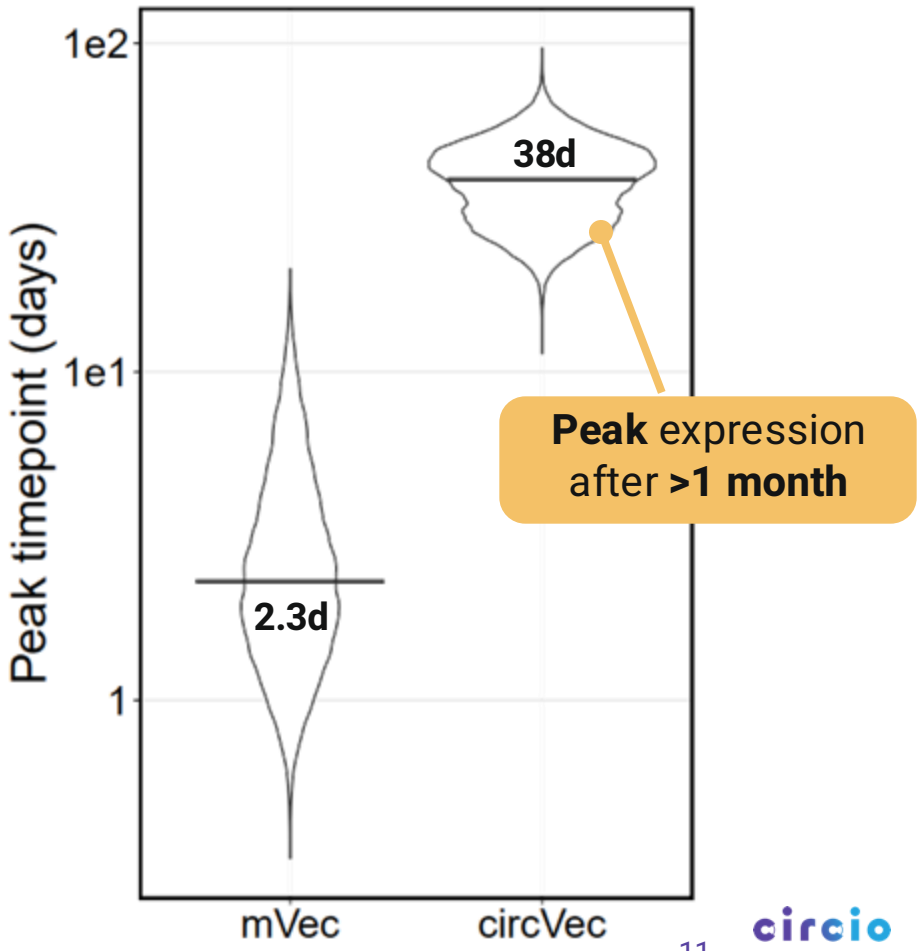


Immuno-compromised mice



