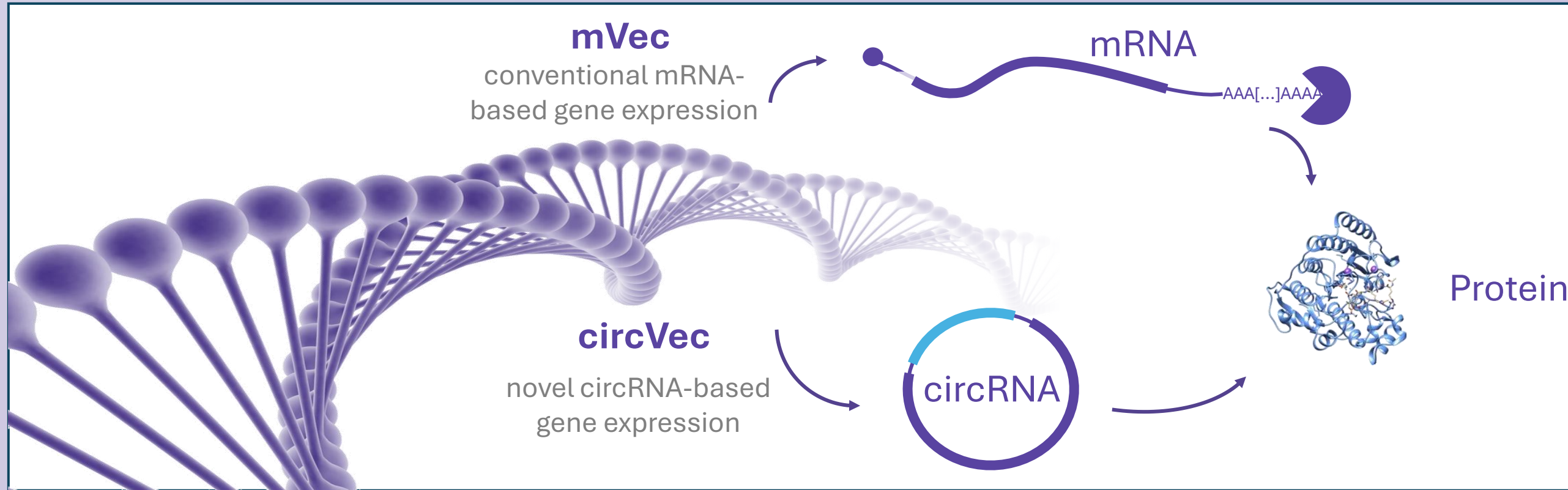
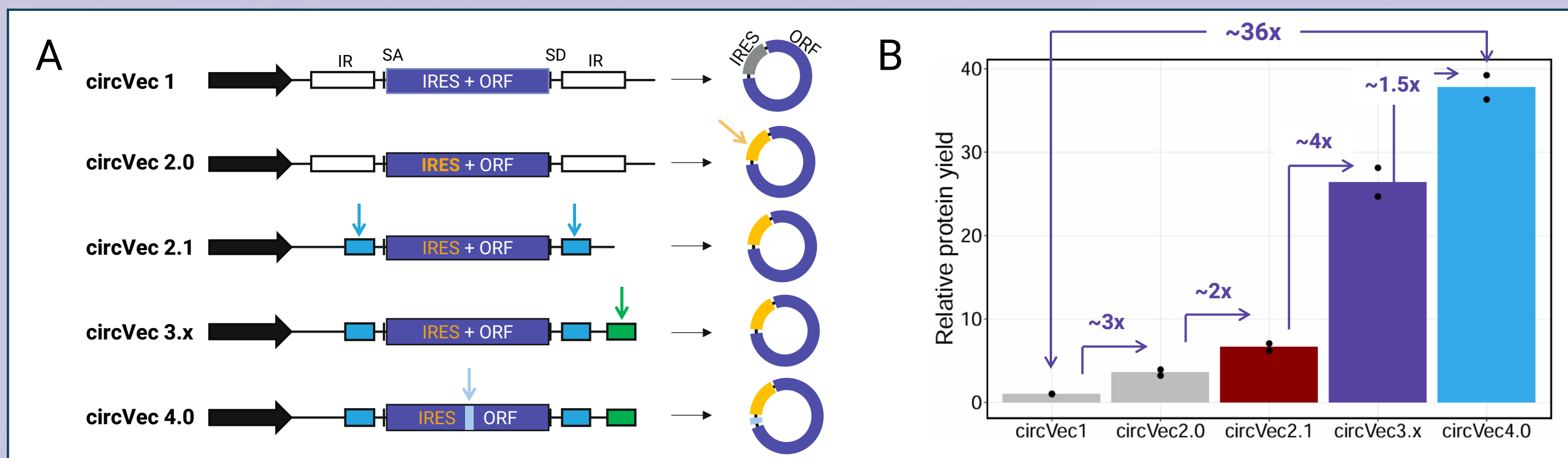


