



Machine-learning guided rational design of cardiotropic capsids that detarget liver and DRG



Sherry Cao, Ph.D.

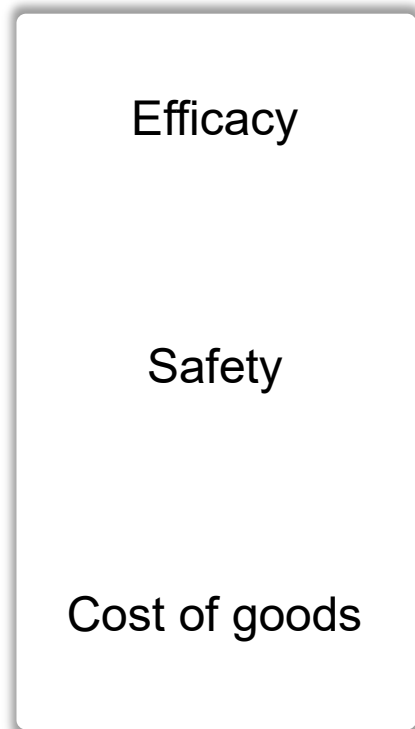
27th Annual Meeting of the American Society of Gene and Cell Therapy, Baltimore, Maryland

May 11th, 2024

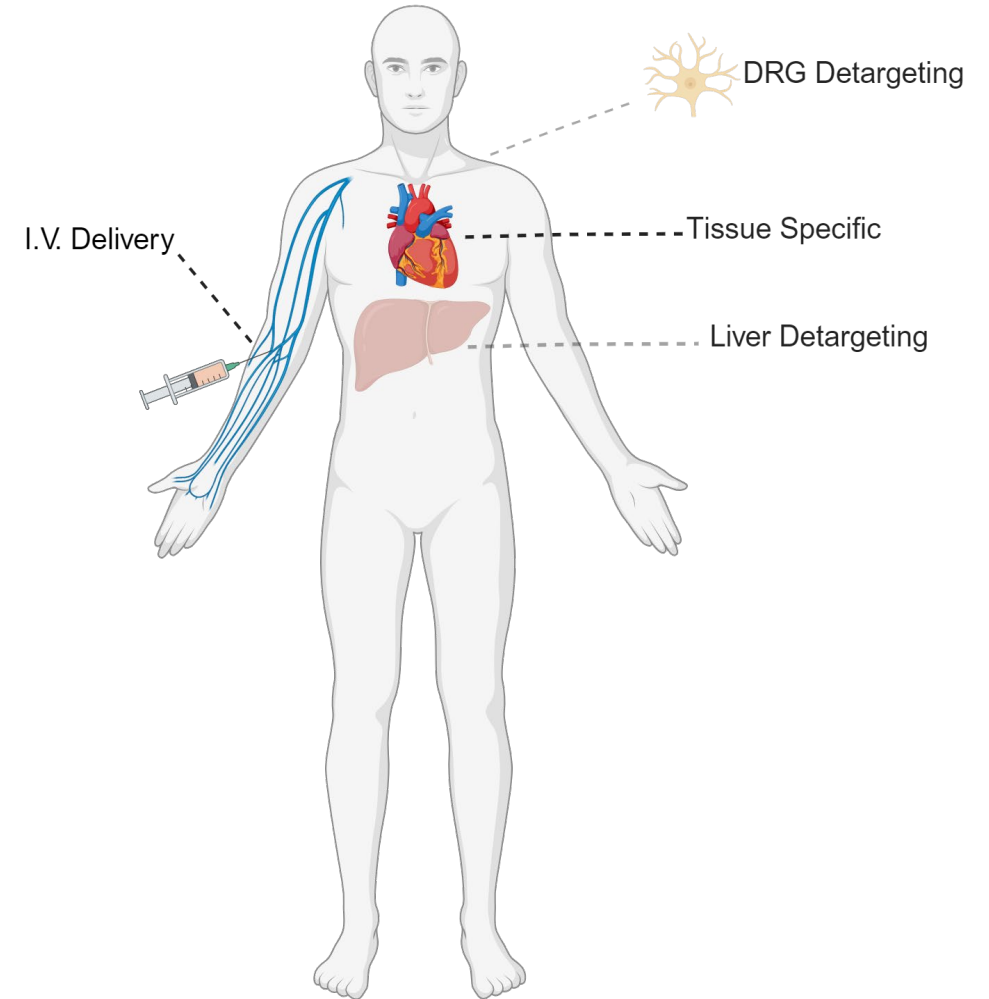
Disclosures

- I am an employee of Affinia Therapeutics

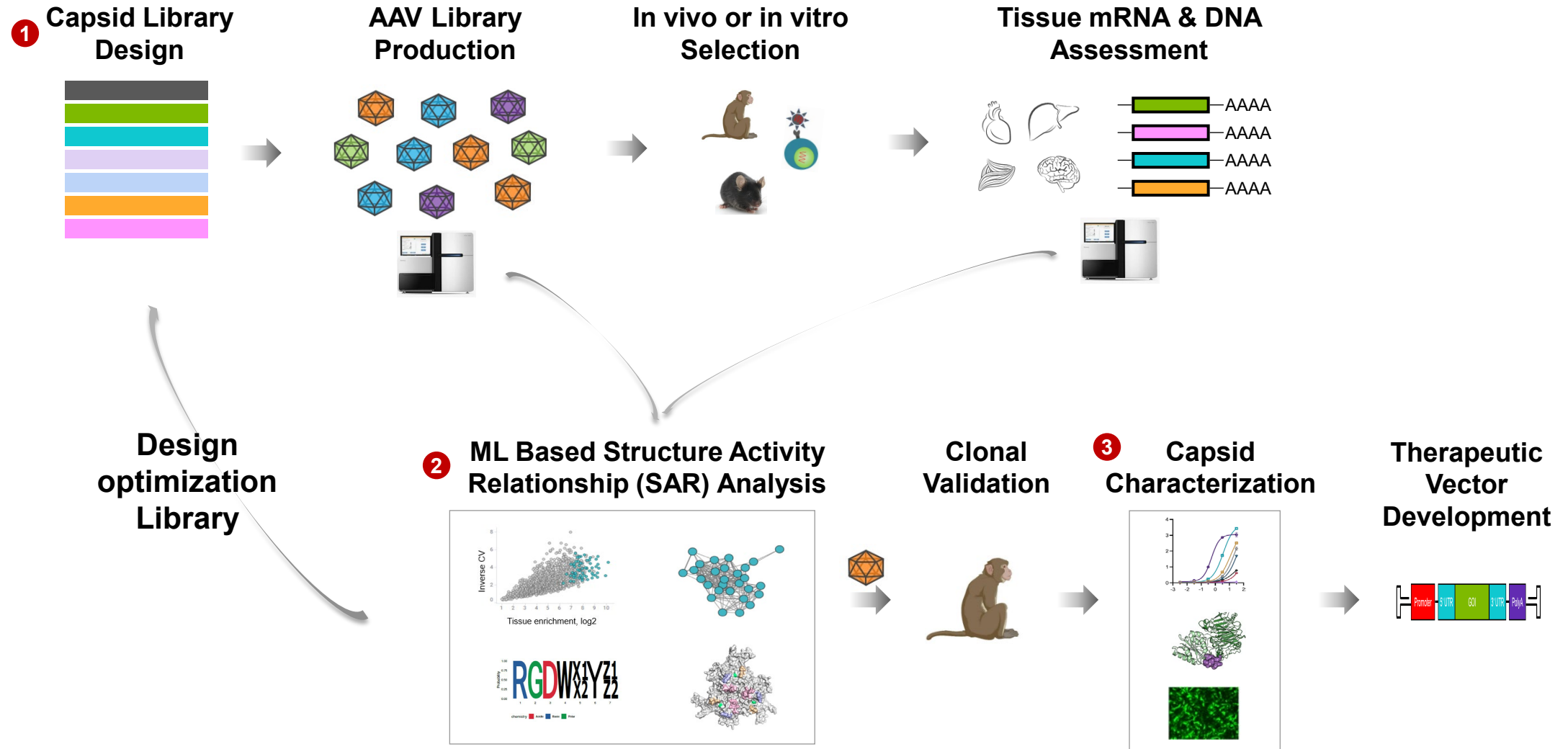
Improvements are needed to use recombinant AAVs to treat systemic diseases such as cardiovascular diseases



- Increased on-target tropism
- Decreased liver and DRG tropism
- Decreased dose
- Improved manufacturability



Affinia's machine-learning guided, rational design capsid discovery platform

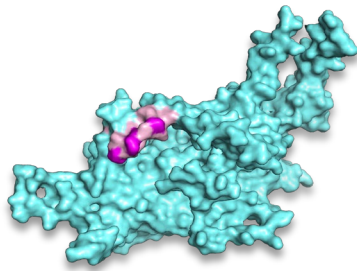


MOA based modular strategy allows fine tuning of multiple capsid tissue tropisms

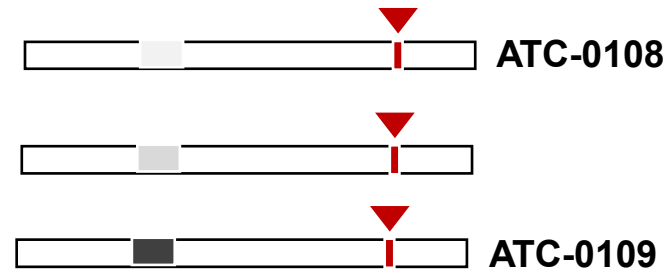
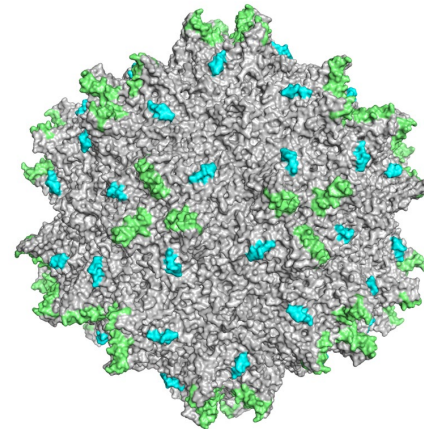
Case study: Cardiotropic capsids that detarget liver and DRG

Liver detargeting

Designed and screened VR1 substitution libraries for liver detargeting

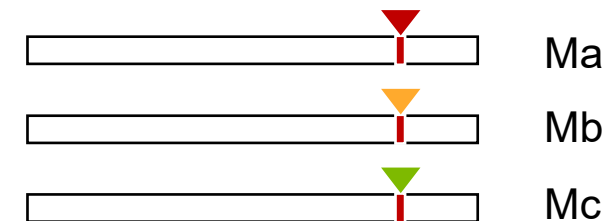
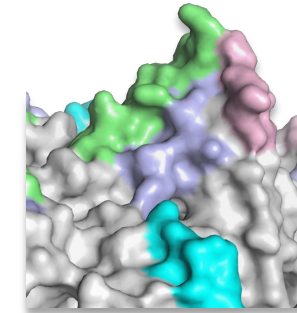


