circio

Enhancing AAV gene therapy through circular RNA-based gene expression

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Circular RNA–based gene expression: an improved format for vector-based therapeutics

circular RNA

- Naturally occurring
- Formed by back-splicing
- Resistant to degradation
- Engineerable & versatile

In vivo gene expression:

mRNA

½-life ~10hrs

VS.

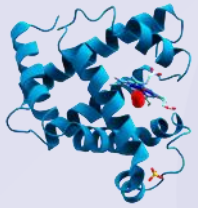
circRNA

???

½-life >600hrs

Circio has developed a novel circular RNA-based alternative to the central dogma of molecular biology

The circVec gene expression platform:

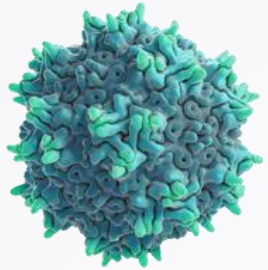


DNA → circular RNA → Protein

- **circVec** is a platform technology for vector-based gene delivery
- **circVec** enables enhanced and prolonged gene expression
- **Circio** has unique IP & know-how in circRNA gene expression

Deploying circVec to enhance AAV gene therapy

AAV vector

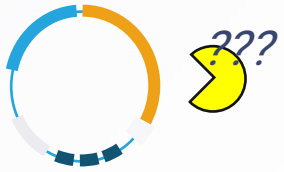


mVec – conventional mRNA approach

RNA

Protein

circVec – novel circRNA approach

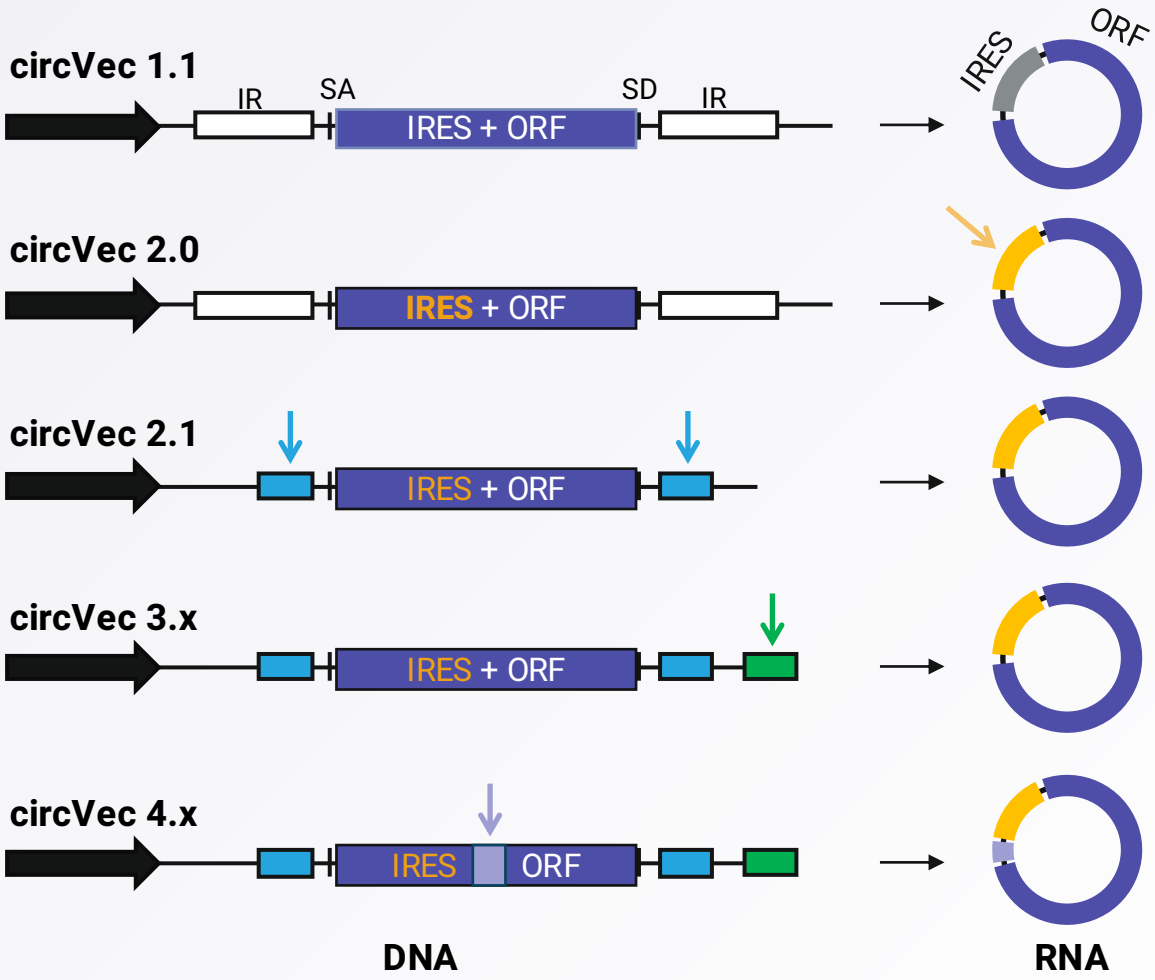


75x prolonged half-life vs. mRNA

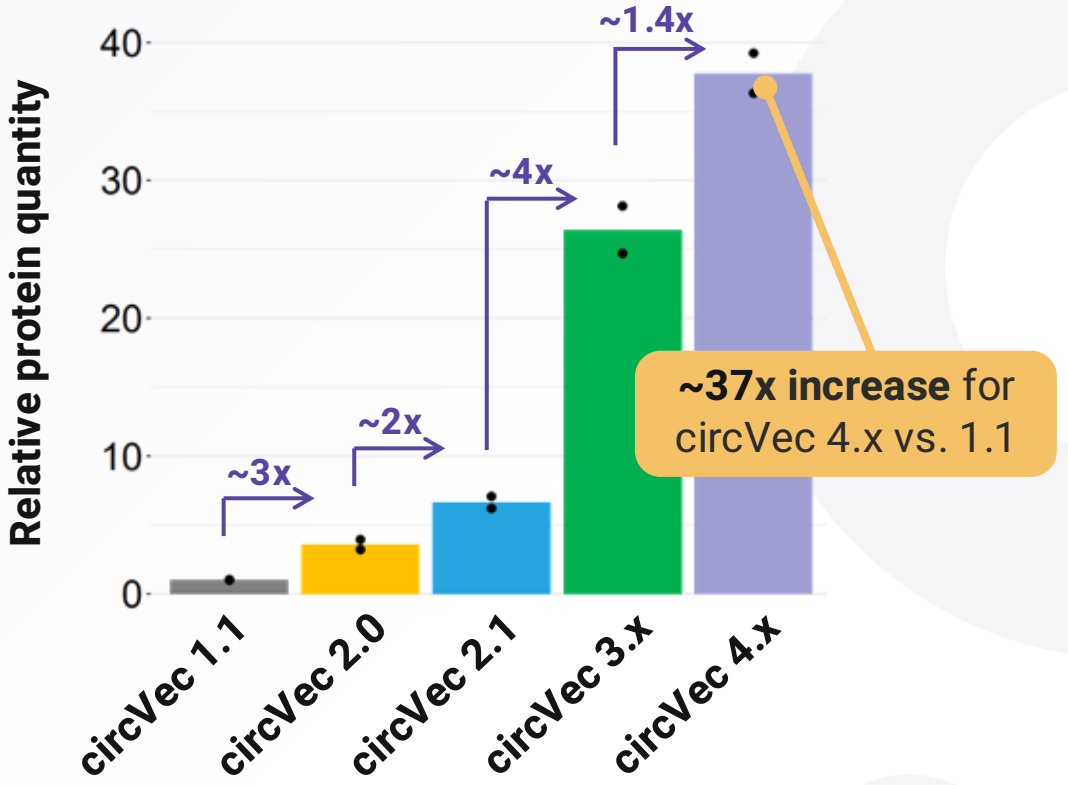
Up to 50x enhanced protein expression

circVec construct design and evolution from Gen 1 → 4

circVec generation 1.X – 4.X, design schematics

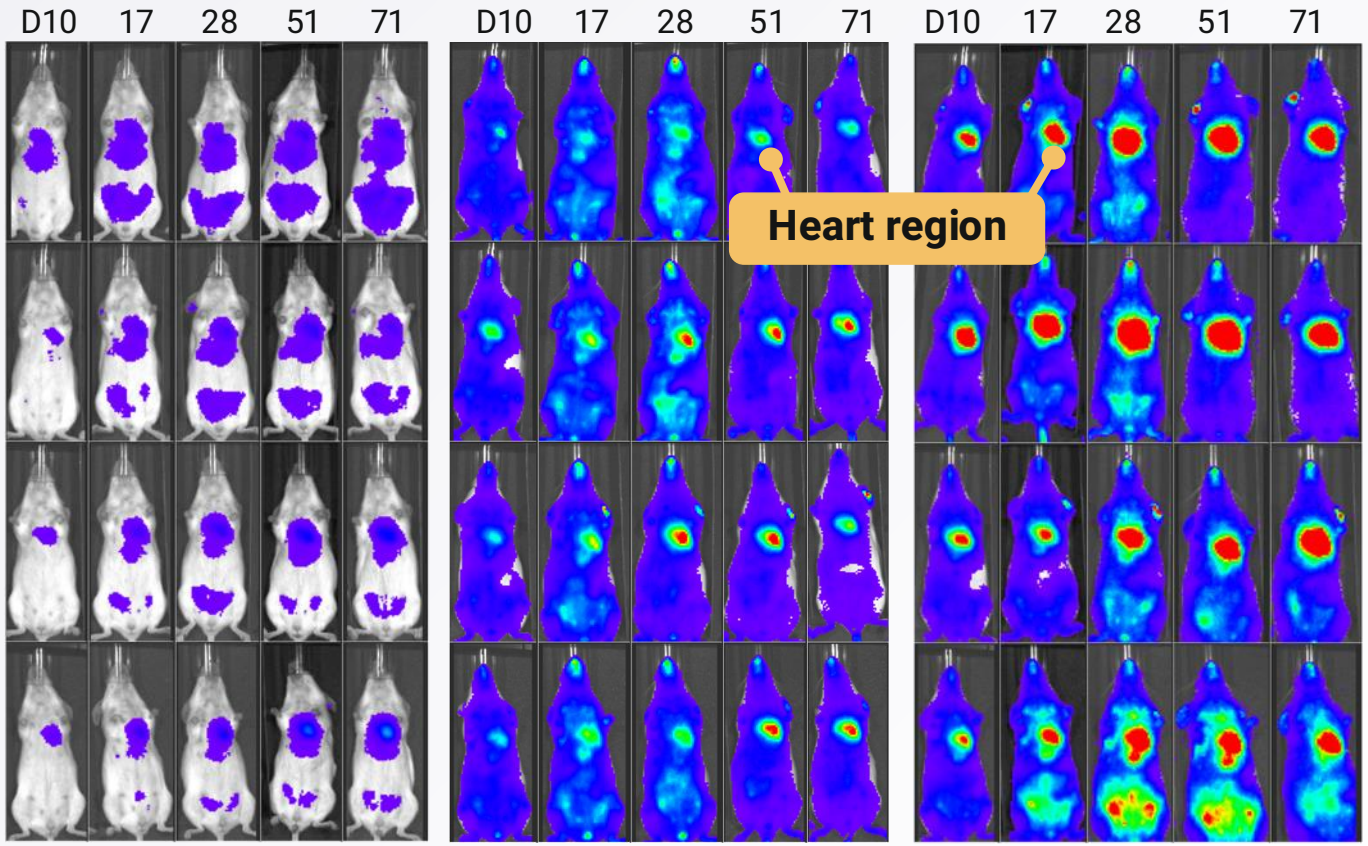


circVec protein quantification, Western blot

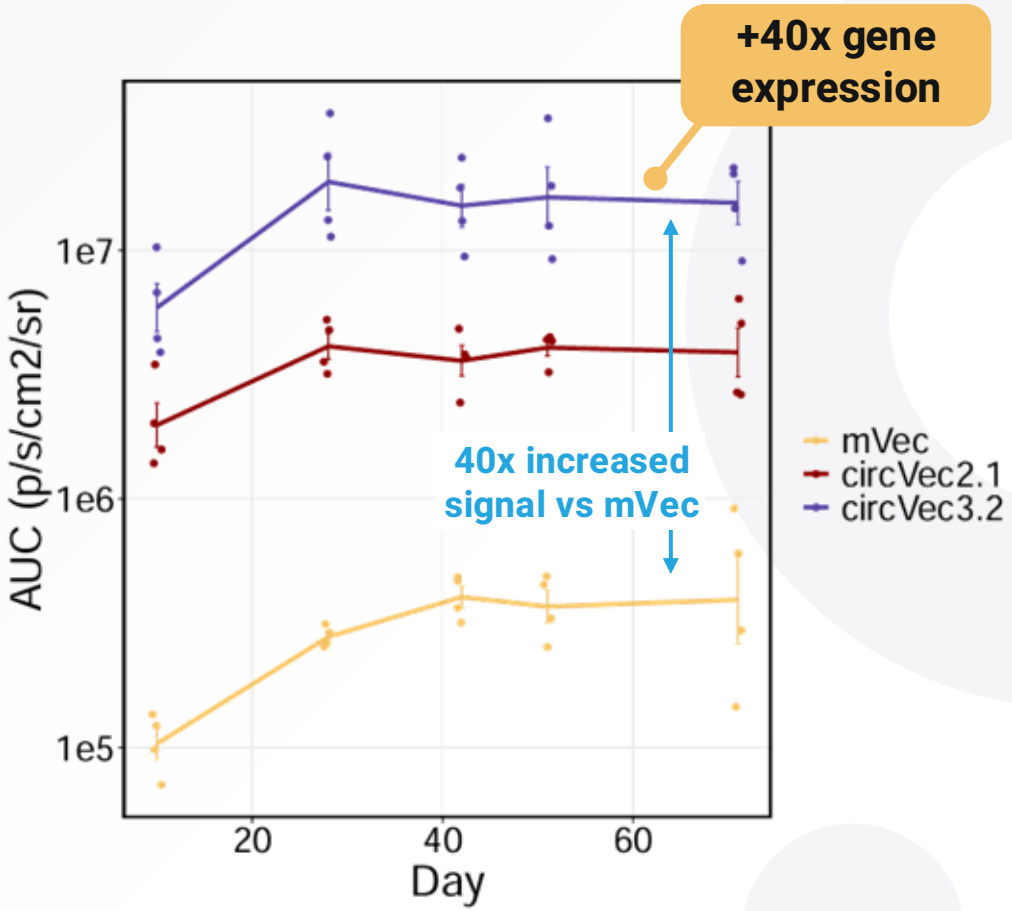


40-fold enhanced expression in heart for circVec-AAV vs. conventional mRNA-based AAV