circVec technology introduction

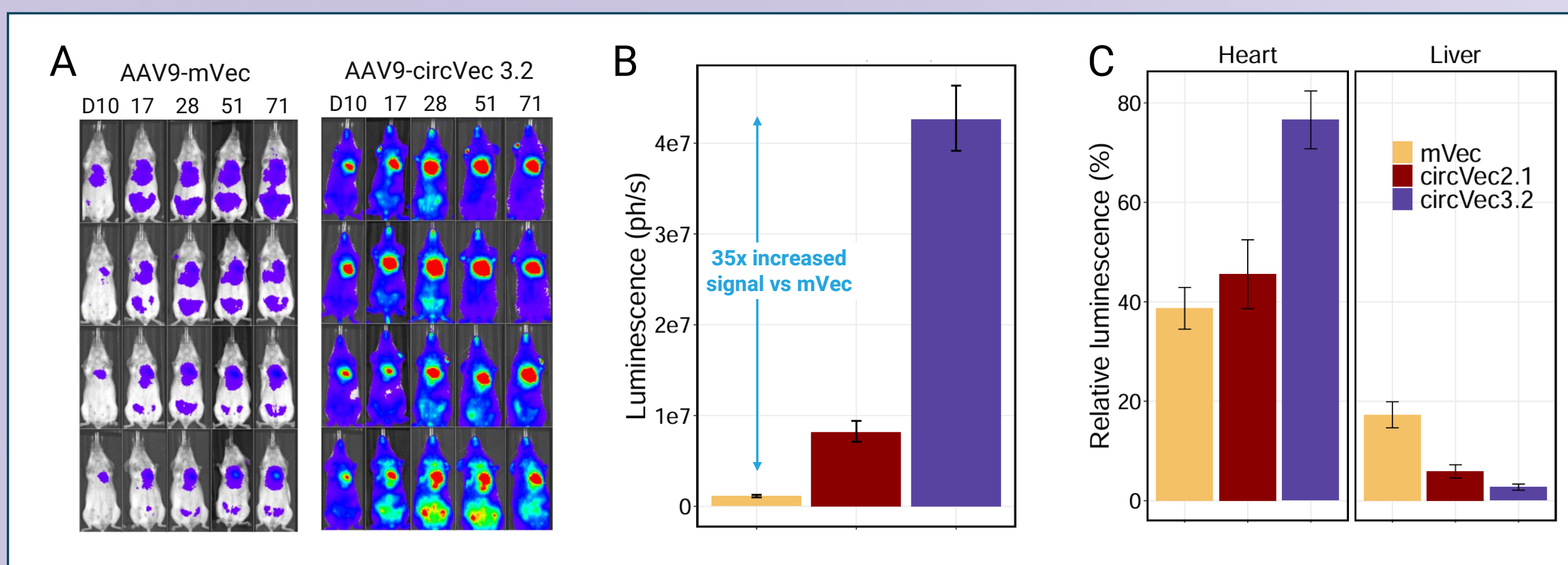
Circular RNA (circRNA) is a novel class of endogenously expressed RNA. CircRNAs are generated by a non-linear splicing event, known as **backsplicing**, where an upstream splice acceptor attacks a downstream splice donor. circRNAs are **resistant to exonucleolytic decay**, which results in **increased intra-cellular stability and persistence compared to mRNA**. Circio has developed a circRNA-based gene expression platform, **circVec**, which utilizes this natural stability advantage to **increase and prolong protein expression from viral and non-viral vectors**