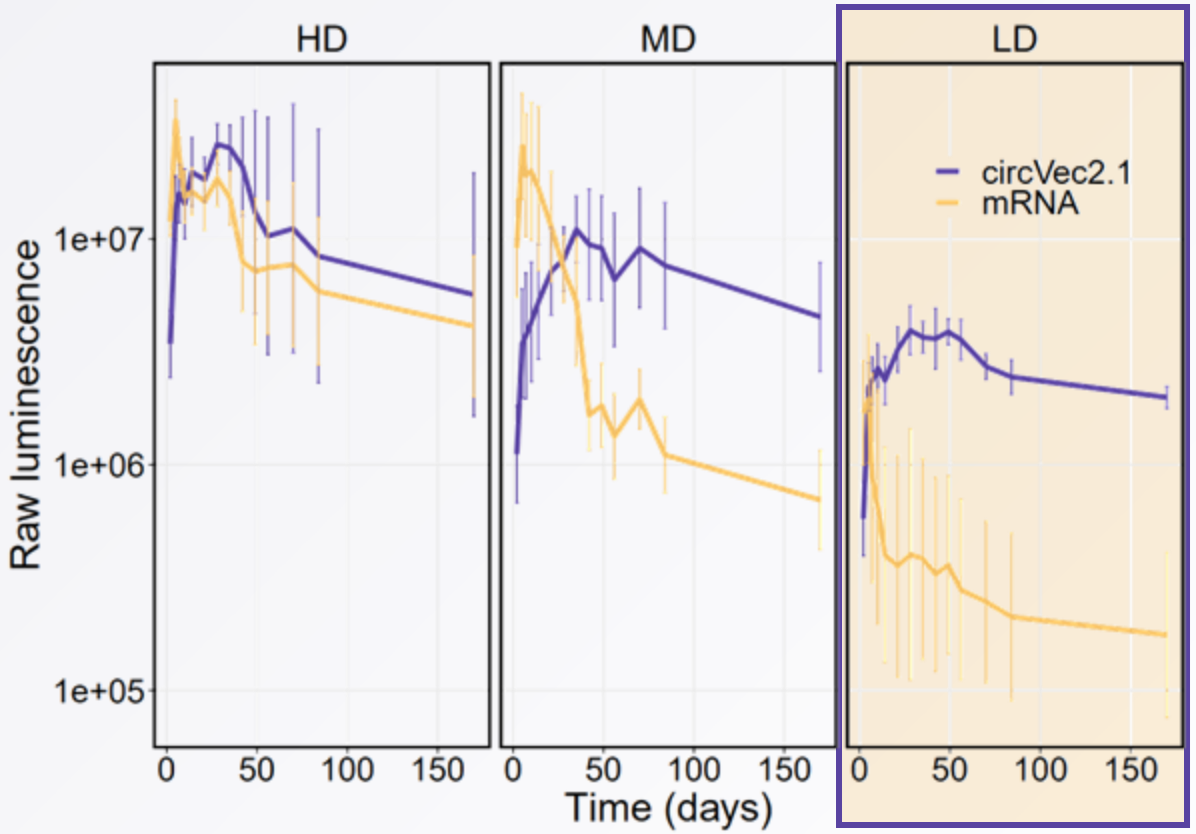
Immuno-competent mice

Inferred in vivo peak expression (days)

