Structure-activity relationships guided engineering of AAV capsids with optimized skeletal muscle, cardiac muscle, and CNS tropism

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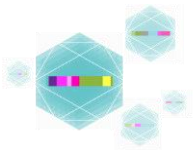
*, Equal contributions

Disclosures

- All authors are employees of Affinia Therapeutics

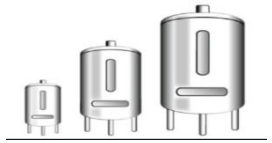
Our objectives: Best-in-class capsids for muscle and CNS

Novel capsids



Novel promoters

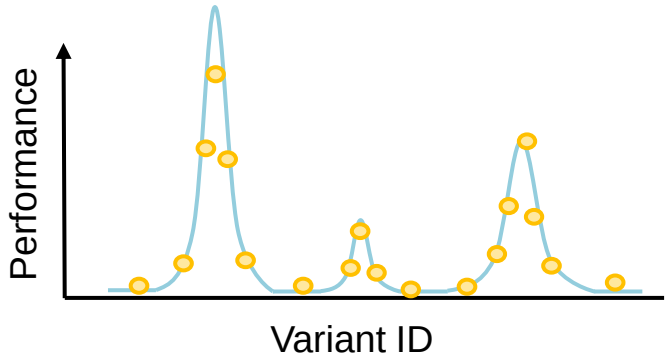
Novel manufacturability approaches

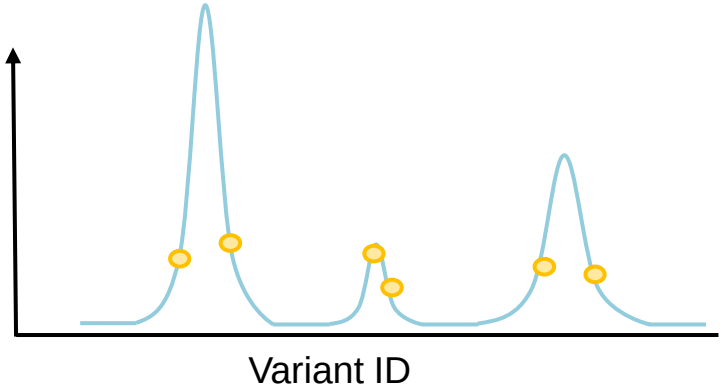


- Capsids with increased expression and fraction of cells expressing
  - Skeletal and Cardiac muscle
  - Skeletal muscle
  - Cardiac muscle
  - CNS
- Capsids that detarget the liver and DRG
- Capsids with acceptable manufacturing and seroprevalence

Structure-activity relationships (SAR) guide capsid design

	Biased library (Capsid Optimization)	Random library (Capsid Discovery)
Library design	Rational design and random (AAV and peptide)	Random (AAV and peptide)
Search space	Narrow per each library	Wide
Library size	≤50k	Theoretically large, for example 1.3 billion for 7-mer
Library coverage	>90%	<0.1%
Variant performance assessment	Tissue Enrichment: Tissue levels normalized for input concentration	Variant concentration
Advantages	▪ Deep coverage of the entire library, including winners and losers ▪ Built-in amino acid redundancy compensates for data variability and allows statistics ▪ Machine learning possible (ML)	▪ Samples large variant sequence space ▪ Uses data from “winners”, not losers ▪ ML difficult