AAV9



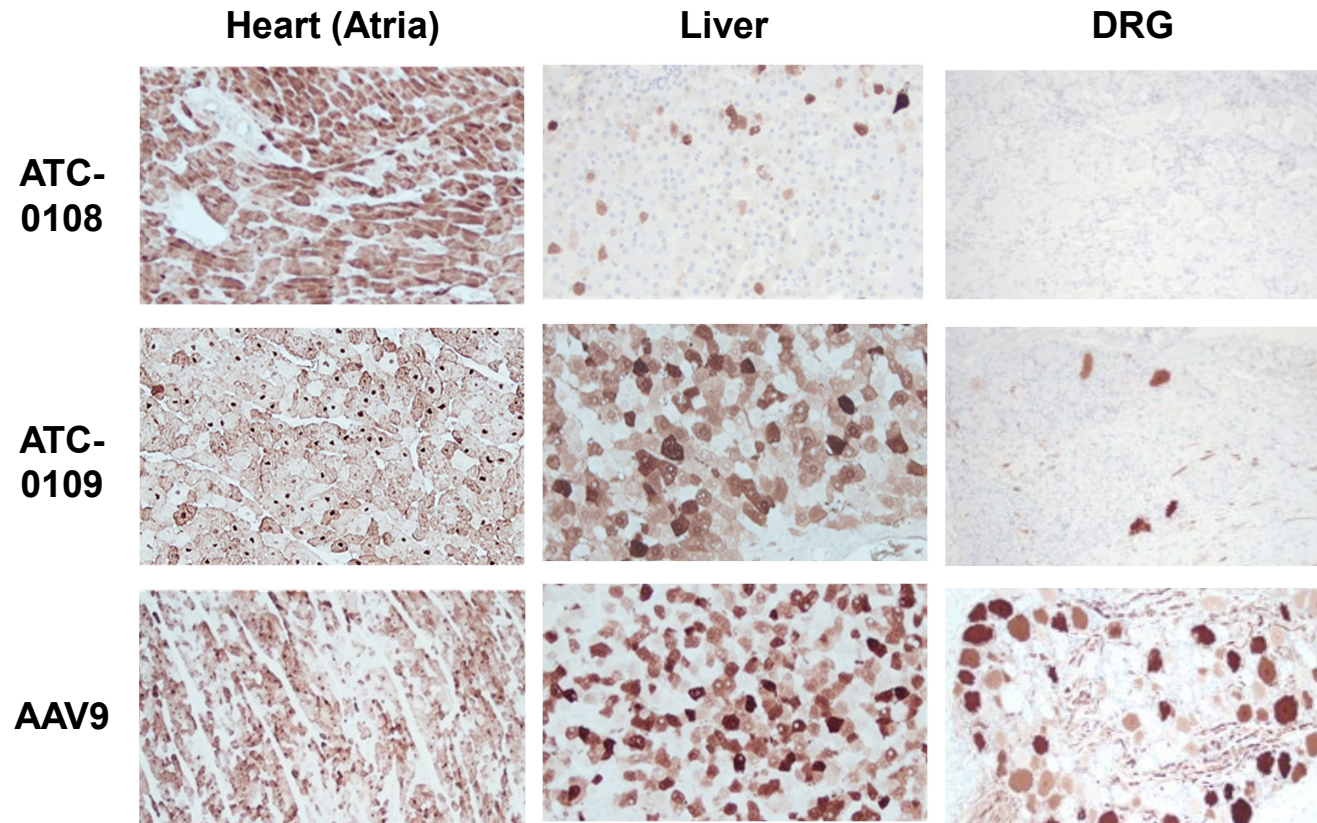
Cardiac tropism

Designed and screened VR8 peptide insertion libraries for cardiac tropism

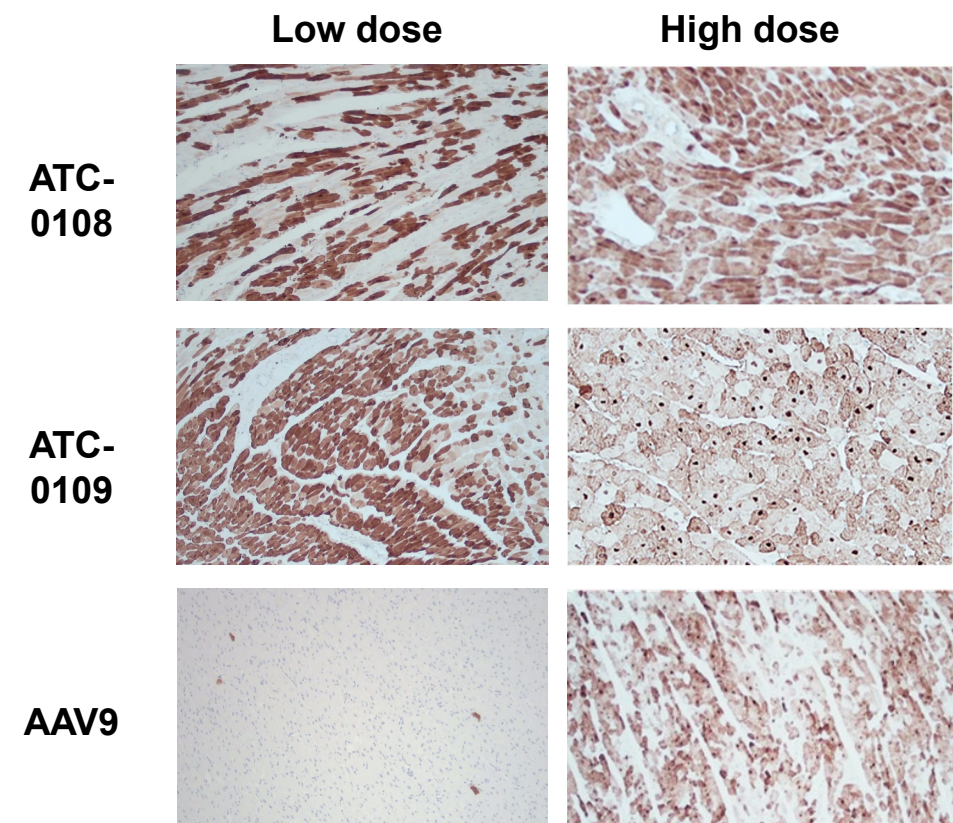


Affinia novel capsids: Superior cardiotropism vs. AAV9 in NHPs, with ATC-0108 de-targeting the liver and DRG