1 Optimized circVec architecture improves circRNA gene expression by ~36-fold



2 circVec increases AAV9 gene expression by over 30-fold compared to benchmark AAV



3 Circular RNA accumulation drives enhanced gene expression and reduces cellular stress response

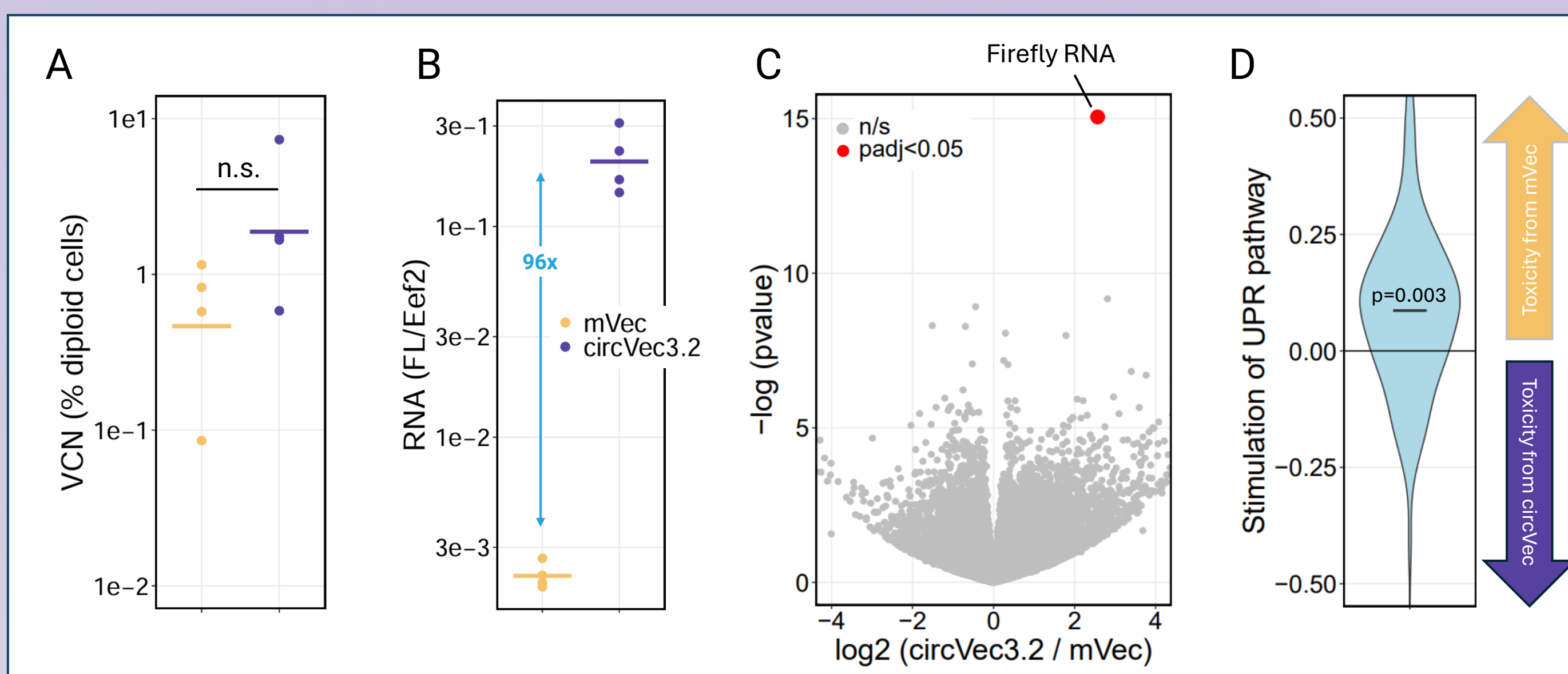


Figure 3: Viral genome and transcriptomic analysis on heart samples. A-B) AAV genome (A) and Firefly RNA (B) quantification on heart tissue 92 days after i.v. injection of 5e12vg/kg AAV9-circVec3.2 or AAV9-mVec, as denoted. T-test on log-transformed values is used to determine significance, n.s.: not significant. C) Volcano plot based on whole transcriptomic analysis of heart tissue (n=4/group). Only Firefly RNA shows a significant change in expression (red dot, BH-adjusted p-value (padj) = 0.01). D) Unfolded protein response (UPR) genes as a class are significantly upregulated in mVec heart tissue compared to circVec (p=0.003, Wilcoxon rank sum test).