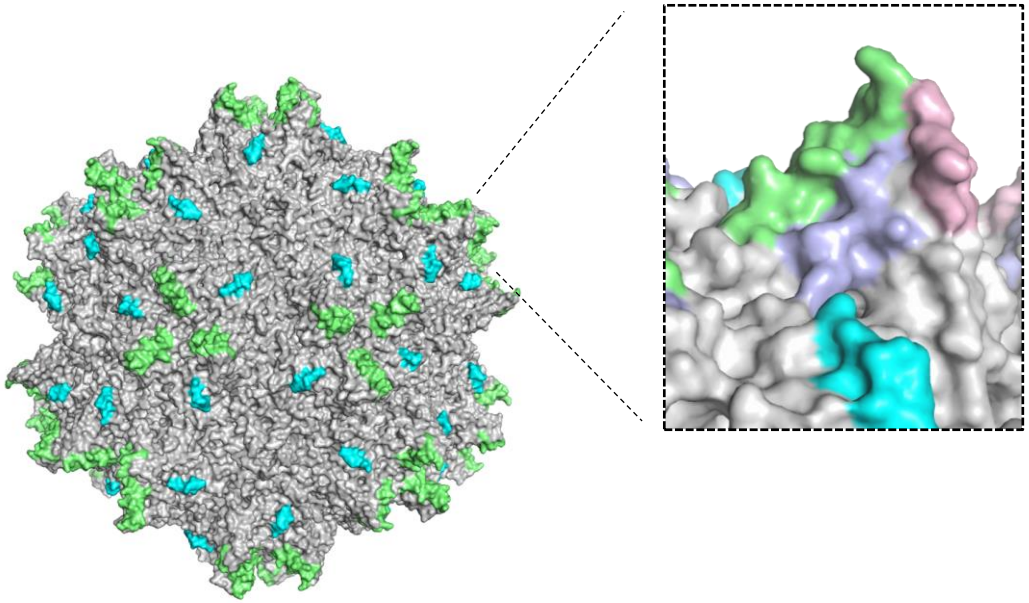


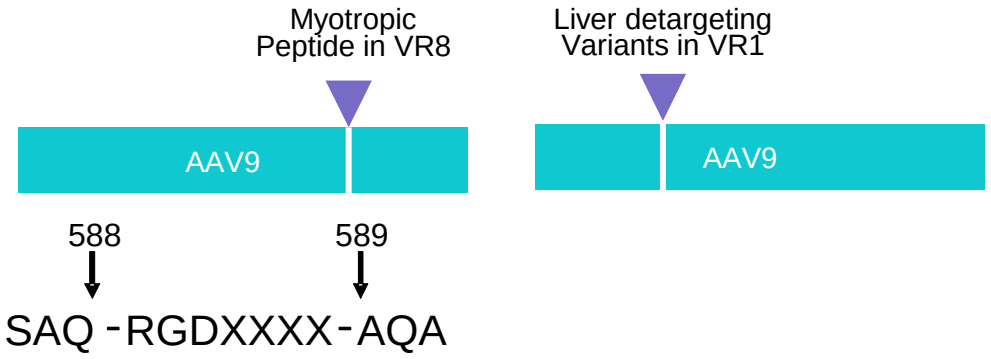
Application example: Liver detargeted myotropic capsid

Dual strategy of employing AAV + peptide sequences increases capsid diversity and range of performance

AAV backbone changes

Peptide insertions

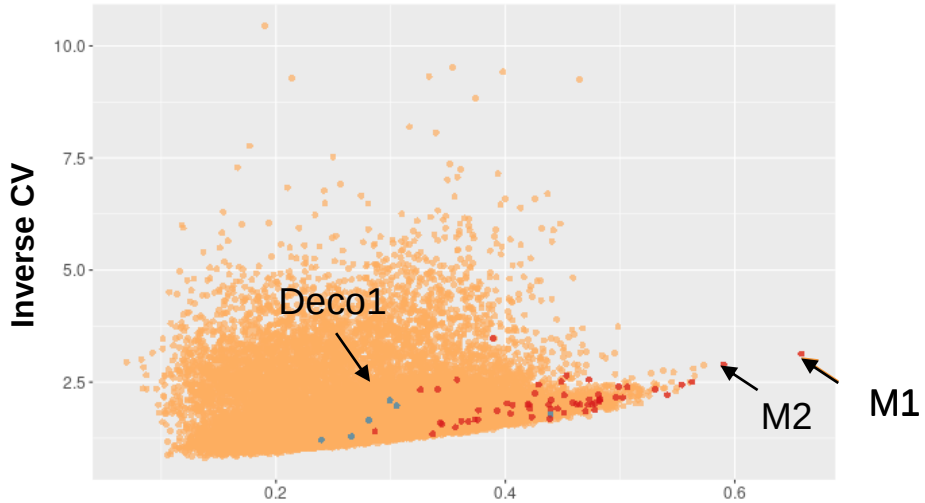




- Designed and screened a peptide library for myotropism
- Designed and screened an AAV capsid backbone library for liver detargeting

Identifying myotropic peptides using SAR from VR8 library

Myotropic peptide screen based on VR8 library

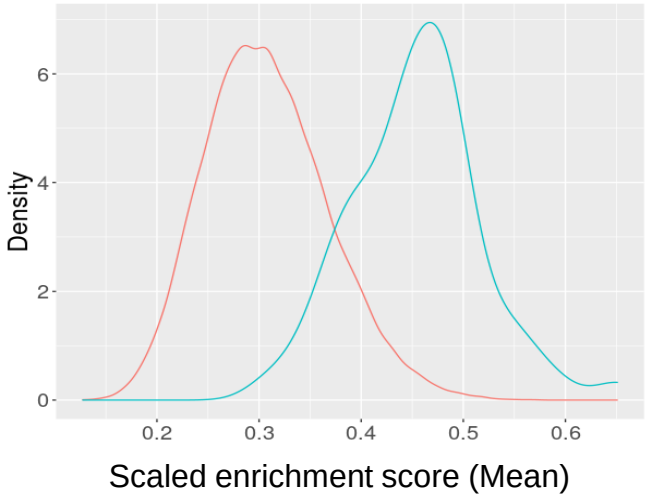


Scaled muscle enrichment score (Mean)