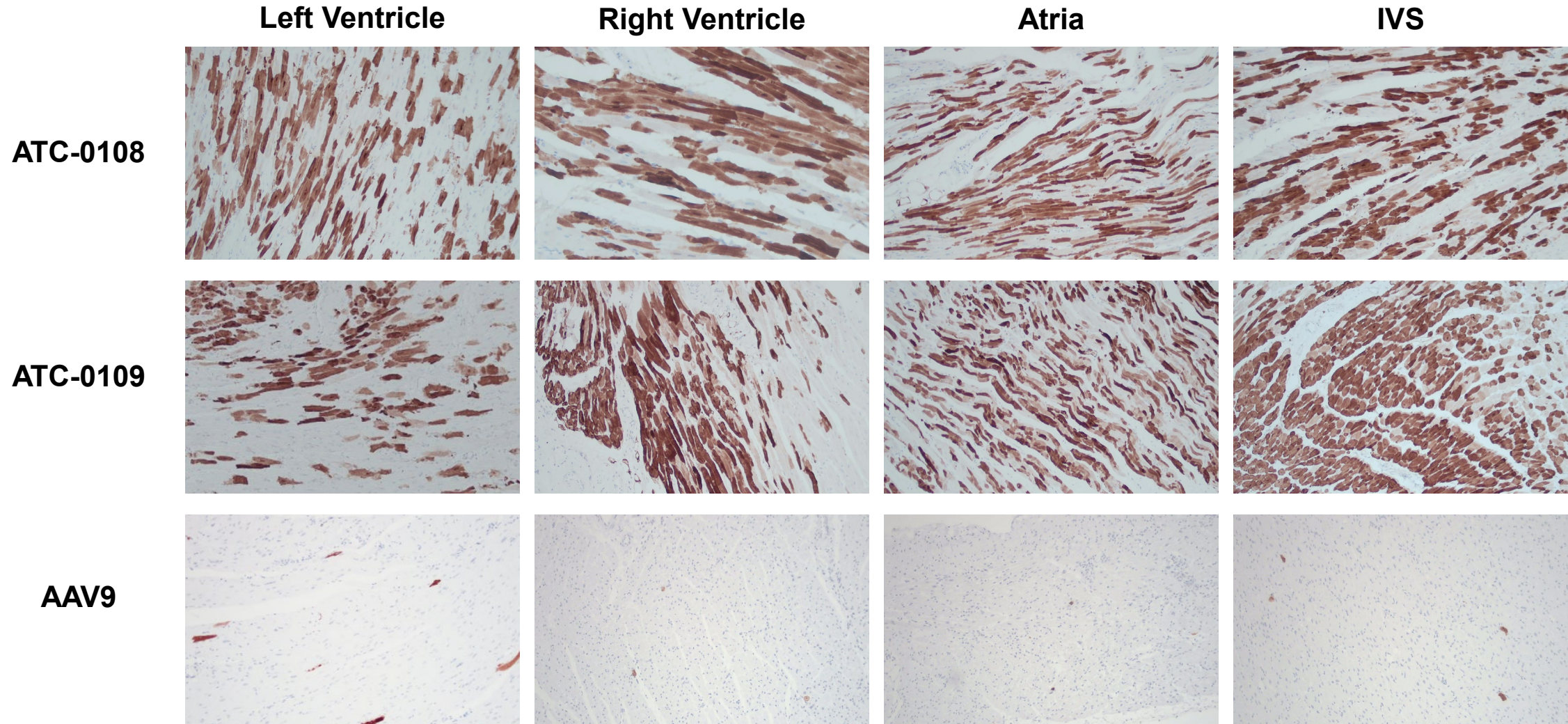
Heart, liver, and DRG at high dose



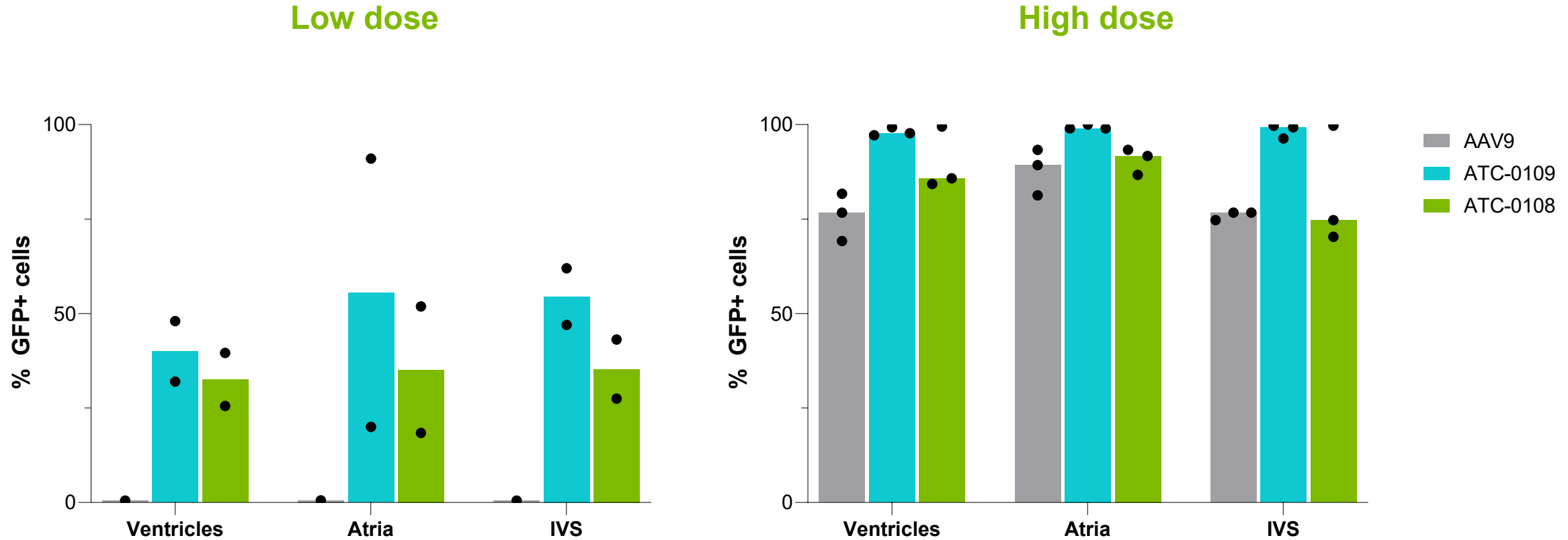
Heart at low dose vs. high dose



Uniform cardiac transduction at low dose in NHPs

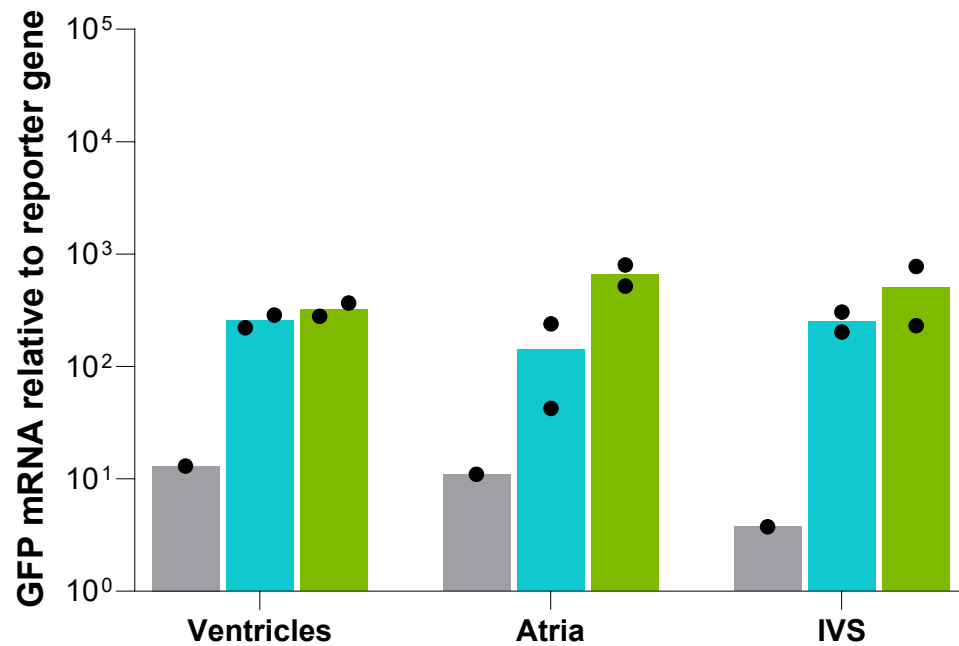


Superior transduction of cardiomyocytes vs. AAV9 across NHP heart regions

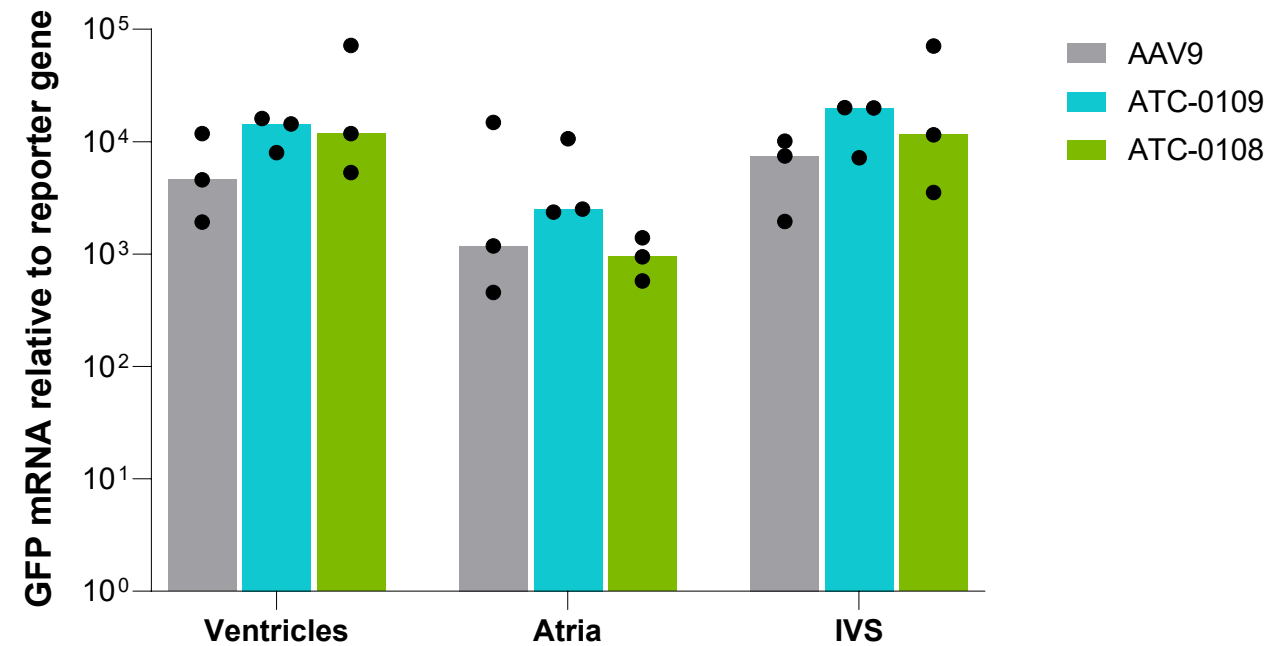


Superior RNA expression vs. AAV9 across NHP heart regions

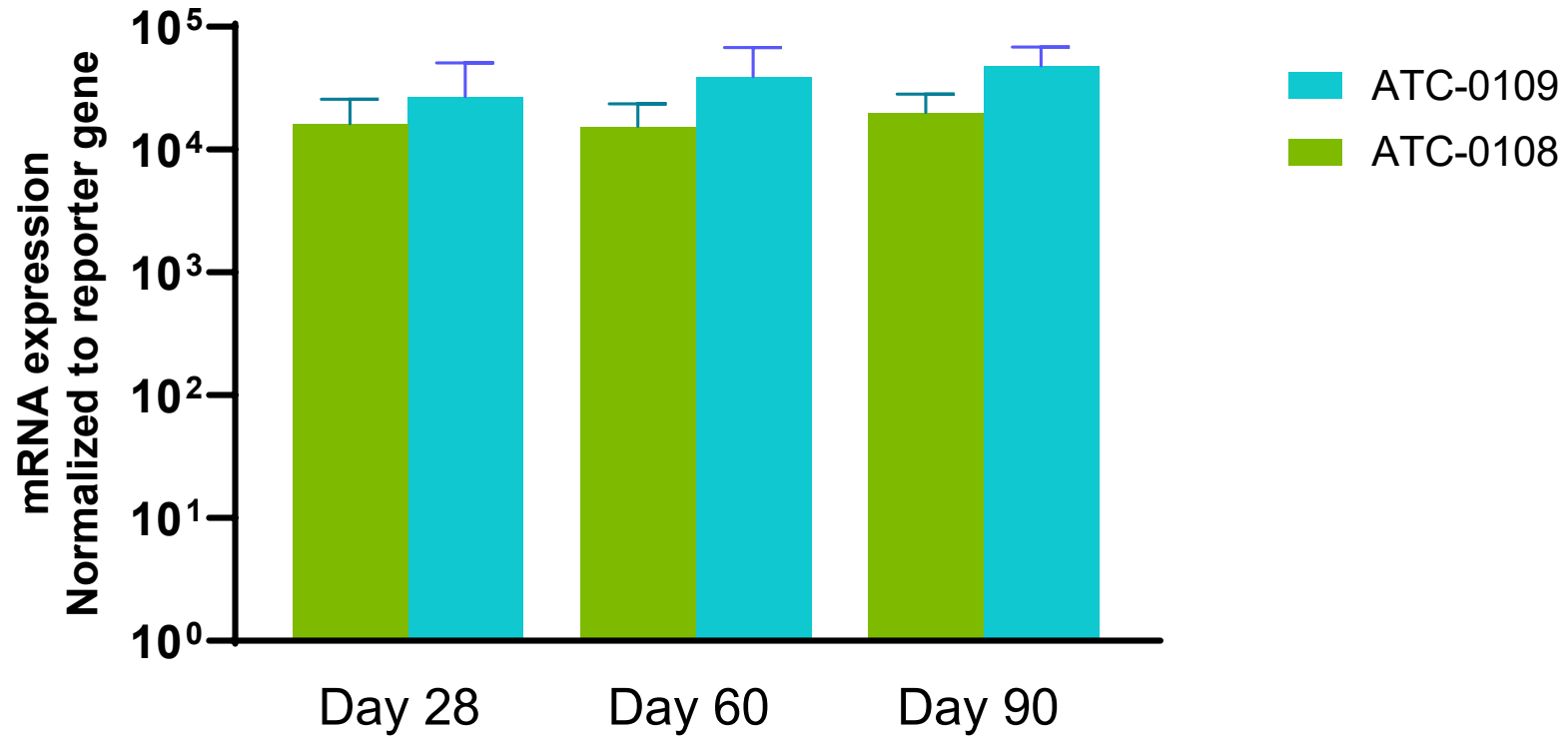