AAV-mVec | **AAV-circVec 2.1** | **AAV-circVec 3.2**



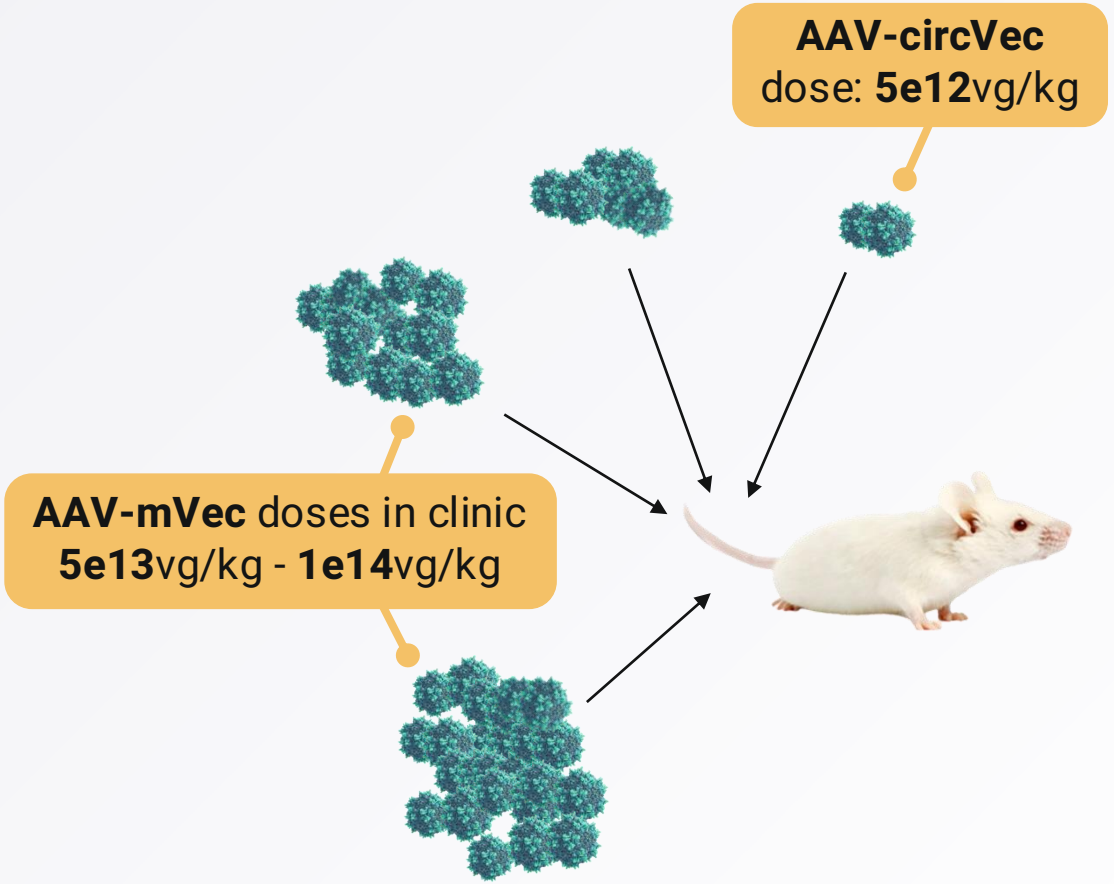
Gene expression quantification, f-luc IVIS signal



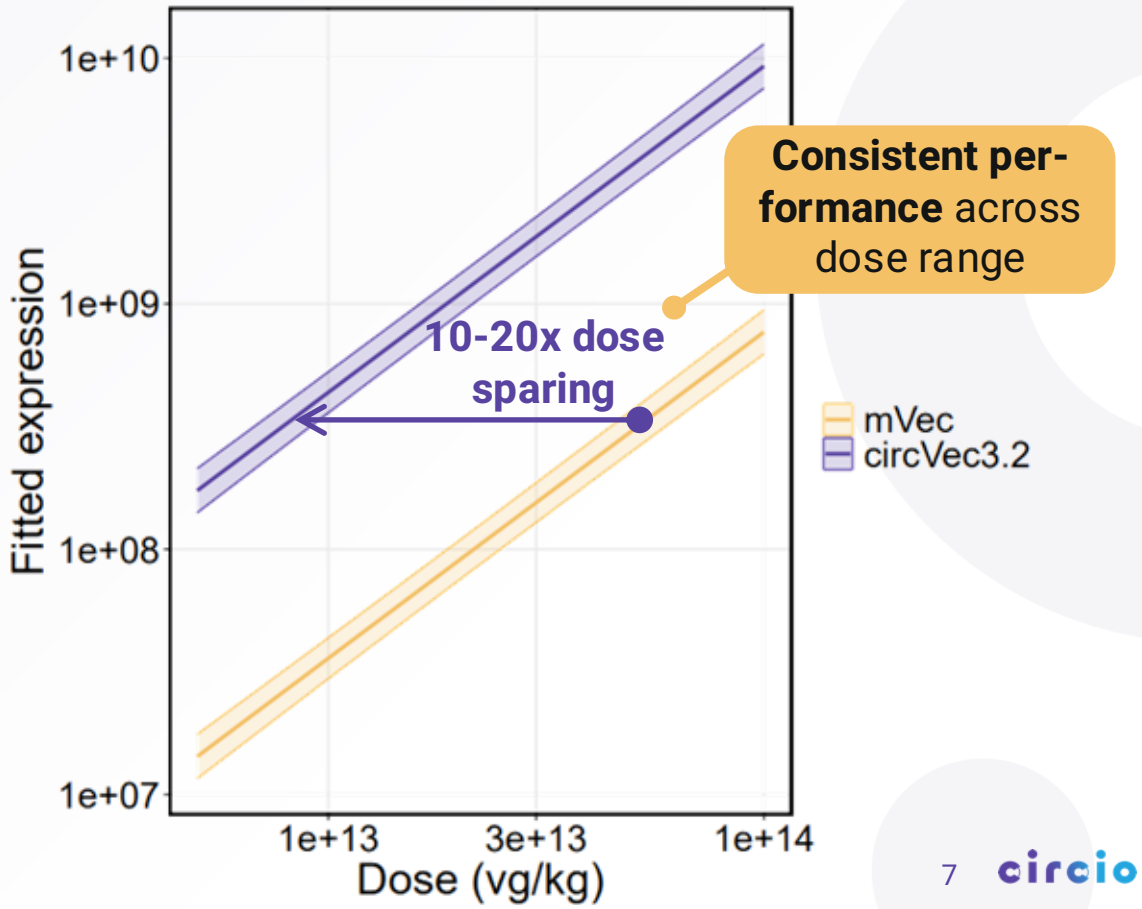
Systemic delivery, AAV9 vector, 5e12 vg/kg dose

Dose-response in vivo: opportunity for at least 10x dose reduction by switching to circVec expression