4 RNAscope analysis shows 80% circRNA positive cardiomyocytes at 5e12 vg/kg dose

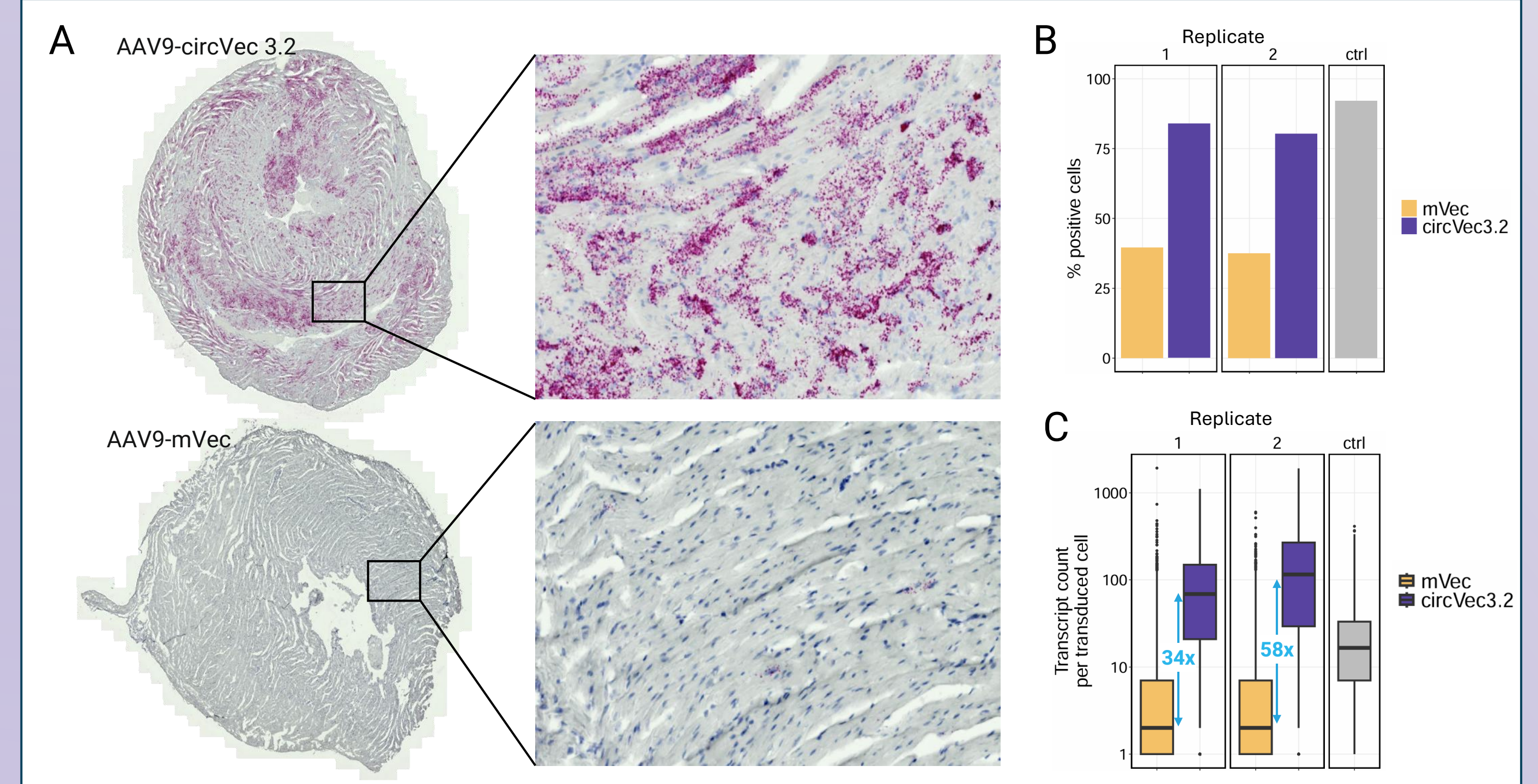


Figure 4: RNAscope analysis on mouse heart subjected to intravenous injection of AAV9-circVec and AAV9-mVec (5e12vg/kg). A) Representative heart sections from AAV9-circVec3.2 or AAV9-mVec treated mice (as denoted) probed for Firefly RNA (violet) using RNAscope technology and stained with Dapi (blue). B) Using the QuPath software, number of positive cells were extracted. *Ppib* (peptidyl prolyl isomerase B) is used as the positive control marker (ctrl). C) Distribution of Firefly transcript copy-numbers in positive cells showing 34x-58x more RNA from circVec3.2 compared to mVec.