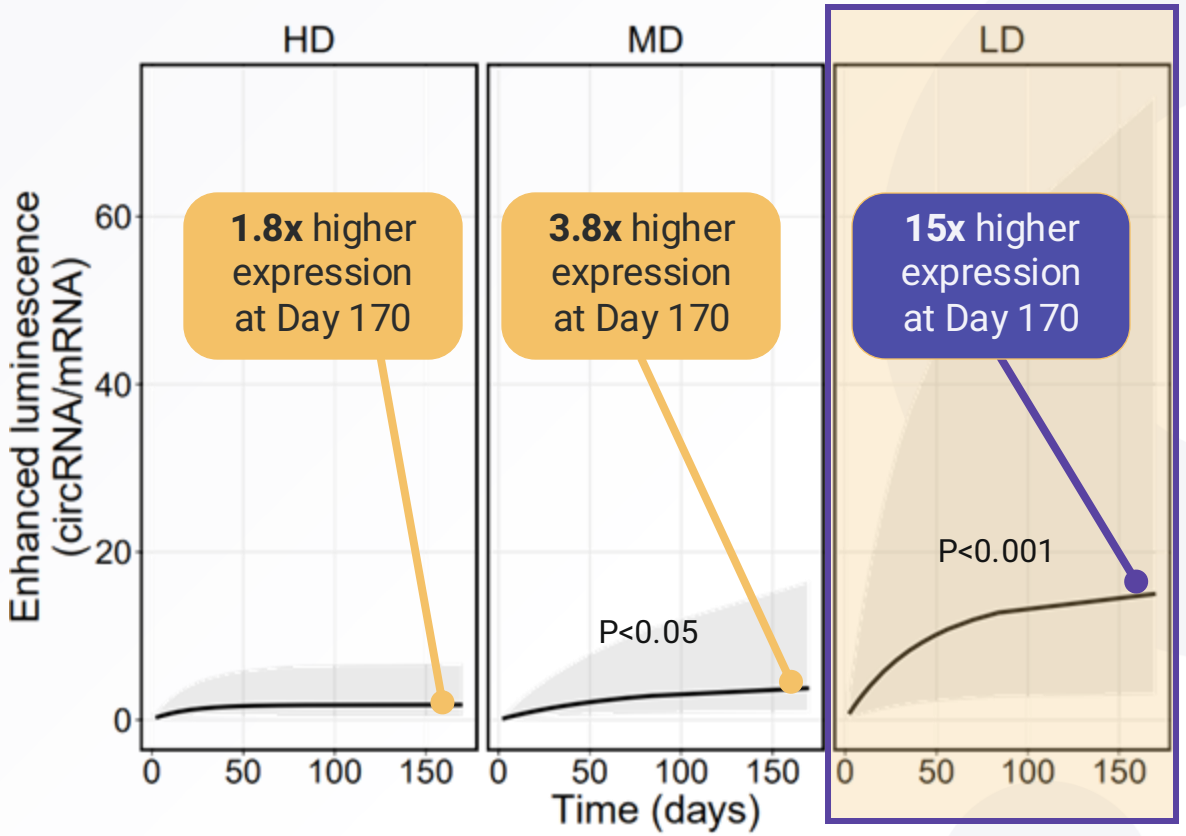


circVec 2.1 dose response in vivo - strongest advantage vs. mRNA observed at low dose, high therapeutic relevance

Absolute expression (luminescence)
circVec 2.1 vs. mRNA pDNA vector expression



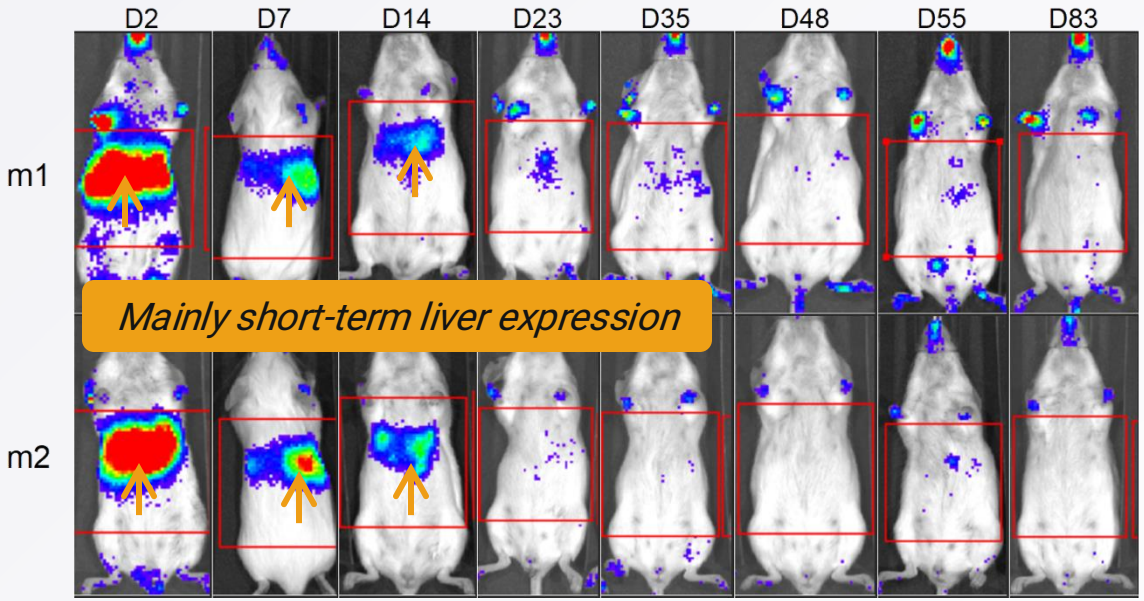
Relative expression (luminescence)
-fold change circVec 2.1 vs. mRNA expression