Low dose



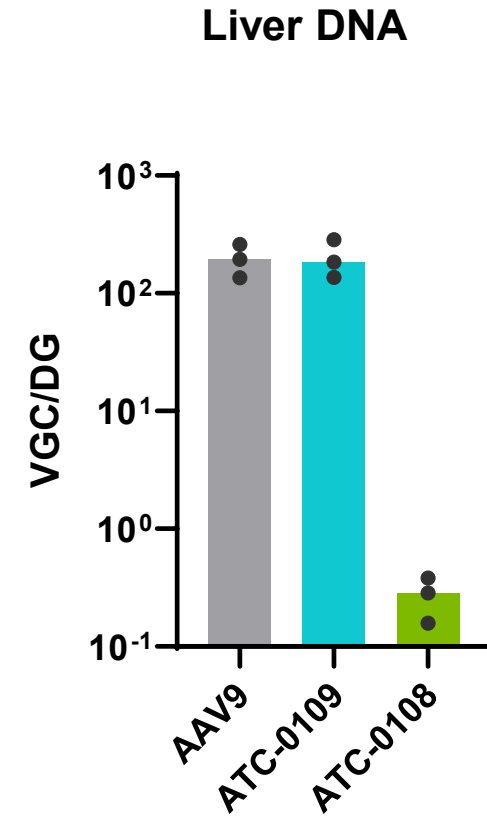
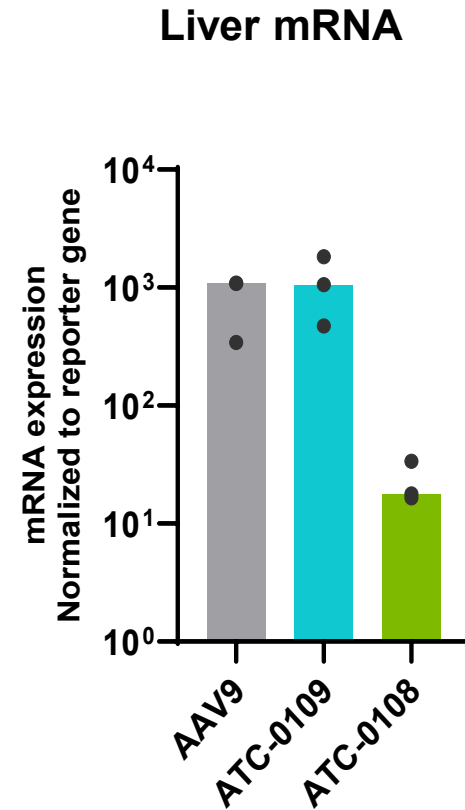
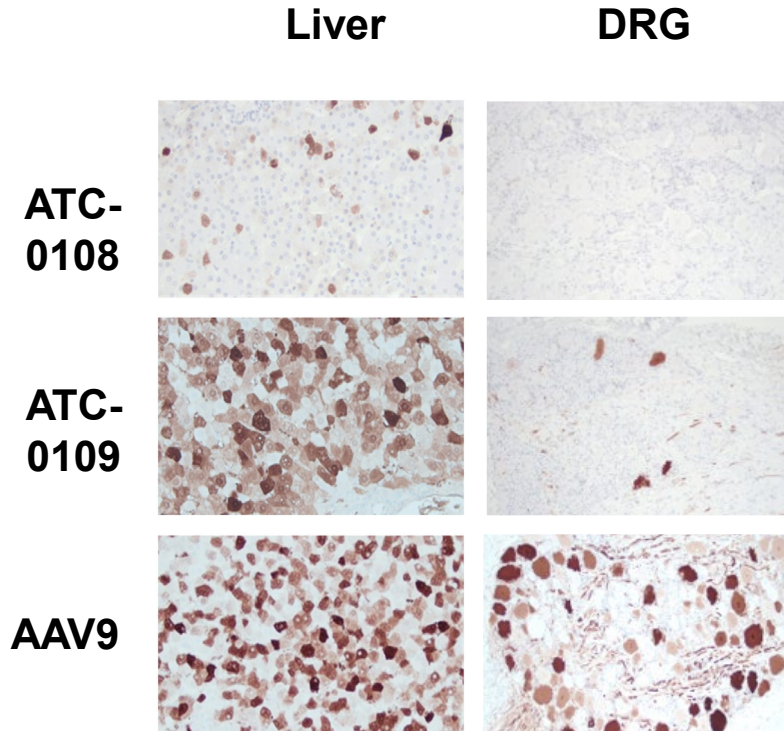
High dose



Durable mRNA expression of novel capsids in mouse heart tissue

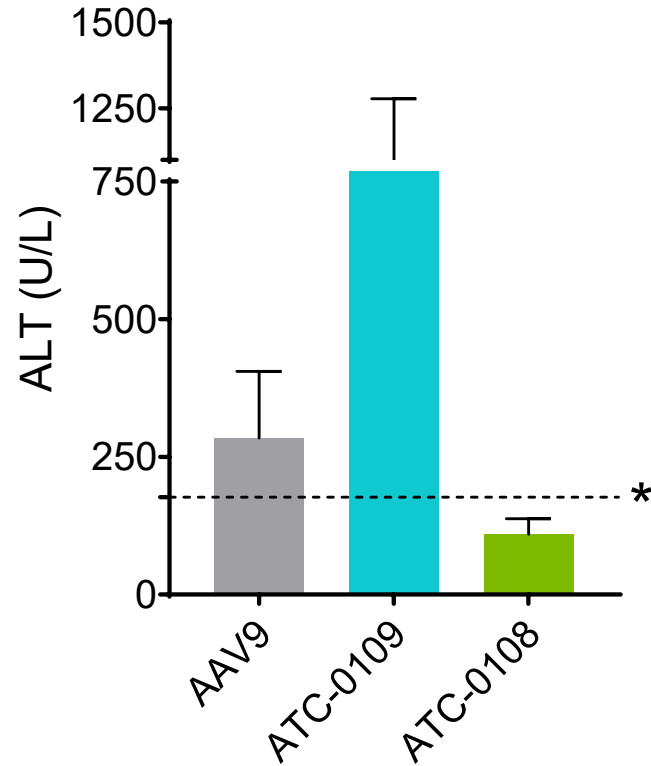


ATC-0108 reduced mRNA expression and DNA levels significantly in liver and DRG in NHPs

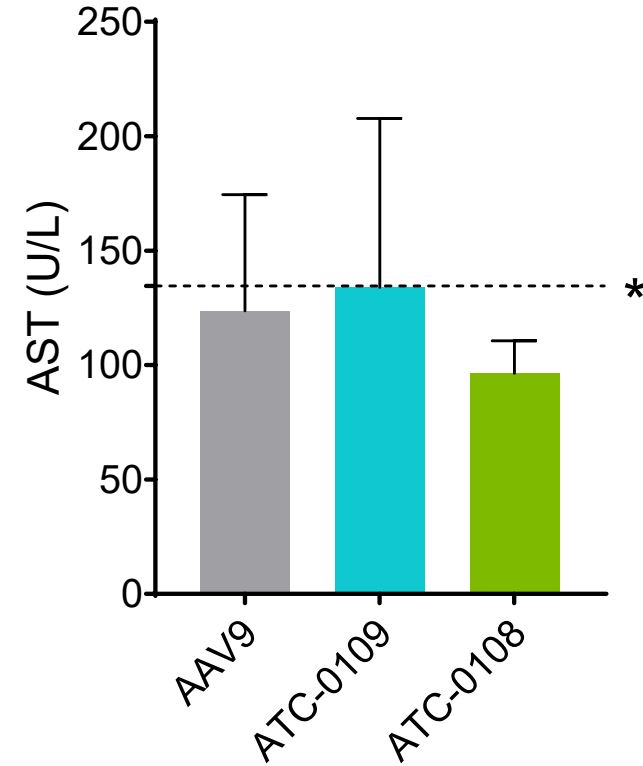


Liver enzyme elevation in serum was not detected after ATC-0108 treatment in NHPs