AAV circVec vs. mVec dose escalation study
Dose range from 5e12 to 1e14 vg/kg

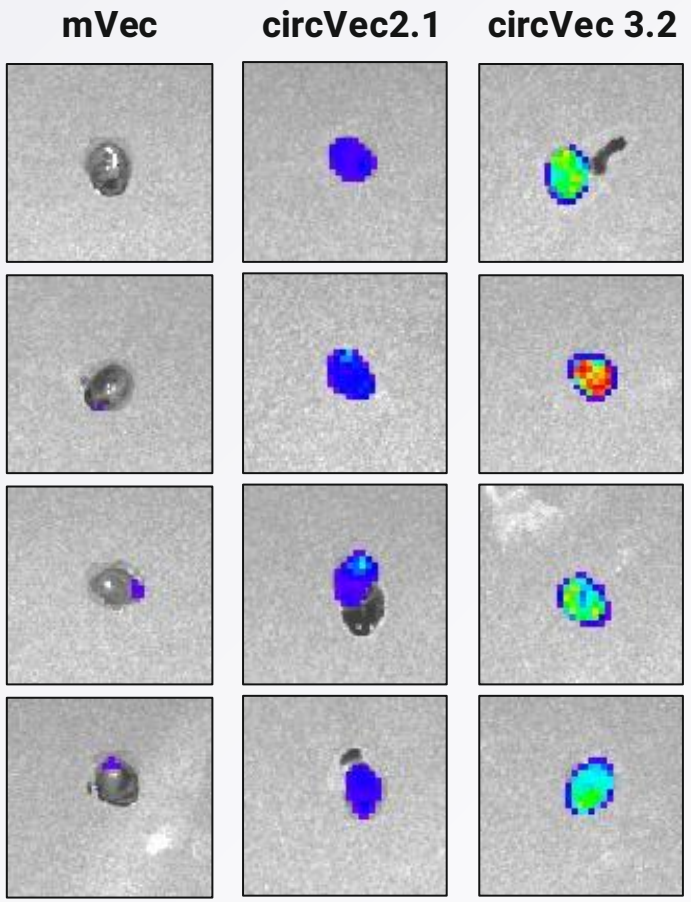


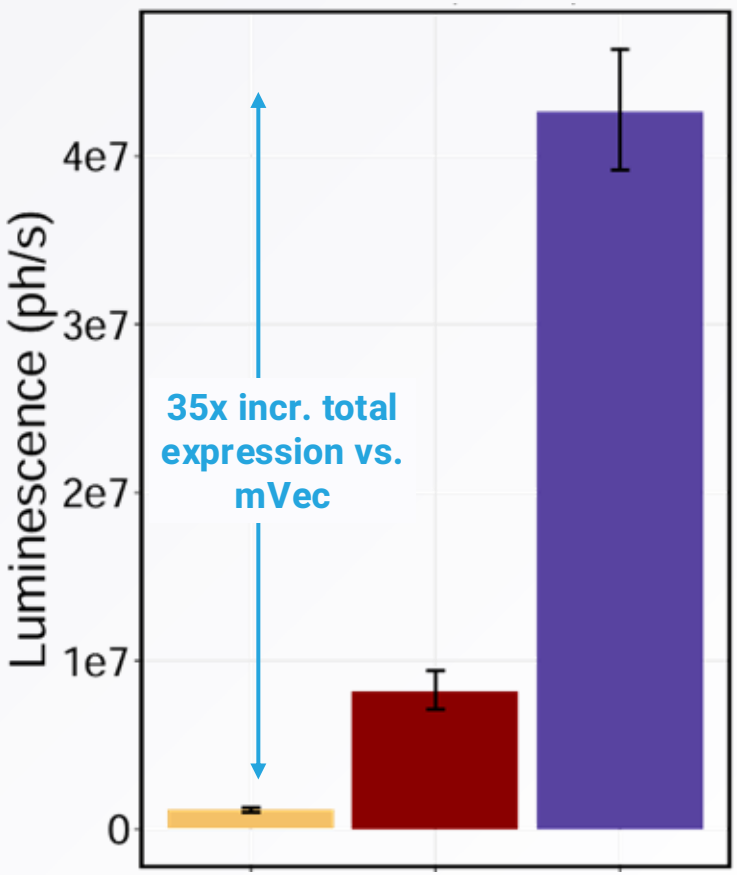
Gene expression level AAV circVec vs. mVec
Luminescence signal by dose, mixed model



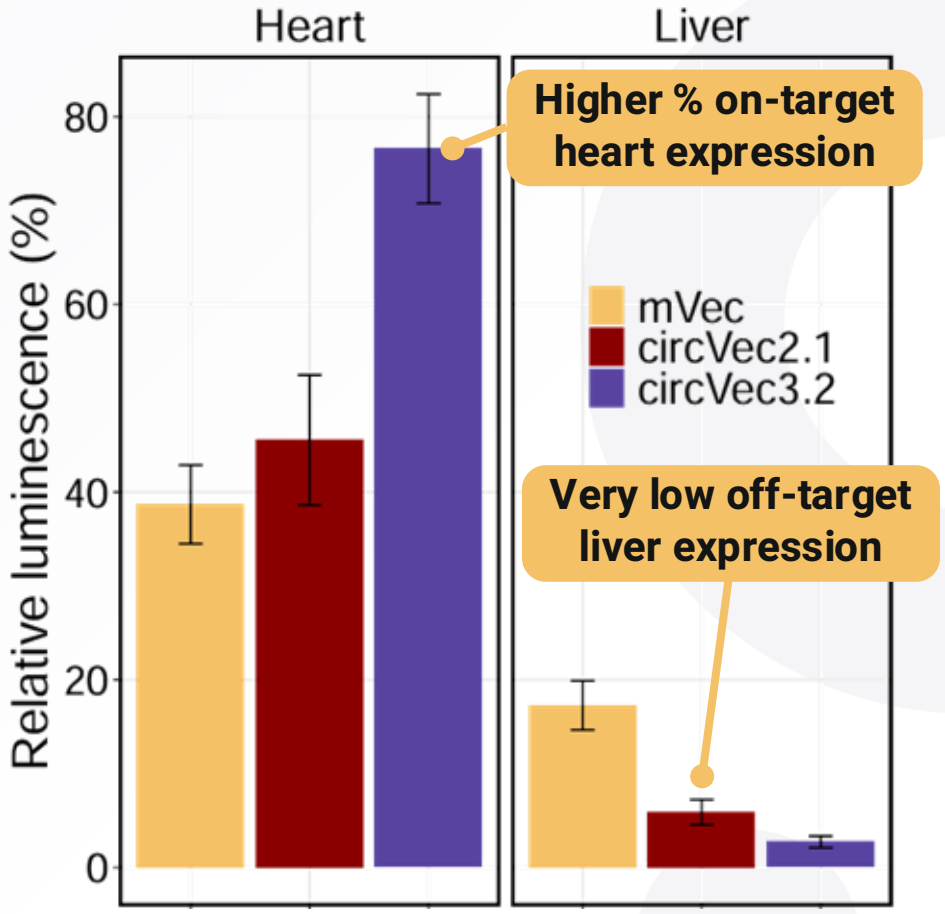
circVec shows increased on-target heart activity and substantially reduced off-target liver expression