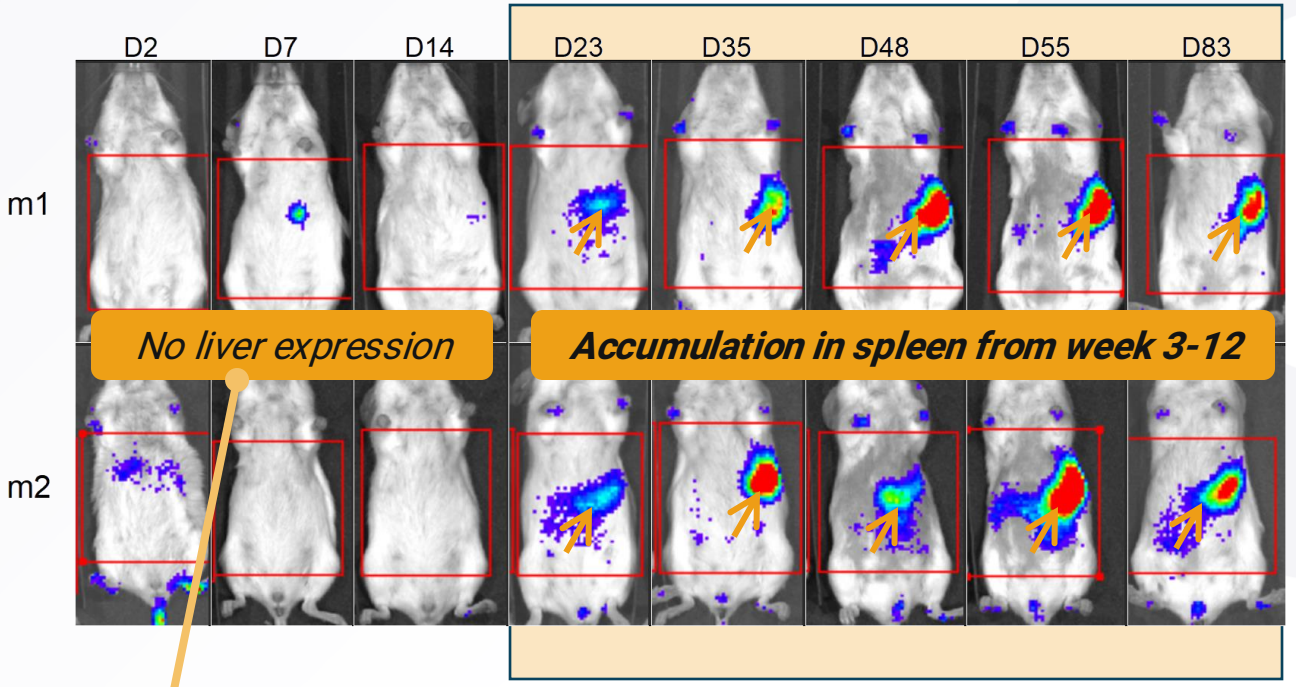
HD: High dose MD: Mid dose LD: Low dose

LNP-formulated circVec 2.1 accumulates in spleen with >12 week durability, minimizes liver expression

LNP-mVec (mRNA), luminescence
Systemic I.V. delivery, single dose on Day 0



LNP-circVec 2.1 (circRNA), luminescence
Systemic I.V. delivery, single dose on Day 0



circRNA durability adv. does not apply in liver

3

circVec therapeutic development

The circVec platform can be deployed in multiple disease areas and therapeutic settings