ALT level at Day 8 after treatment



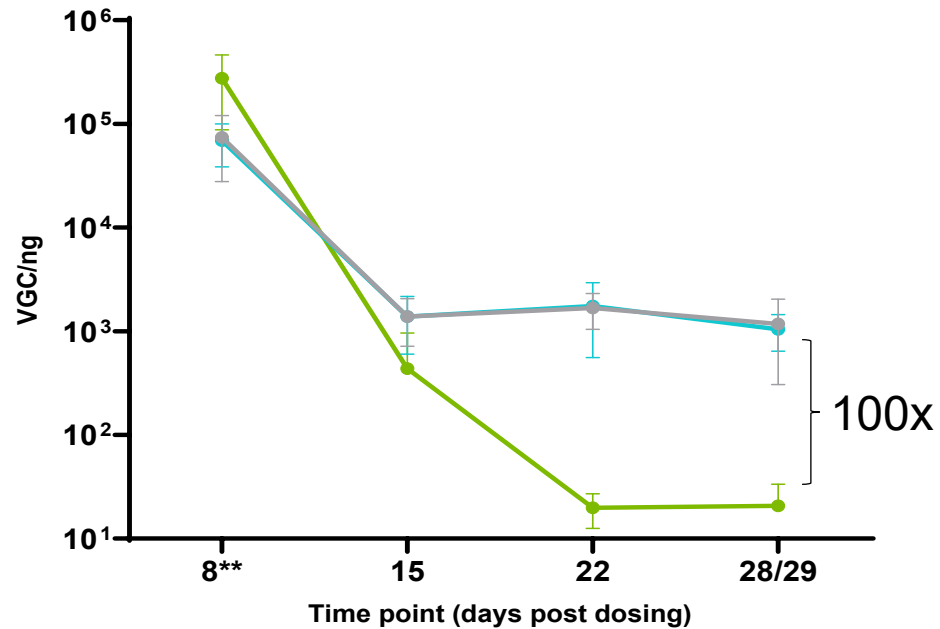
AST level at Day 8 after treatment



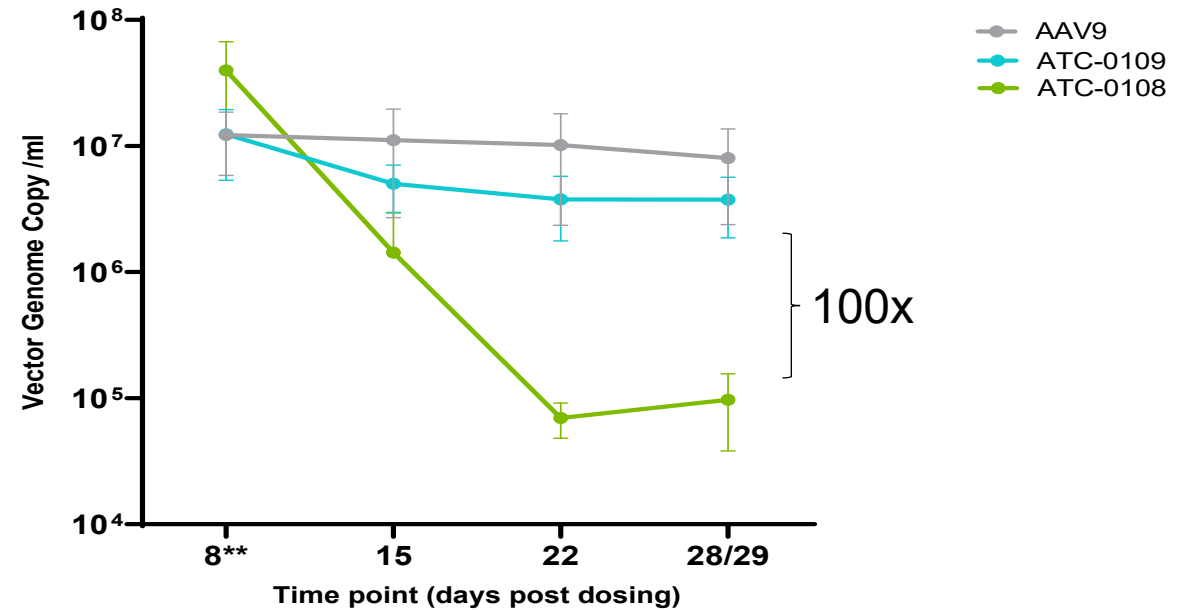
* Maximum levels reported in healthy cynomolgus macaques in *Park et al 2016 Lab Anim Res 32(2), 79-86*

ATC-0108 has reduced levels in blood than AAV9 in NHPs at later times

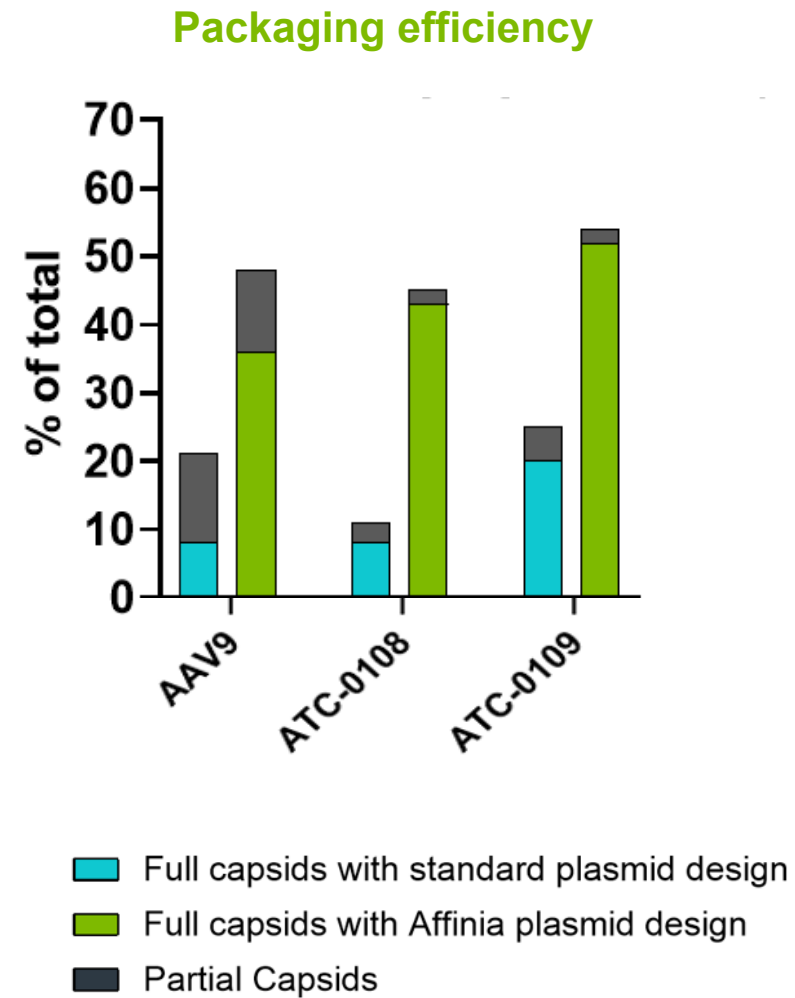
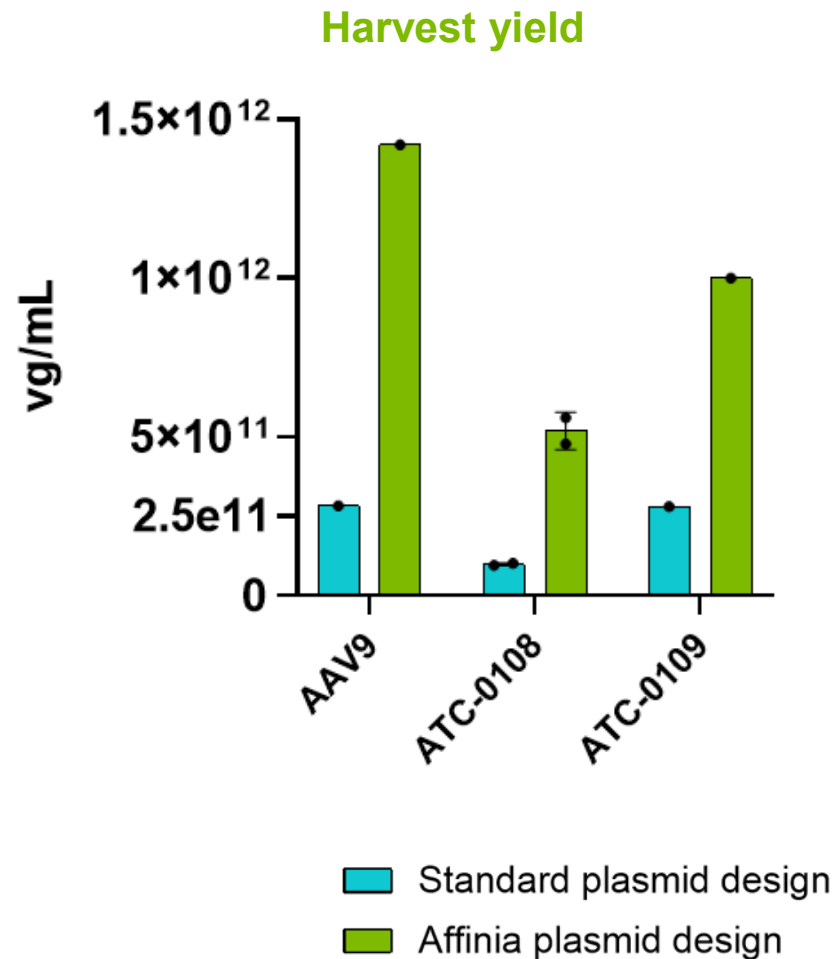
VGC/ng-DNA over time post dosing



VGC/ml over time post dosing



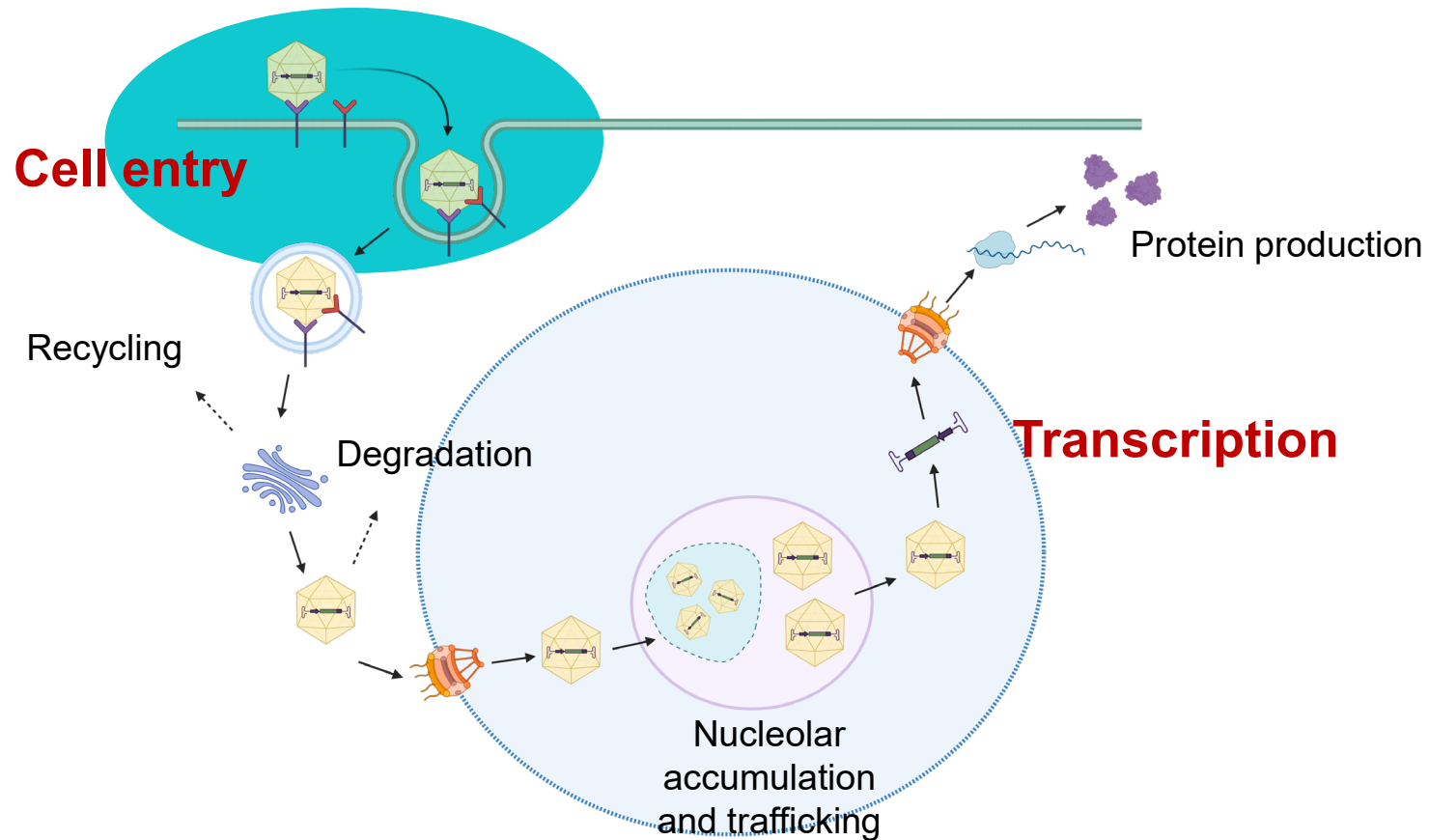
Affinia novel capsids are manufactured with high yields and high % full packaging efficiency