5 Systemic delivery of AAV9-circVec shows ~10-20x dose-sparing potential in heart

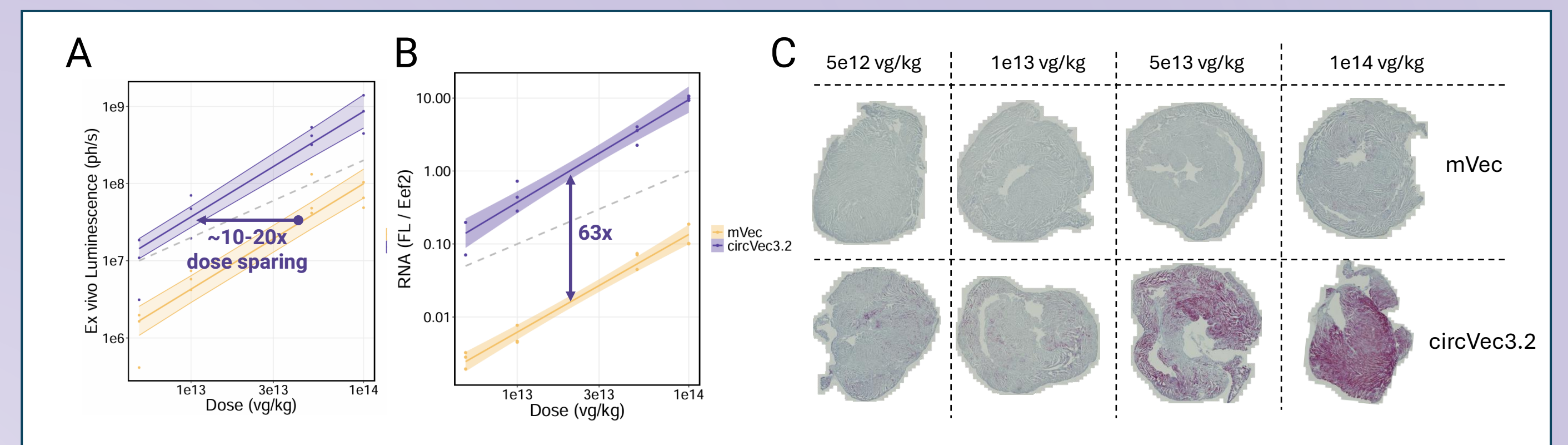


Figure 5: Dose-response relationship for AAV9-circVec and AAV9-mVec in heart. A-B) Super-linear dose response curve on ex vivo luminescence (A) and Firefly RNA quantities (B) from heart 54 days after injection of AAV9-mVec or AAV9-circVec3.2 using 4 different dose levels: 5e12, 1e13, 5e13, and 1e14 vg/kg. Dashed line represents linear dose-response with a slope of 1. C) RNAscope images on representative hearts from mice subjected to the different doses of AAV9-mVec or AAV9-circVec as denoted.