Lead program, in house focus

Gene therapy

Genetic muscular dystrophies

Enhanced and durable expression

´Remove & Replace´ concept

AAV and DNA format

Next step / partnership

Cell therapy

Auto-immune disease

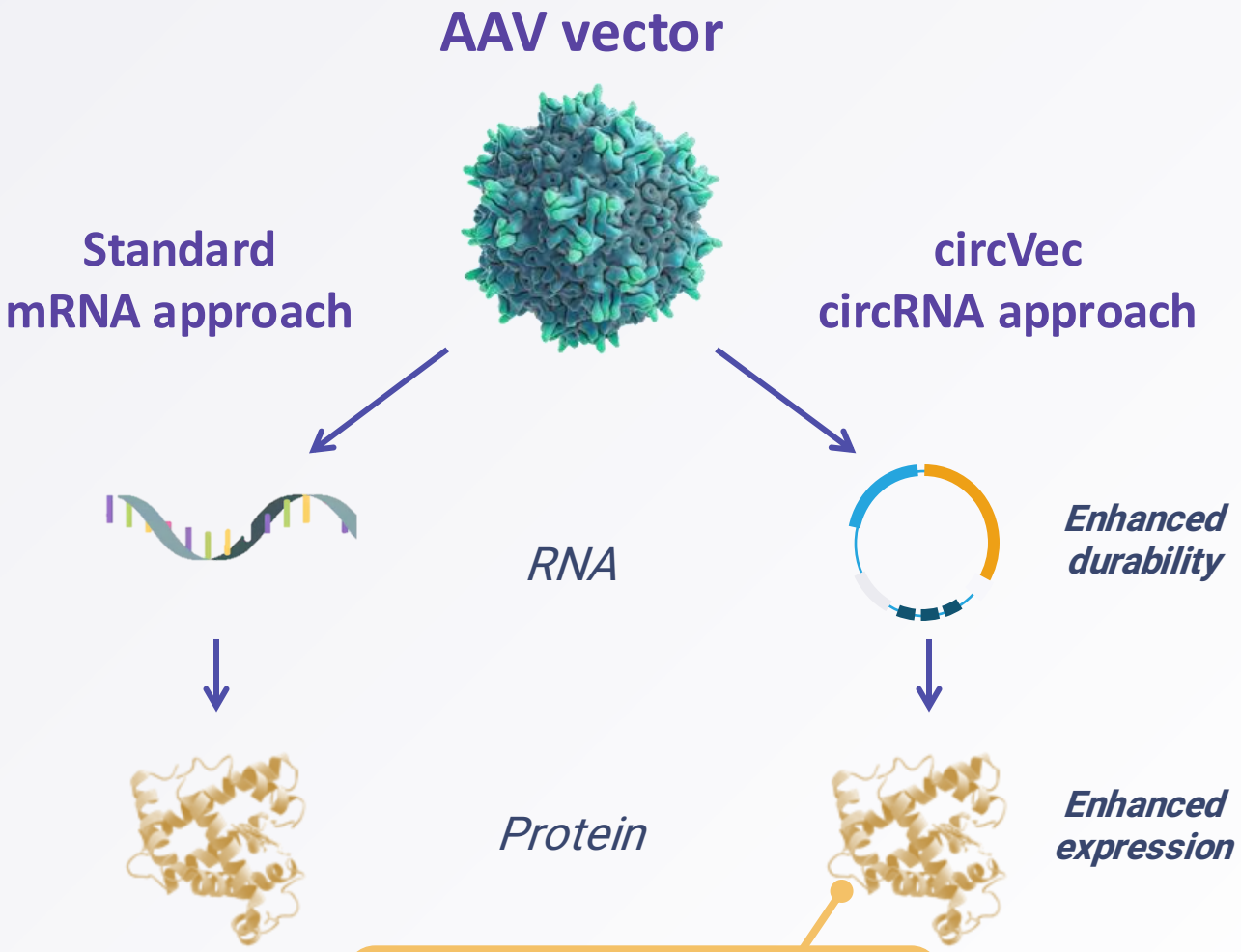
Non-integrating transduction

In vivo and ex vivo approach

DNA and VLP format