See Oral Abstract #290

MOA of novel capsids trafficking and transduction

Multi-step pathway used by AAV from cell entry to protein production

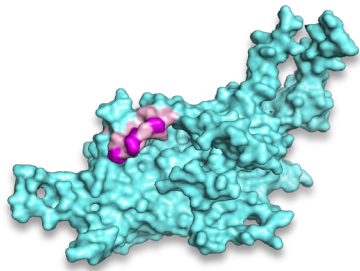


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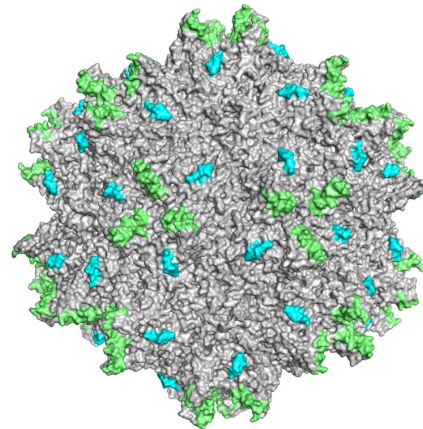
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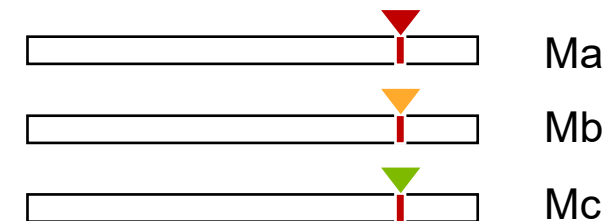
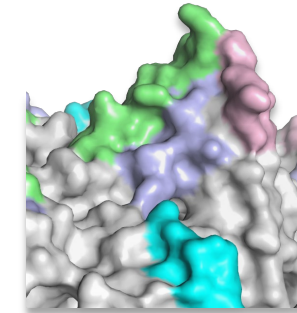


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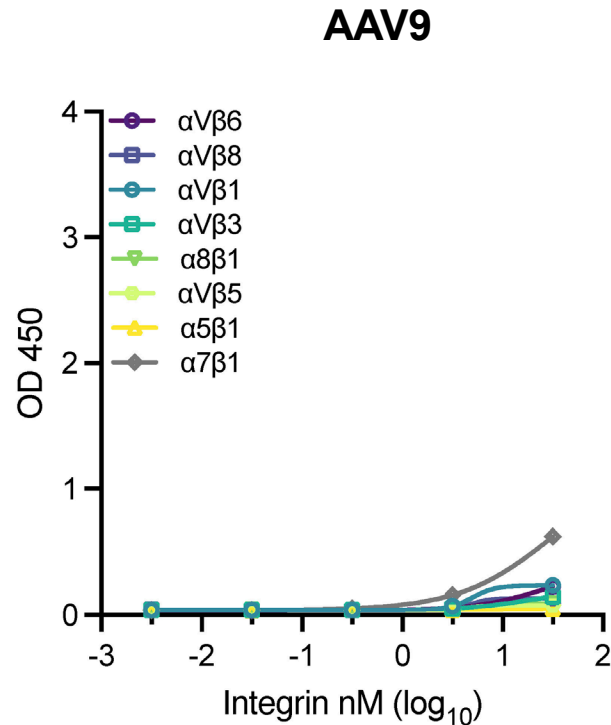
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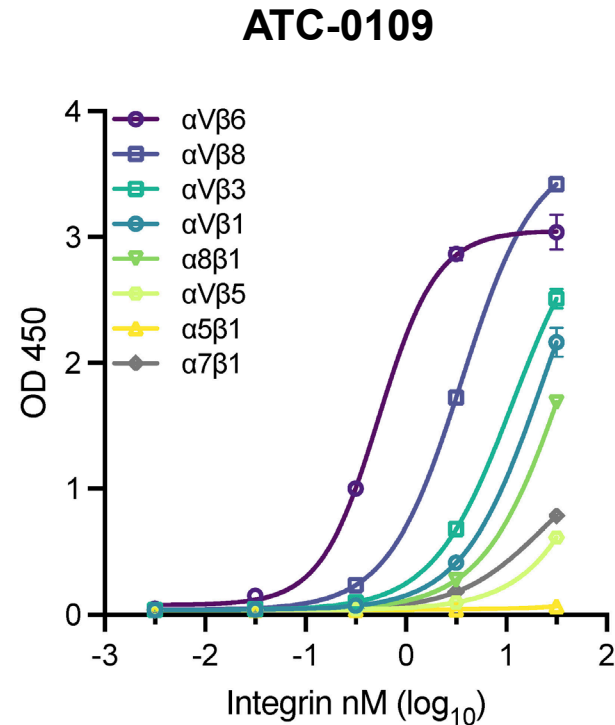


Novel capsids bind $\alpha V\beta 6$ integrin at sub-nanomole level

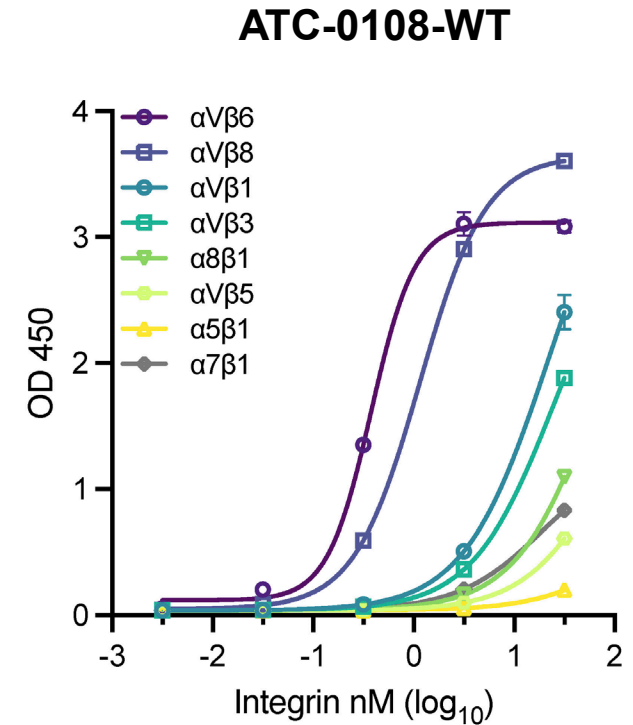
Capsid Integrin binding ELISA assay



$\alpha V\beta 6$ EC50



0.54 nM

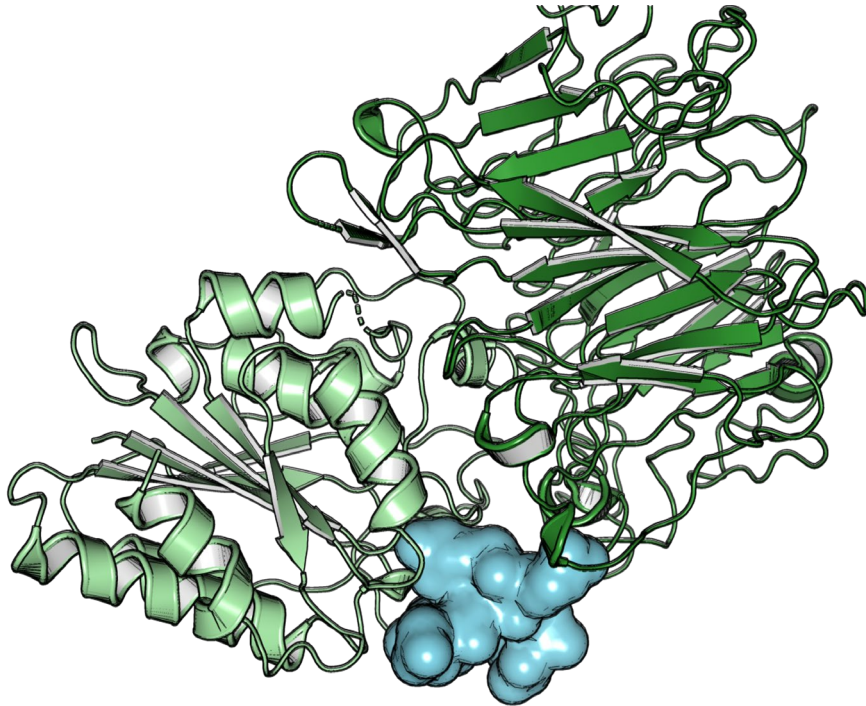


0.37 nM

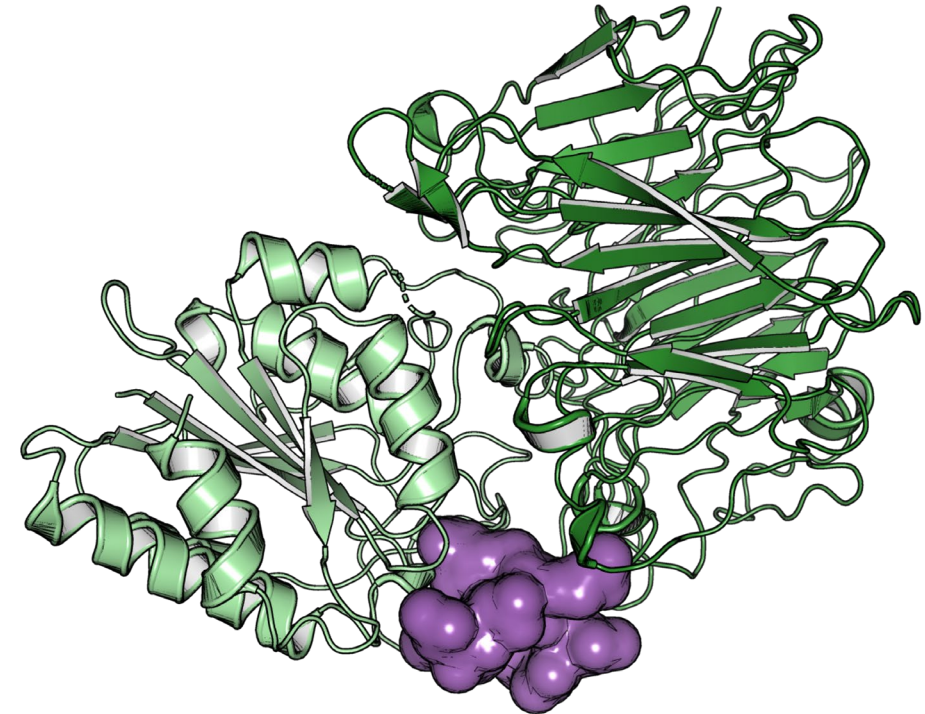
Data generated by Lars Clark (Vertex Pharmaceuticals)