6 AAV-circVec advantage also applies to a therapeutically relevant gene (LAMP2) in vivo

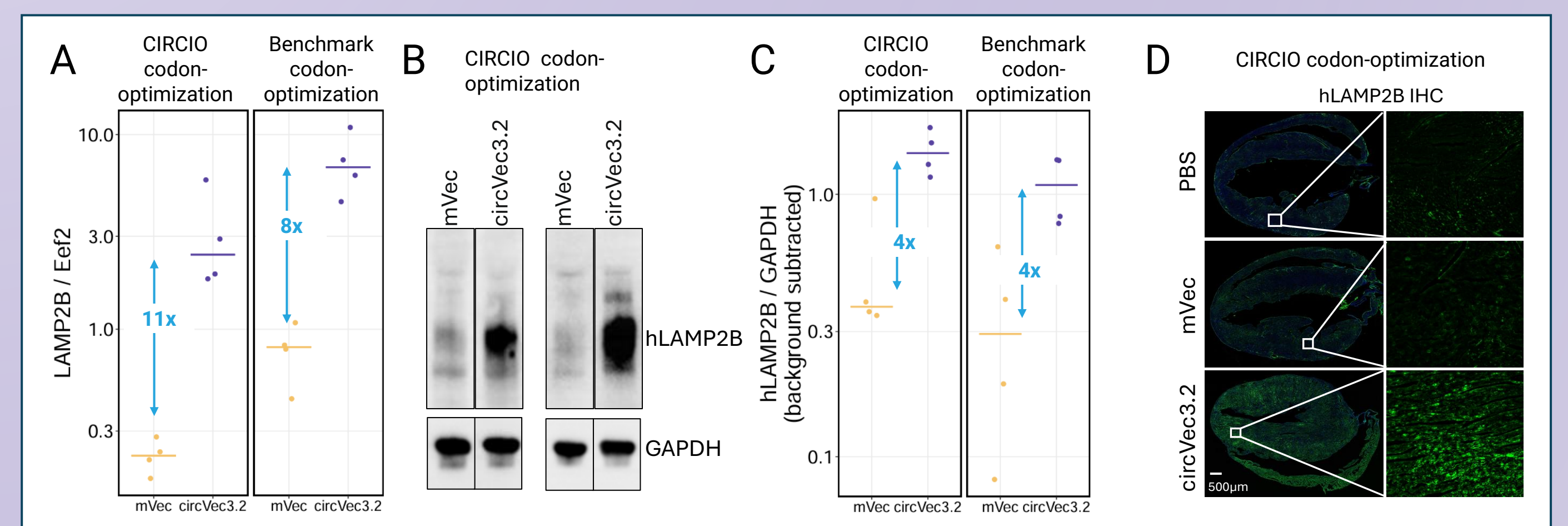


Figure 6: Comparison of hLAMP2B expression in heart from AAV9-circVec3.2 and AAV9-mVec using 5e13vg/kg. A) RNA quantification by RT-qPCR on heart samples 42 days after injection of CIRICIO and industry benchmark codon-optimized hLAMP2B expression vectors (AAV9-circVec3.2 and AAV9-mVec) with a heart specific promoter. B-C) Representative western blots (each mVec/circVec pair cropped from same blot, B) and quantification of relative hLAMP2B protein expression in heart (C). D) IHC analysis of hLAMP2B protein expression in mouse heart 42 days post AAV injection.

7 Circio pipeline

Technical concept	In vitro PoC	In vivo technical PoC	In vivo disease model	IND enabling	Expression	Cross-correction	Stress	Off-target expression
	Genetic cardiomyopathy - systemic delivery				↑	↑	↓	↓
	Ophthalmology - local delivery				↑	tbd	tbd	tbd
	CNS - local & systemic				↑	↑	tbd	tbd
	B-cell malignancies & autoimmunity				↑	tbd	tbd	↓
	Oral presentation: Wednesday 8.30-10.30 - Non-viral vectors/nanotechnology/RNA therapeutics I							

Conclusions

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- 40x enhanced protein expression in heart from AAV9-circVec: Prolonged circRNA stability leads to accumulation of RNA and enhanced protein expression compared to conventional mRNA-expressing AAV
- Reduced stress response and off-target liver expression from circVec: Significantly lower activation of UPR pathway by as well as lower reduced off-target expression adds to the safety and specificity of circVec-based gene therapy in heart
- 80% circRNA penetrance in heart using low dose (5e12vg/kg) i.v. administration: RNAscope analysis shows higher and broader expression of circRNA compared to mRNA from equivalent vector copy numbers in heart
- Superior LAMP2B expression from circVec: Using two different codon-optimized hLAMP2B ORFs, circVec significantly outperforms mVec on RNA and protein levels in wild-type mice

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