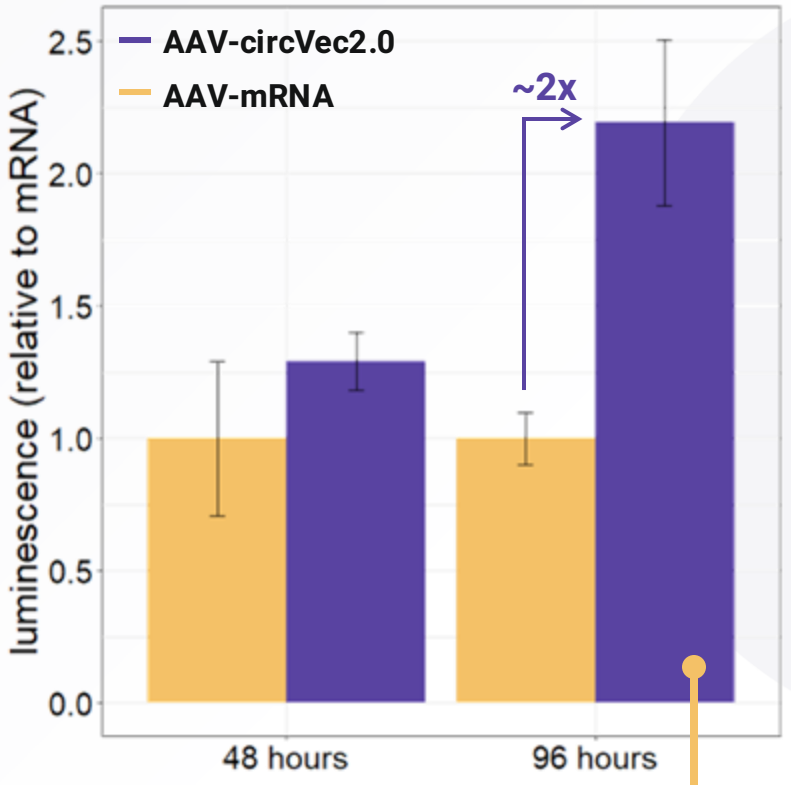
Target, therapeutic format and disease to be prioritized based on data from ongoing in vivo program

circVec is a potentially disruptive novel expression technology for AAV gene therapy



Aim: at least 5x enhanced potency vs. mRNA-AAV

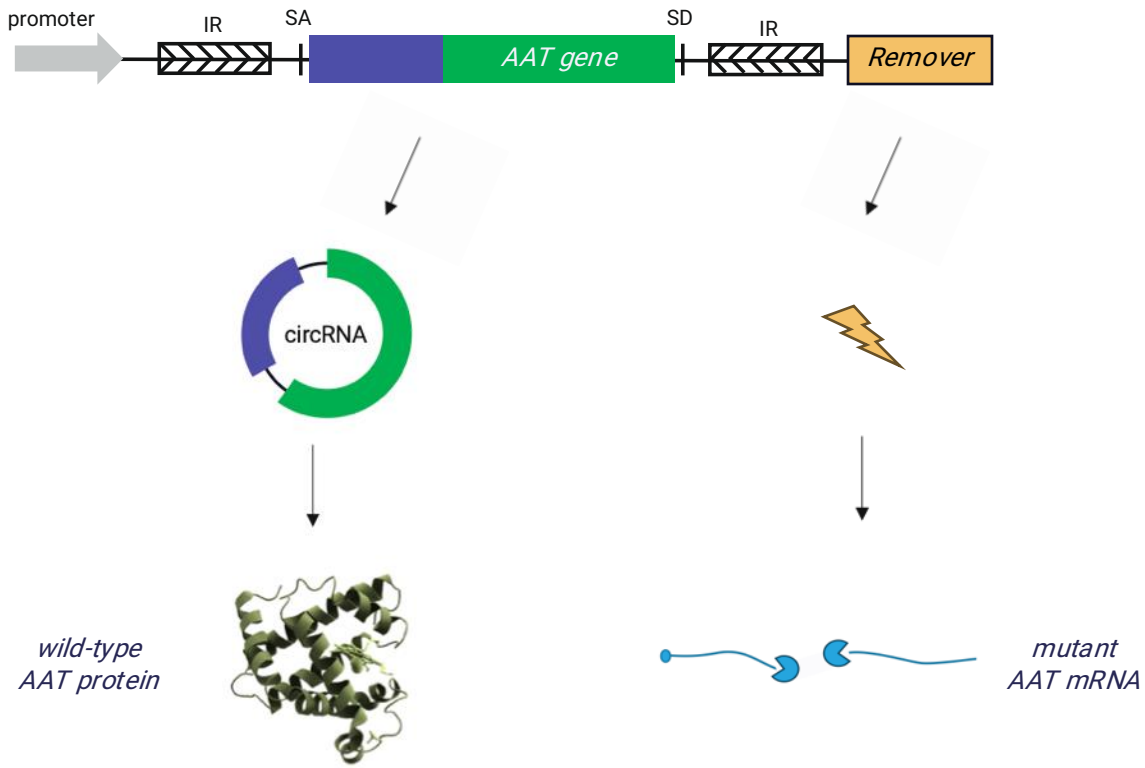
AAV protein expression, *in vitro* f-Luc