Increased expression in heart, ex vivo tissue analysis week 10



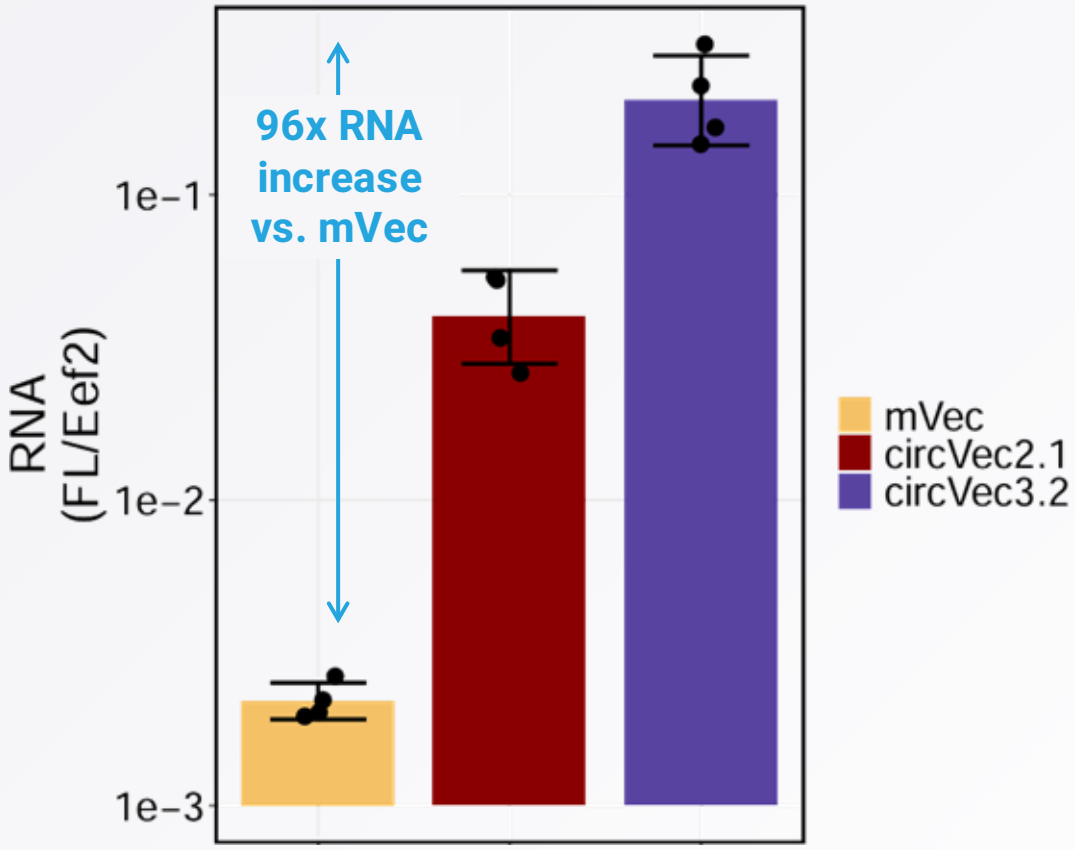


...and reduced off-target liver expression

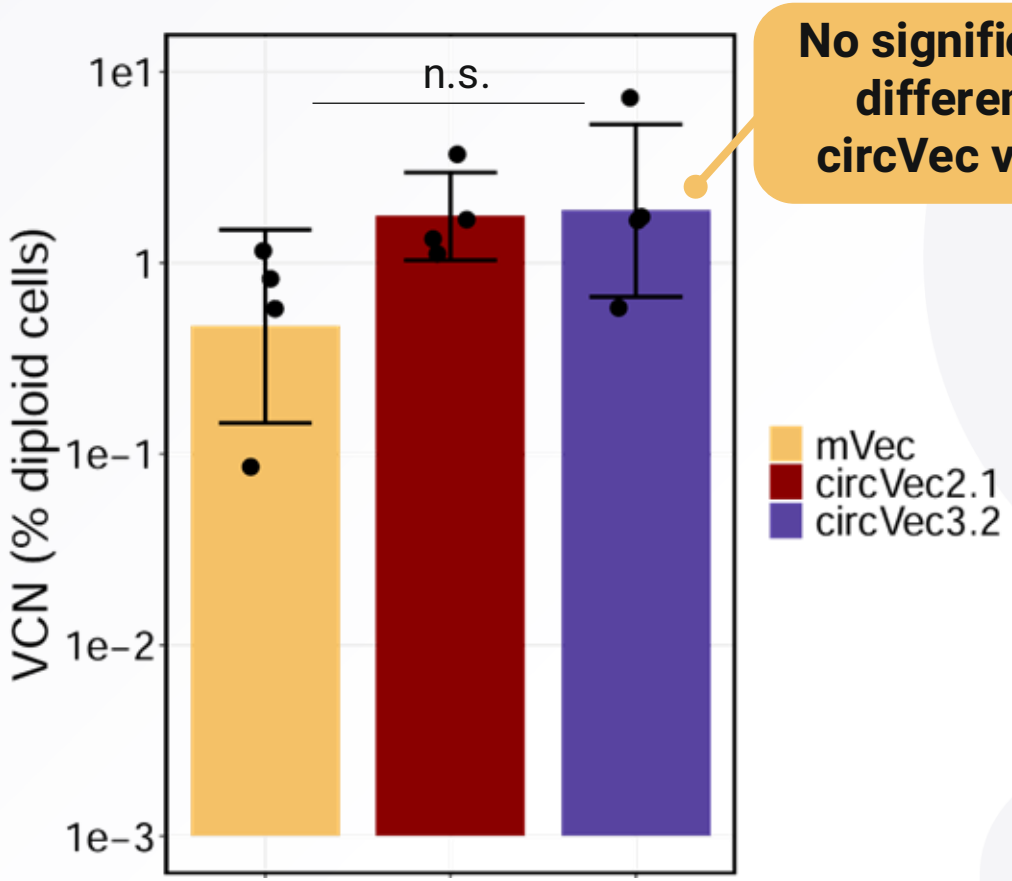


The circVec advantage is driven by RNA transcript level

RNA expression in heart tissue, RT-qPCR



Vector Copy Number (VCN) in heart tissue, qPCR

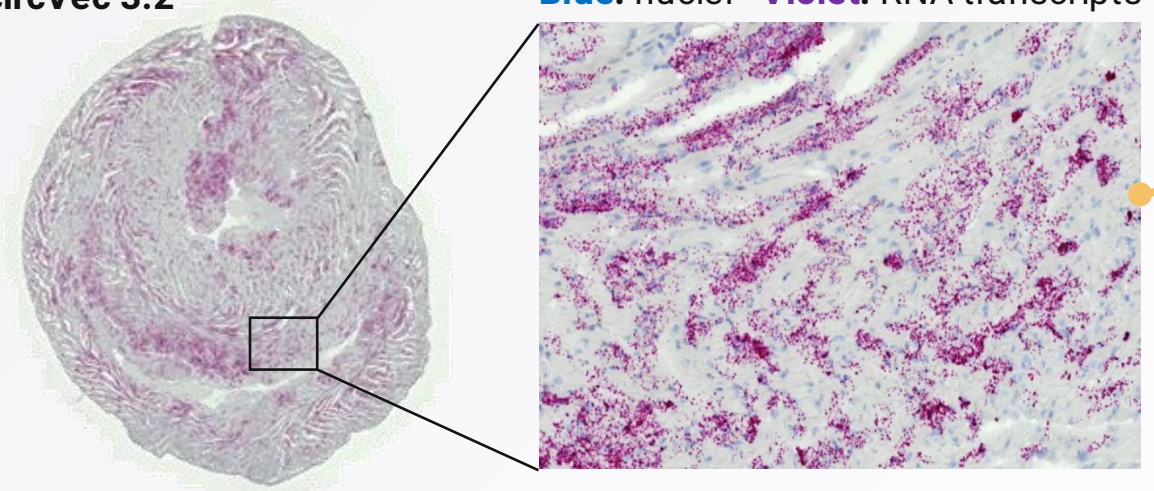


circVec-AAV circular RNA transcripts detected in 80% of heart cells at low dose level

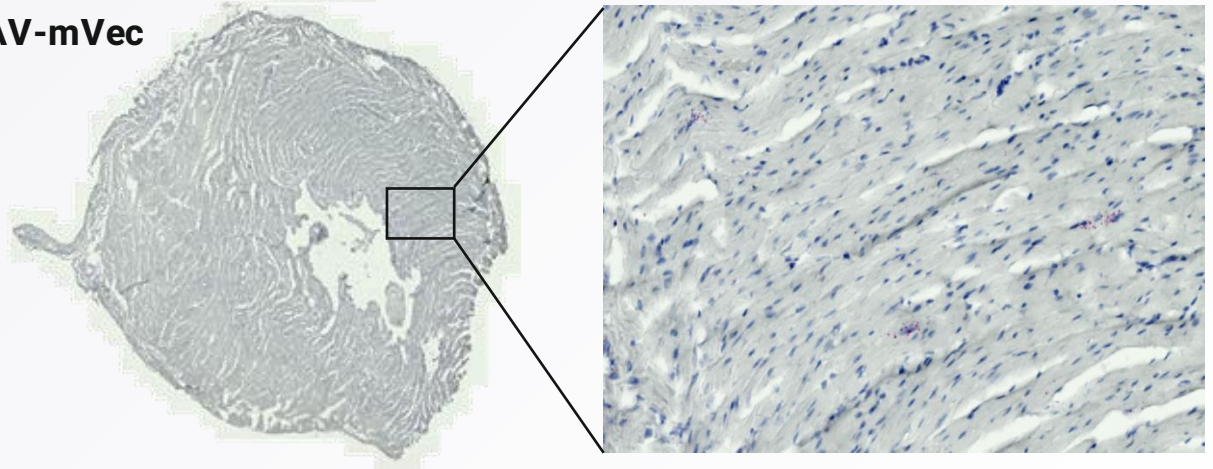
RNA expression in heart at low dose, RNAscope* microscopy ex vivo tissue section, 5e12 vg/kg