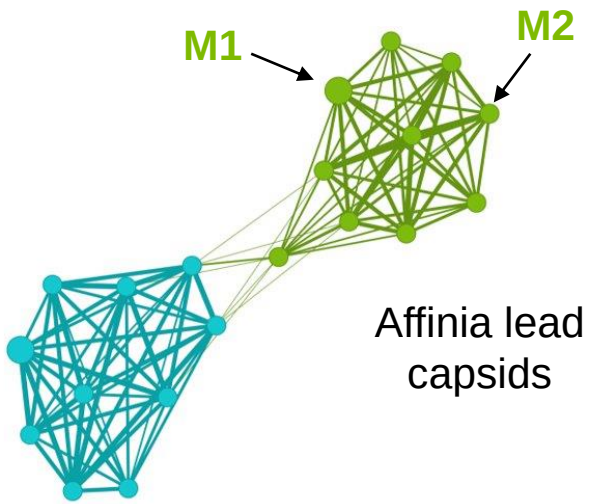
"Tissue Enrichment" equals tissue mRNA normalized for amount of capsid injected

Red dots represent variants with muscle-tropic sequence motif

Linear modeling to identify myotropic peptide SAR



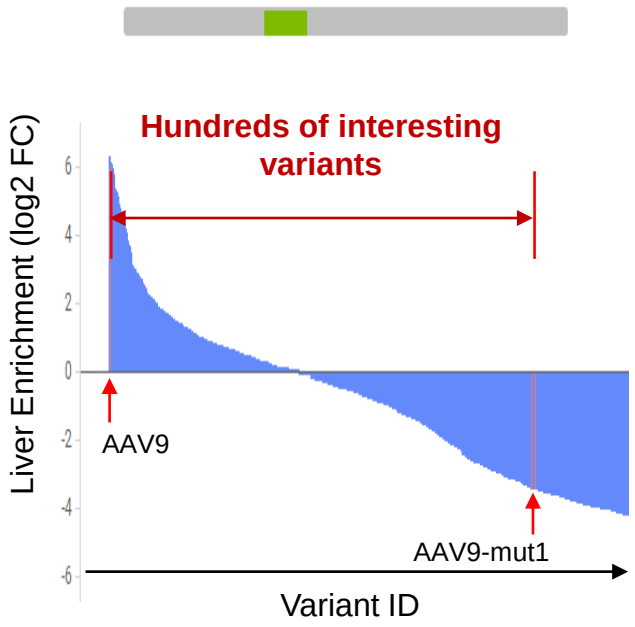
Network analysis and structural modeling to validate findings



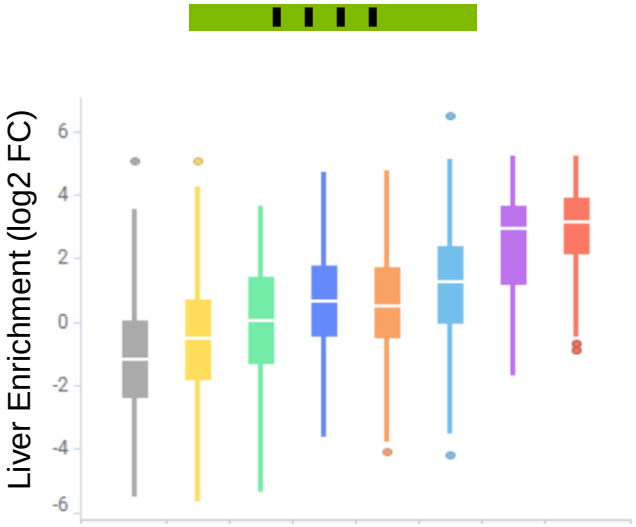
AFT-PR-0014, 3 cynos, IV, 5e13 vg/kg total dose, CMV promoter, day 28