circVec-AAV feasibility validated, testing and optimization of constructs ongoing

circVec ´Remove-&-Replace´ gene therapy concept, AATD case example

circVec AATD R&R design



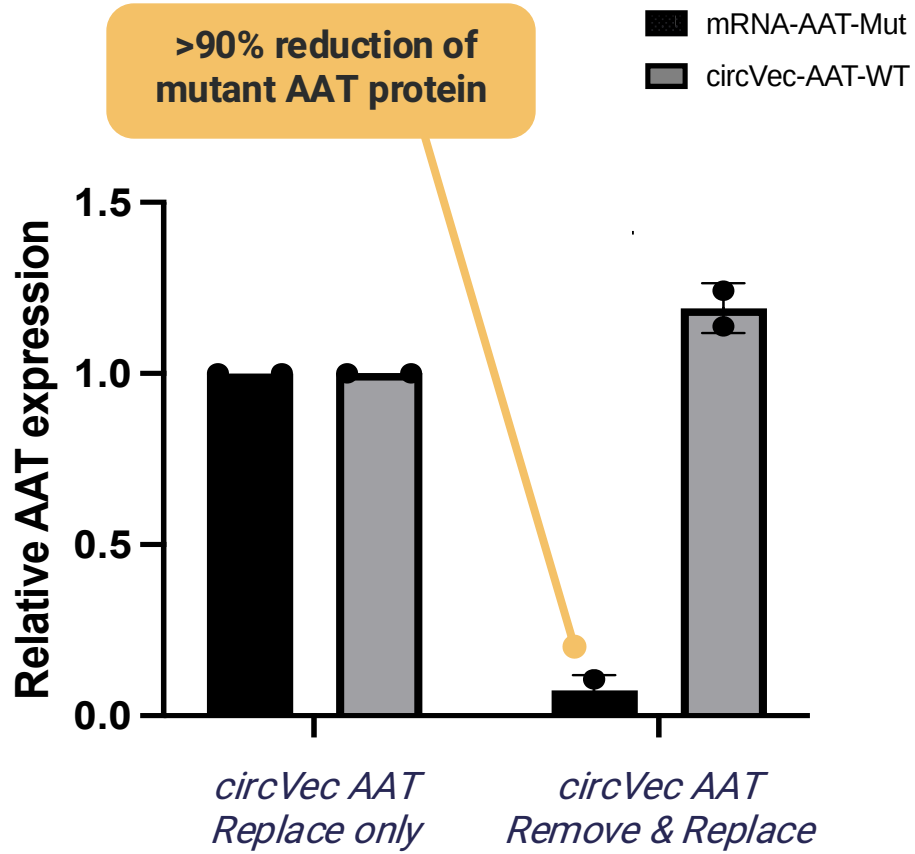
Replace

Expression of functional AAT protein

Remove

Degradation of mutant AAT mRNA

Remove-&-replace *in vitro* PoC



Circio is the leader in DNA-format circular mRNA, take-home messages:

Durability

The circular mRNA format offers **70x durability and 15x protein expression advantage** over conventional linear mRNA-based expression → **further improvements ongoing**

Versatility

circVec can be deployed in any **AAV or DNA format** therapeutic → **lead program in muscular dystrophies**

Platform potential

circVec is a powerful and protected circRNA expression **platform** with **opportunities in multiple disease areas**

"Due to its significant advantages, circRNA systems can be expected to replace mRNA-based expression for DNA format therapeutics in the future – just as synthetic circRNA can be expected to replace current mRNA formats"

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