AAV-circVec 3.2

Blue: nuclei **Violet:** RNA transcripts



AAV-mVec

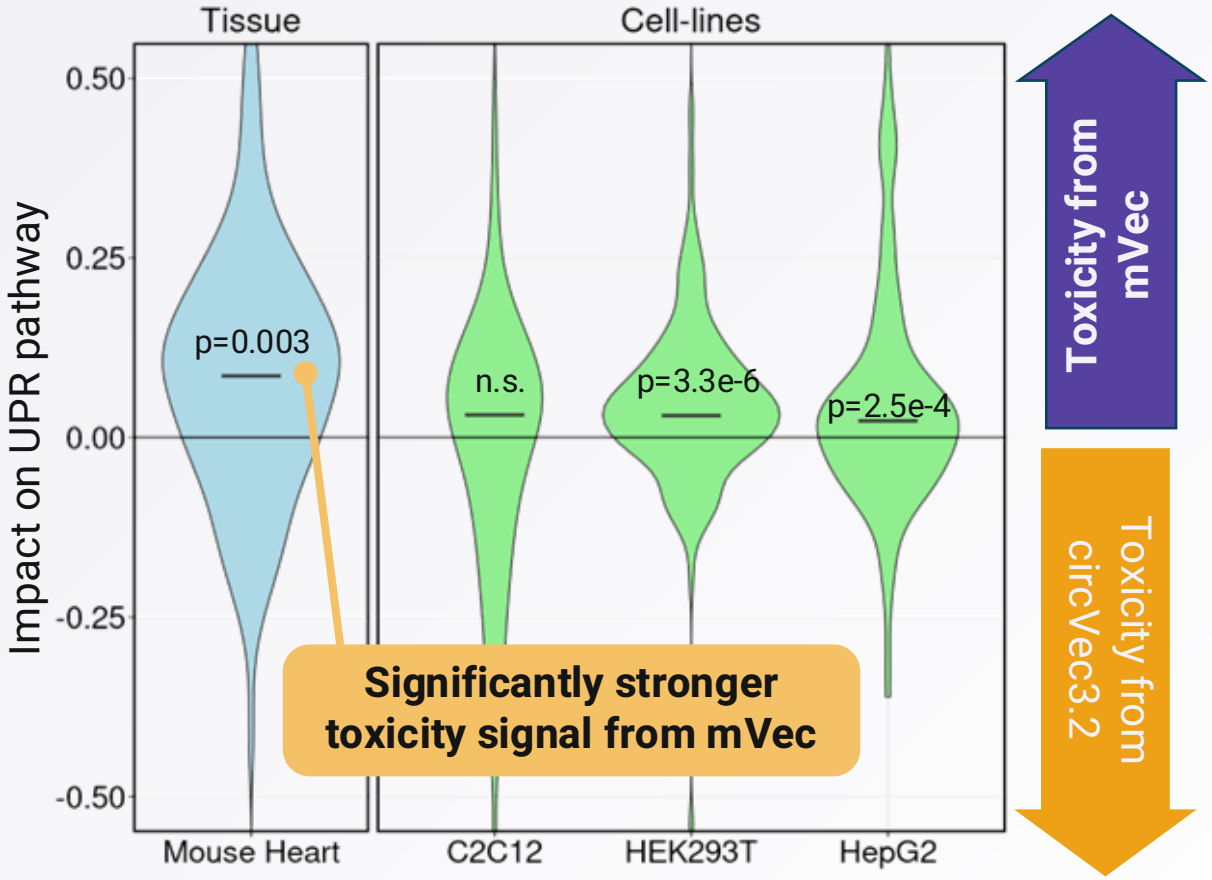


- **80% of heart cells positive for circRNA expression at low dose**
- Substantial improvement vs. AAV benchmarks

*** RNAscope:** single molecule detection of Firefly Luciferase RNA

Consistently reduced cellular stress response observed for circVec-AAV both in vivo and in vitro

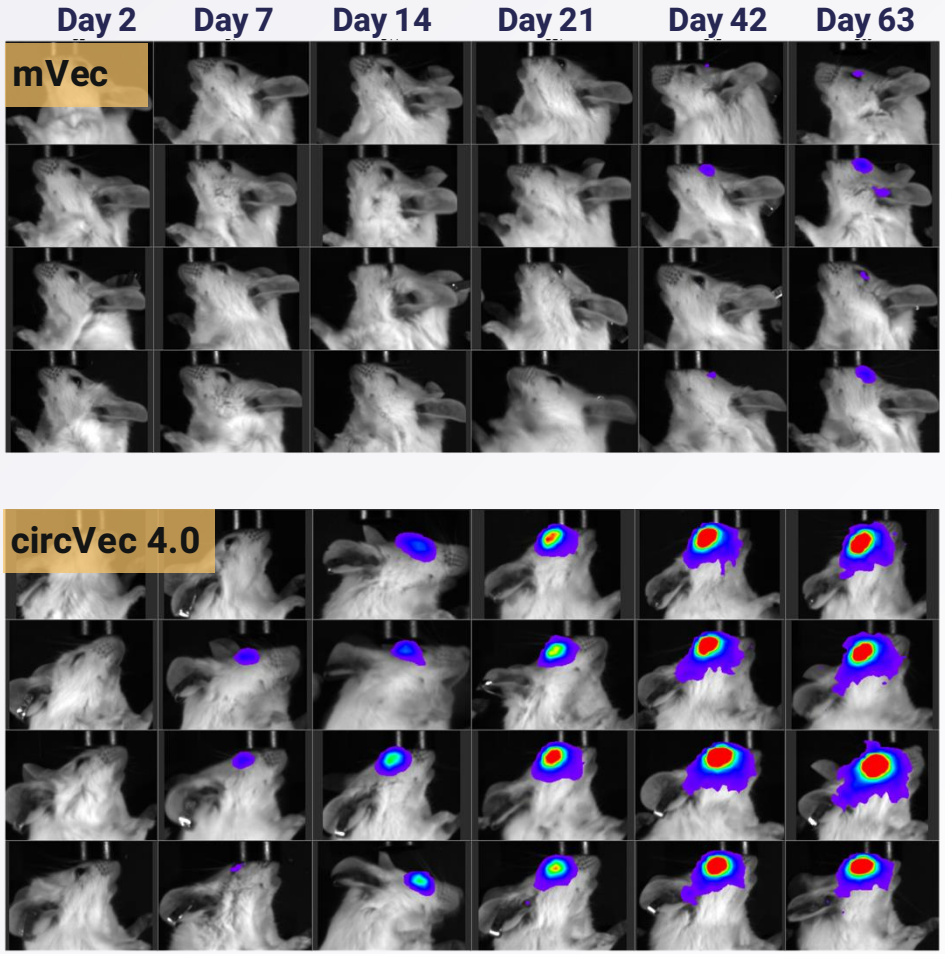
Cellular stress response, UPR pathway activation



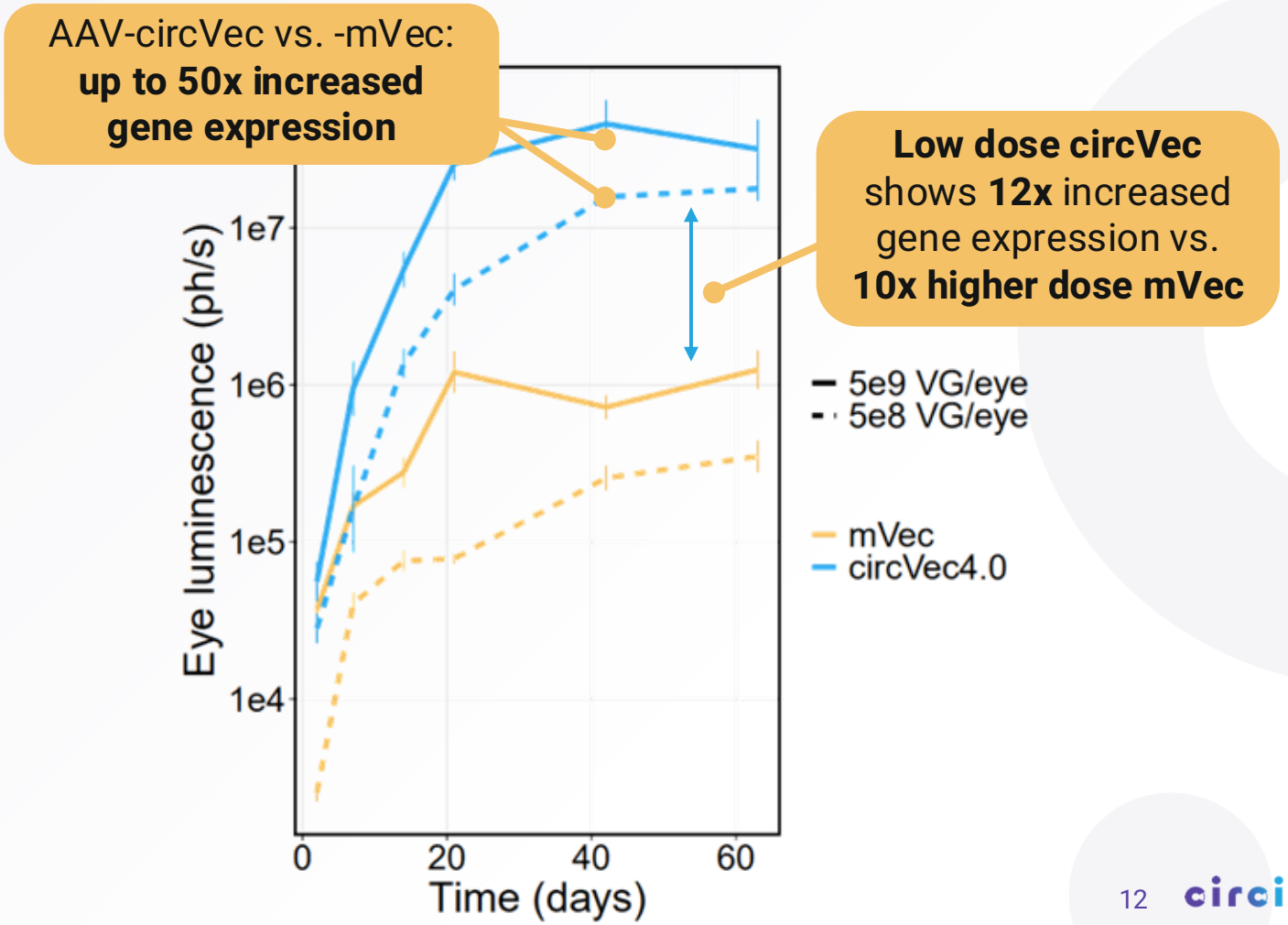
- **Unfolded Protein Response** (UPR) activation is a **major contributor to AAV toxicity** in patients
- AAV-circVec shows **less activation of UPR pathway in heart** than AAV-mVec at **same dose**
  - Despite 40x increased gene expression
  - Confirmed in various cell lines