Capsid- $\alpha V\beta 6$ integrin binding determined by CryoEM structure to help guide capsid optimization

ATC-0108-WT in complex with integrin $\alpha V\beta 6$ fragments



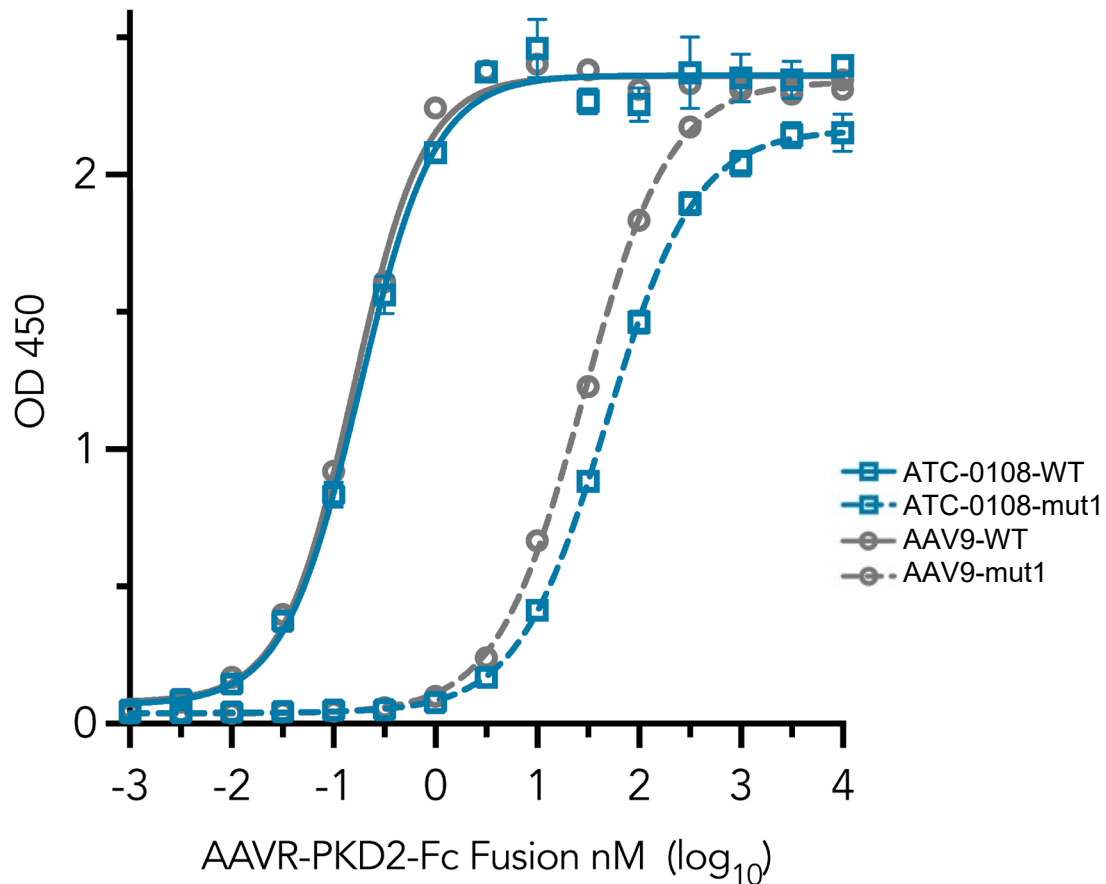
ATC-0109 in complex with integrin $\alpha V\beta 6$ fragments



Data generated by Lars Clark and Adam Johnson (Vertex Pharmaceuticals)

Mut1 significantly decreases binding to AAVR

AAVR-PKD2 binding

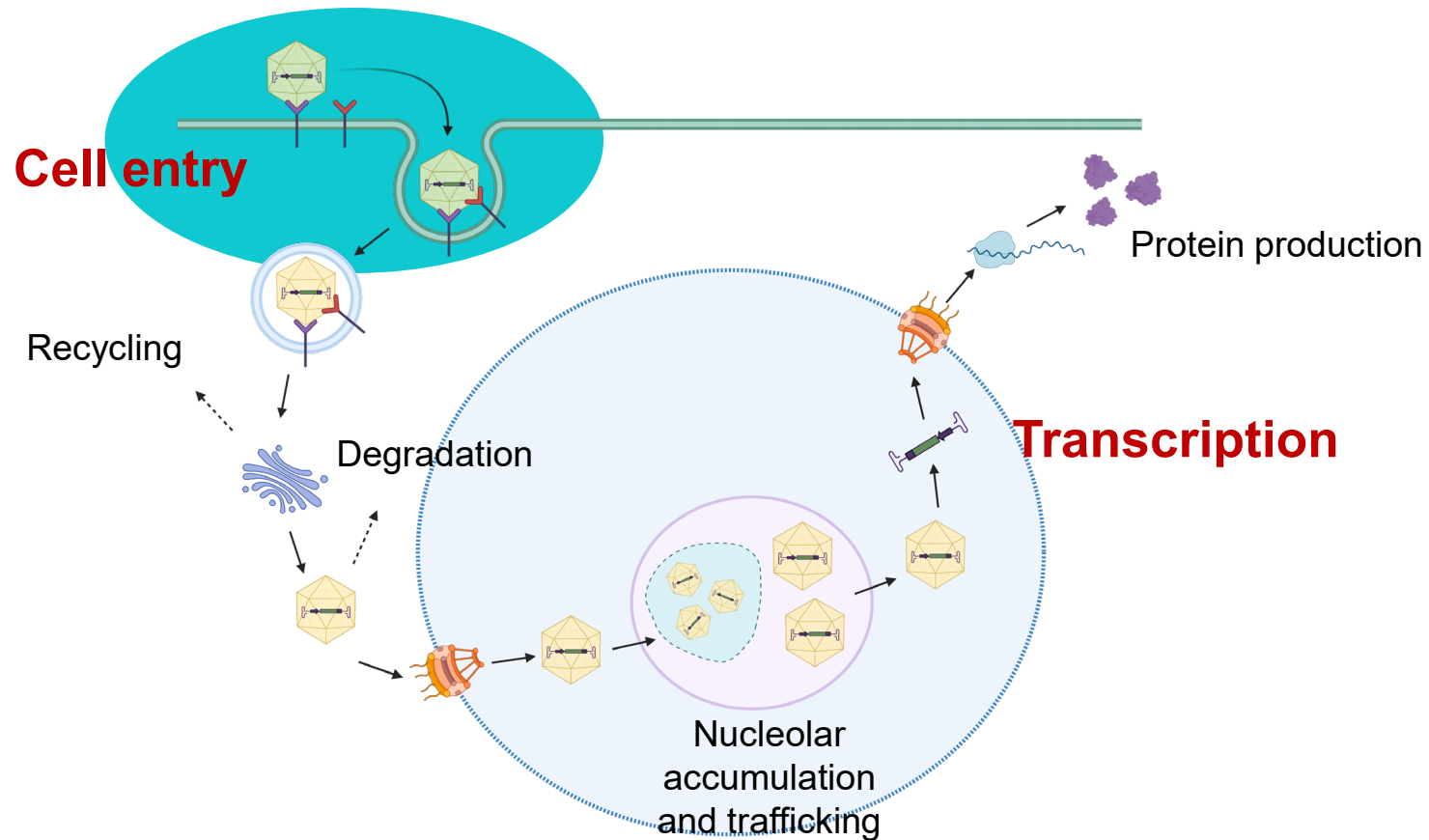


- We hypothesized that Mut1 mutation decrease AAVR binding
- Both ATC-0108 and AAV9-Mut1 have weaker binding affinity to AAVR than ATC-0108-WT and AAV9-WT (>200-fold)
- Biochemical data is consistent with our hypothesis
- Reduced AAVR binding explains decreased tropism of Mut1-containing capsids for other tissues, such as liver

Data generated by Lars Clark (Vertex Pharmaceuticals)

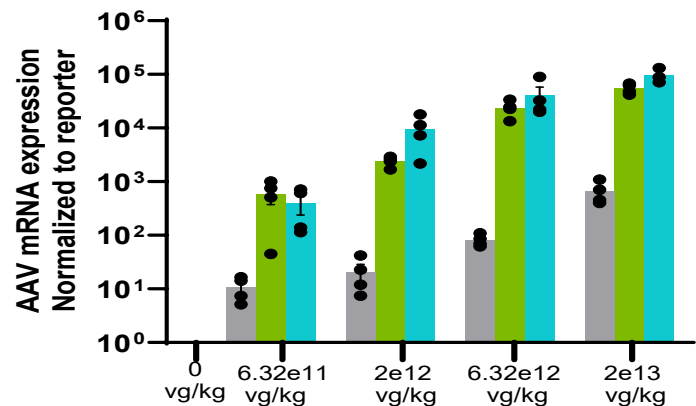
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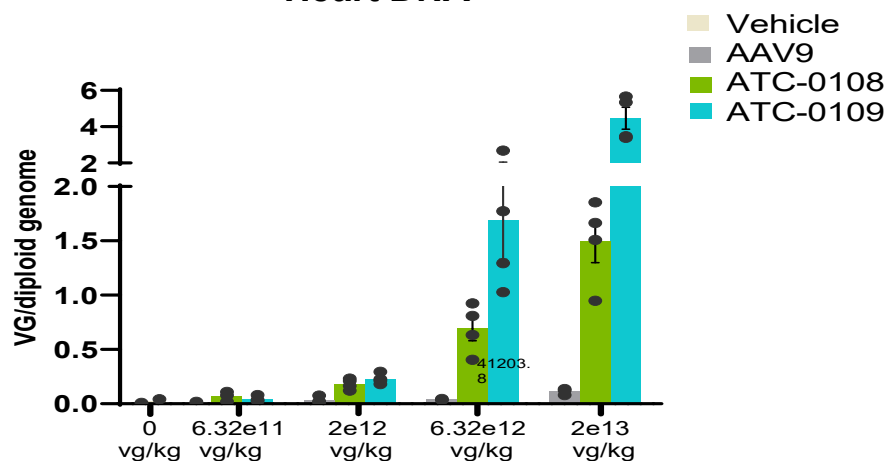