Ophthalmology: local delivery of AAV circVec 4.0 enhances gene expression by up to 50x in eye

IVIS images, low dose mice (5e8 VG/eye)



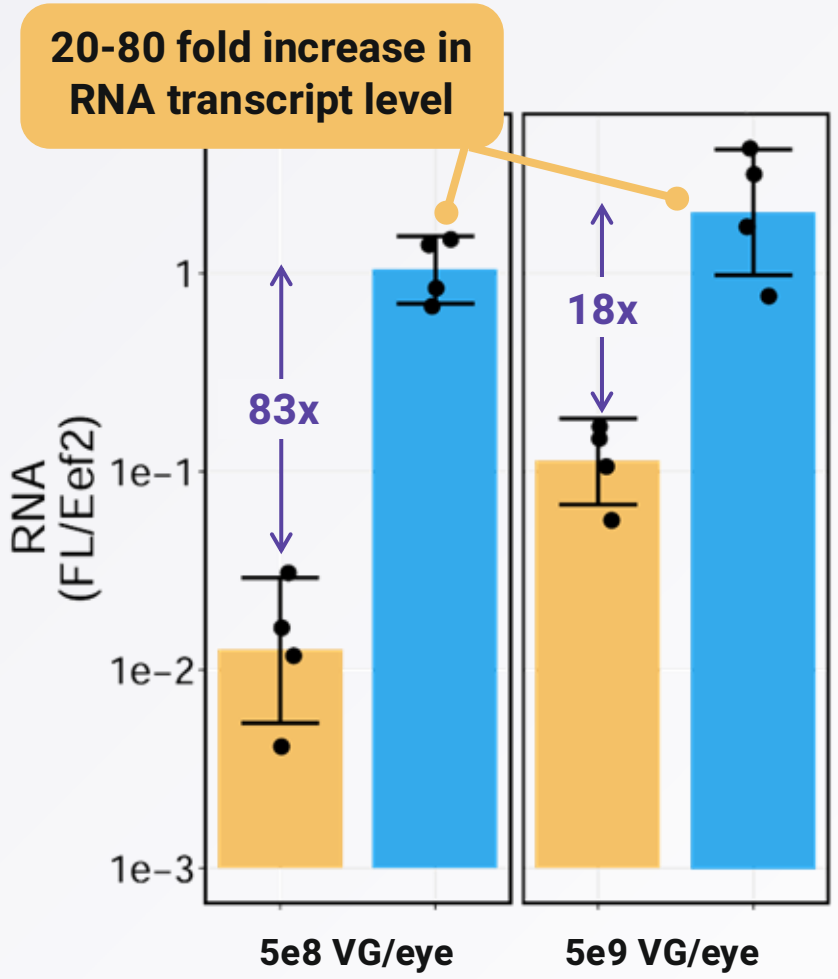
Expression over time, intra-vitreal inj. of AAV-circVec vs. -mVec



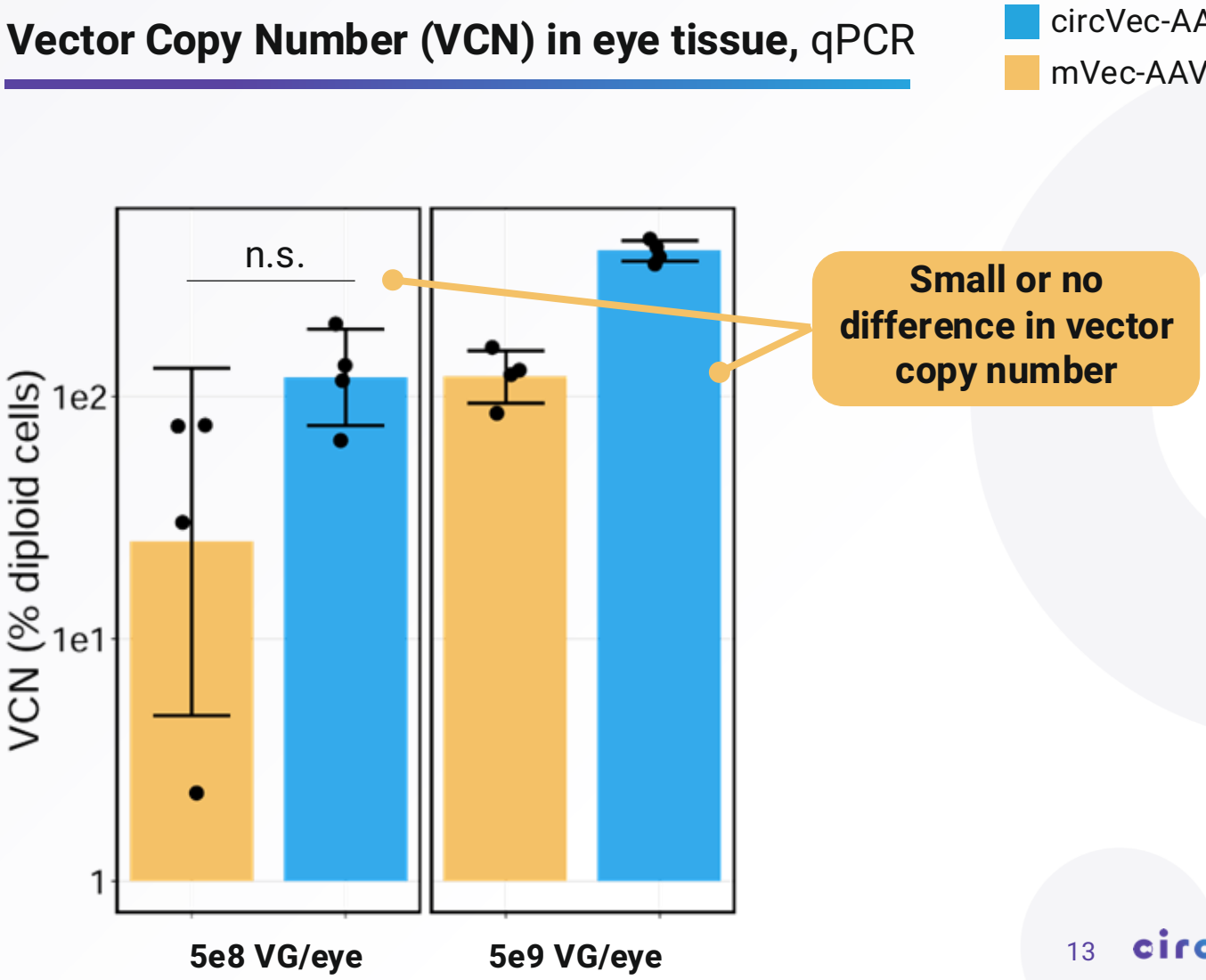
Intra-vitreal delivery, AAV2 vector

The circVec advantage is driven by increased circRNA transcript level also in the eye