Novel capsids deliver 10x transcription enhancement than AAV9

Heart mRNA



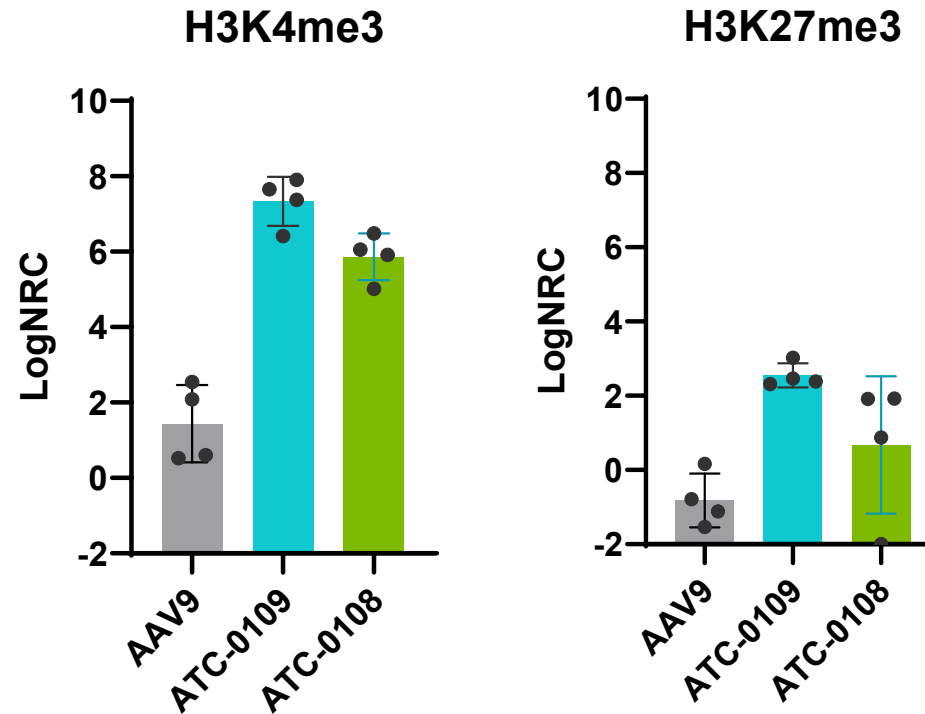
Heart DNA



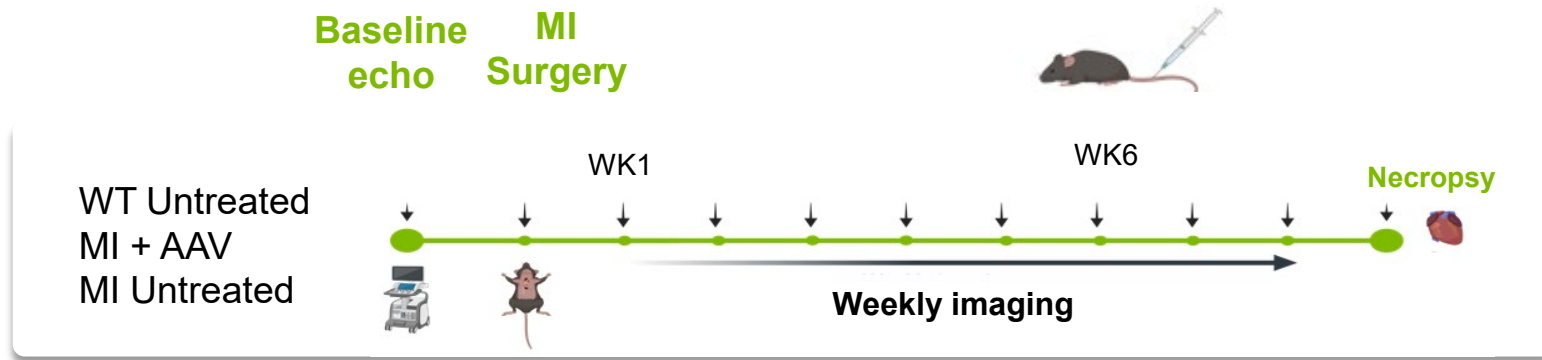
Capsid	Dose	mRNA/DNA over AAV9
ATC-0108	6.32E+11	6.7
ATC-0108	2.00E+12	21.7
ATC-0108	6.32E+12	17.4
ATC-0108	2.00E+13	6.3
ATC-0109	6.32E+11	7.4
ATC-0109	2.00E+12	69.3
ATC-0109	6.32E+12	12.4
ATC-0109	2.00E+13	3.7

Novel capsid-delivered genomes are enriched in active histone marks in mouse heart

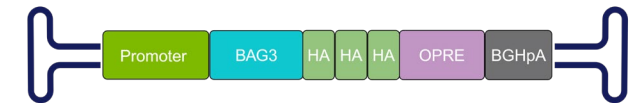
- Literature evidence for capsid influencing epigenetic marks on the vector genome
- Epigenetic marks affect mRNA expression bi-directionally
- Activating histone mark H3K4me3 is enriched in mouse heart tissue transduced by both novel capsids compared to AAV9
- Inhibitory histone mark H3K27me3 is not enriched compared to AAV9



Testing ATC-0108 in a mouse model of Myocardial Infarction (MI)



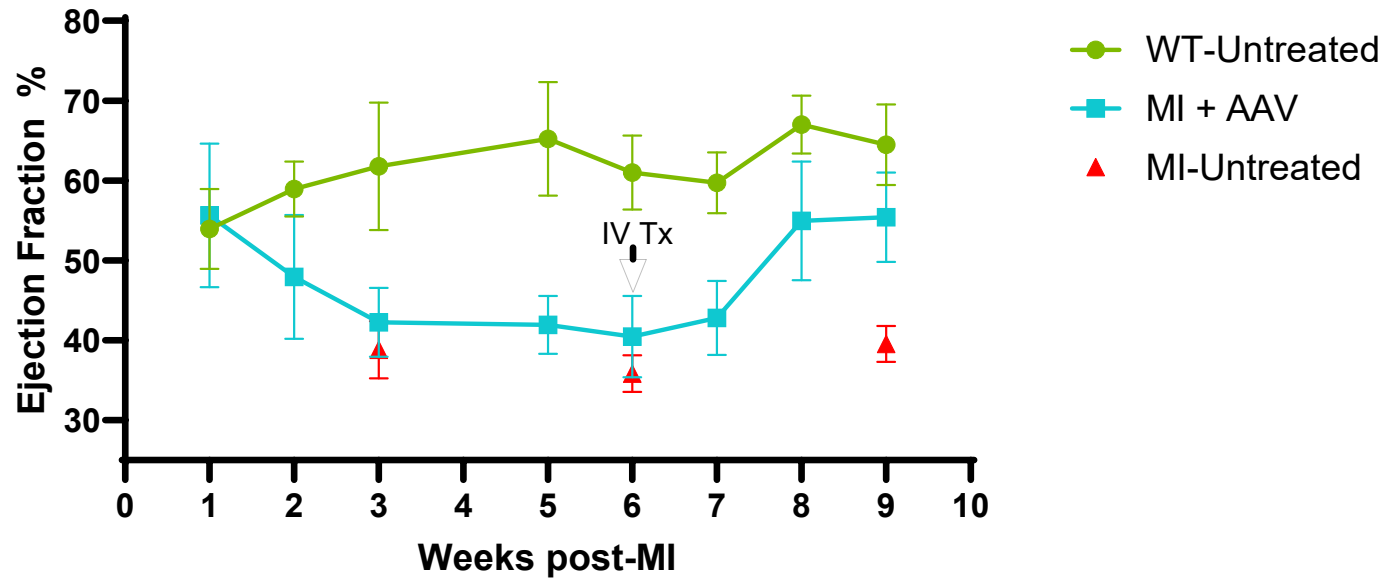
ATC-108 expressing human BAG3
under the control of a cardiac
promoter



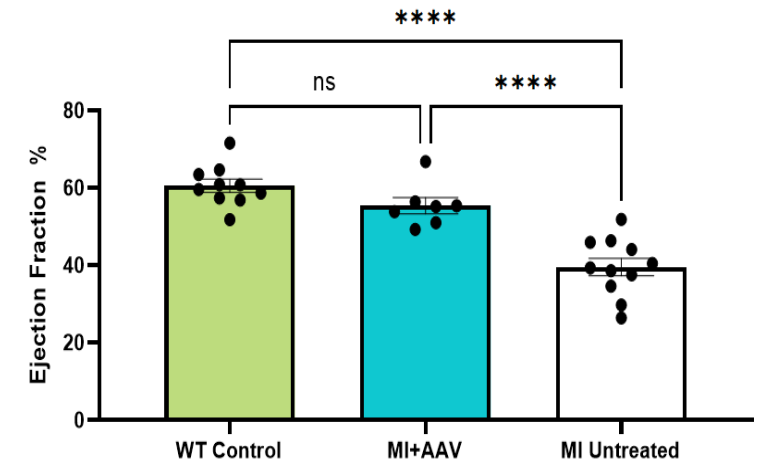
Affinia ATC-0108-BAG3 construct improves cardiac functional deficit in Myocardial Infarction (MI) mice at 2e12 vg/kg dose



Ejection fraction time course



Ejection fraction 3 weeks post injection



See poster #1795

Summary

- We have established a machine learning guided rational design capsid engineering platform at Affinia
- The modular design strategy based on AAV cell surface receptor interaction mechanism enables fine tuning of tissue targeting and liver plus DRG detargeting
- Affinia novel capsids achieved >100 fold in vivo transduction enhancement relative to AAV9 in skeletal and cardiac muscle in mice and NHPs. This improved transduction profile is durable
- Affinia novel capsids achieved meaningful cardiac transduction in NHP at a 10x lower dose than currently used in clinical trials
- Novel capsids bind $\alpha V\beta 6$ integrins
- ATC-0108 capsid is liver detargeted due to reduced binding to AAVR. ATC-0108 treatment does not lead to liver enzyme elevation in serum and there is reduced levels in blood after dosing
- Delivery of BAG3 gene by ATC-0108 improved cardiac functional deficit in MI mice at a dose level of $2e12$ vg/kg

Acknowledgements

Affinia Therapeutics

- Charles F. Albright
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- Laura K. Richman
- Fagun Shah
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- Jie Tan
- Cara West

Vertex Pharmaceuticals

- Lars Clark
- Adam Johnson
- Shen Shen
- Harmon Zuccola
- Raj Maganti
- Dylan Jacobs
- Alison McVie-Wylie
- John Gray
- Mike Cooke



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