RNA expression in eye tissue, RT-qPCR



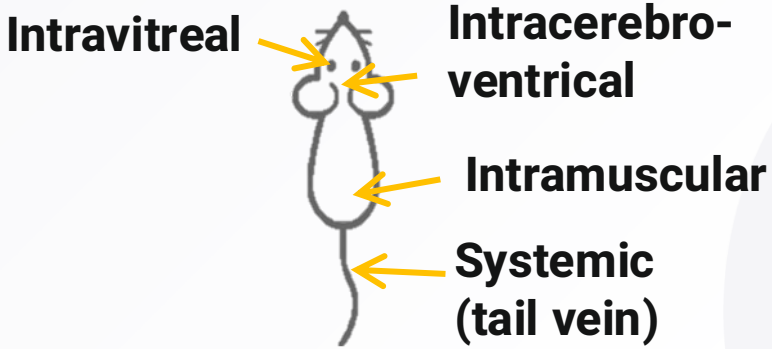
Vector Copy Number (VCN) in eye tissue, qPCR



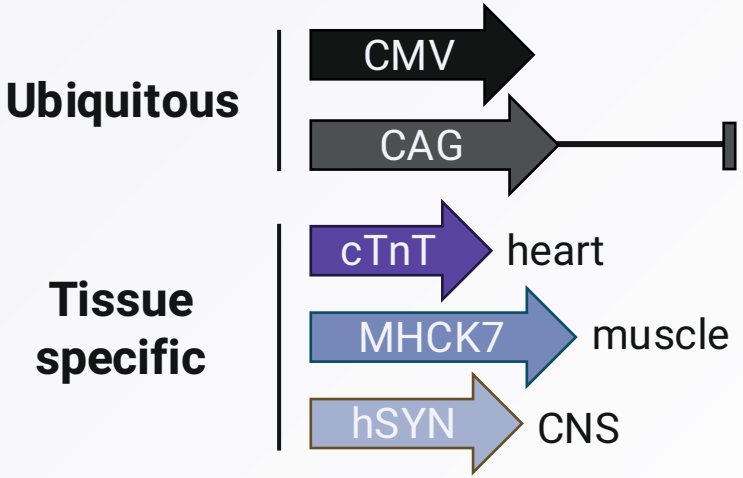
circVec performance has been validated across capsids, tissues and delivery routes

AAV capsids

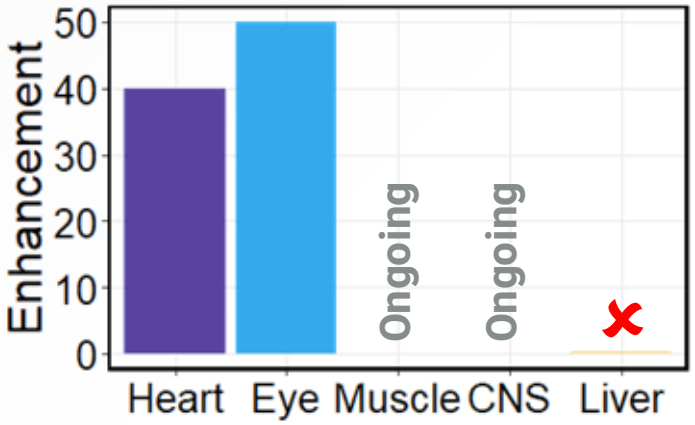
Delivery routes



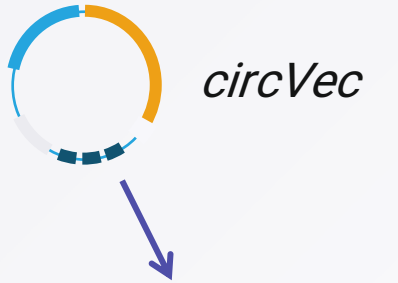
Promoters



circVec in vivo PoC

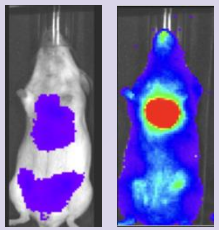


Summary : circVec confers three major advantages for AAV gene therapy

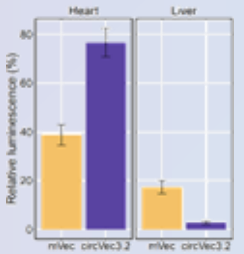


circVec-AAV compared to benchmark mVec-AAV:

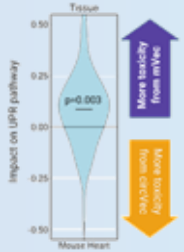
Expression



Specificity



Toxicity



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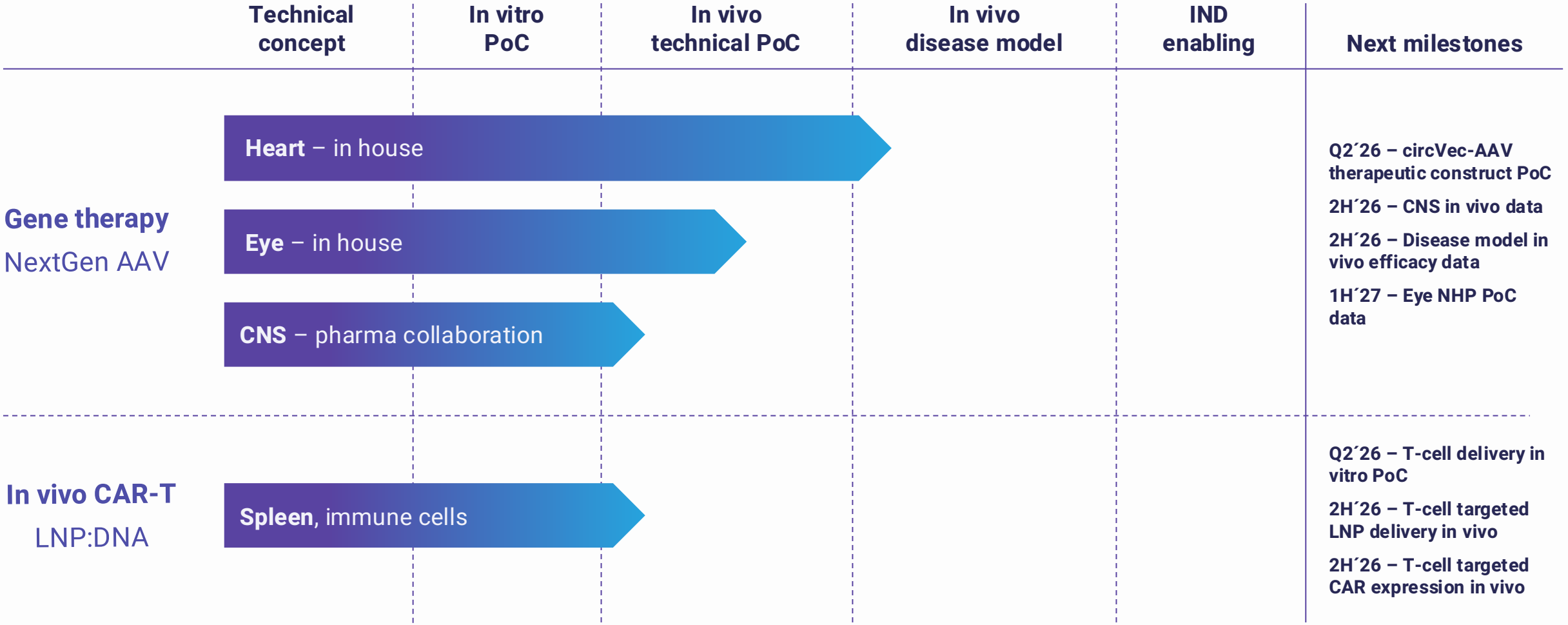
Circio pre-clinical circVec development pipeline



PoC: proof of concept IND: investigational new drug LNP: lipid nanoparticle CAR: chimeric antigen receptor AAV: adeno-associated virus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