Identifying liver detargeted AAVs using SAR from VR1 library

Liver detargeting AAV screen based on VR1 library

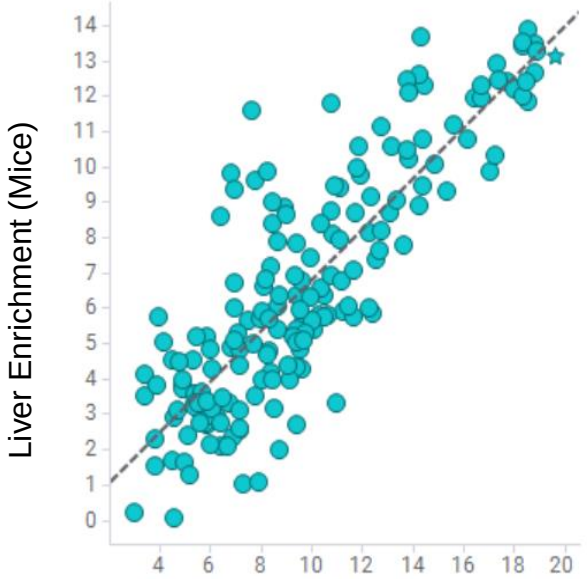


Linear modeling to identify sequence motifs for liver detargeting



Sequence motif at 4 distinct positions

SAR reproducible between mice and NHP



AFT-MR-0026: Mouse study of VR1 library, 3.5K capsids, total dose 5e13 vg/kg, IV, C57BL/6 mice, 7 active + 1 control, day 28
AFT-PR-0018: Pooled capsid study, 3.4e12vg/kg ICM or 5e13 vg/kg IV, 3 cynos per group, day 30.

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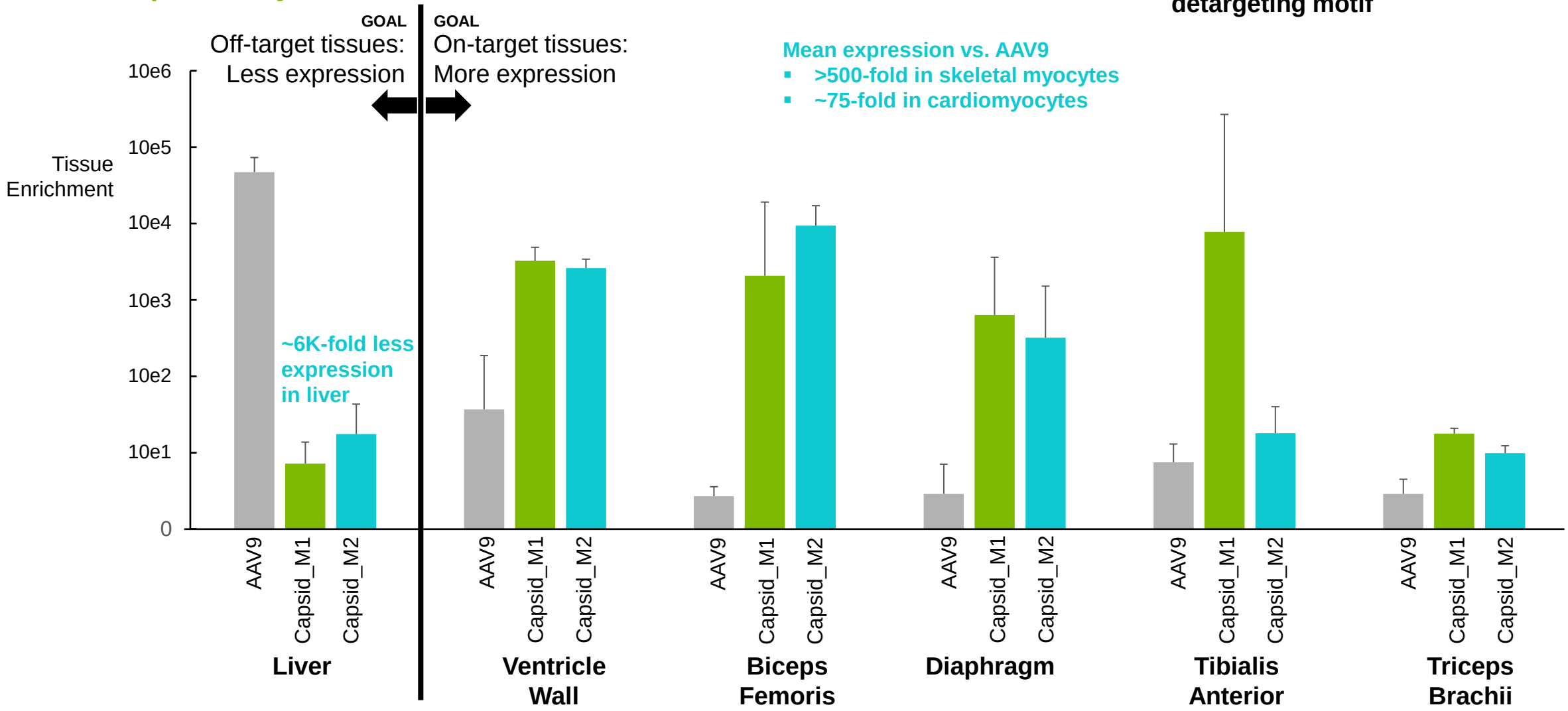
Capsids identified that increase expression in both skeletal & heart muscle while detargeting liver

Pooled capsid study

M1 and M2 have liver detargeting motif

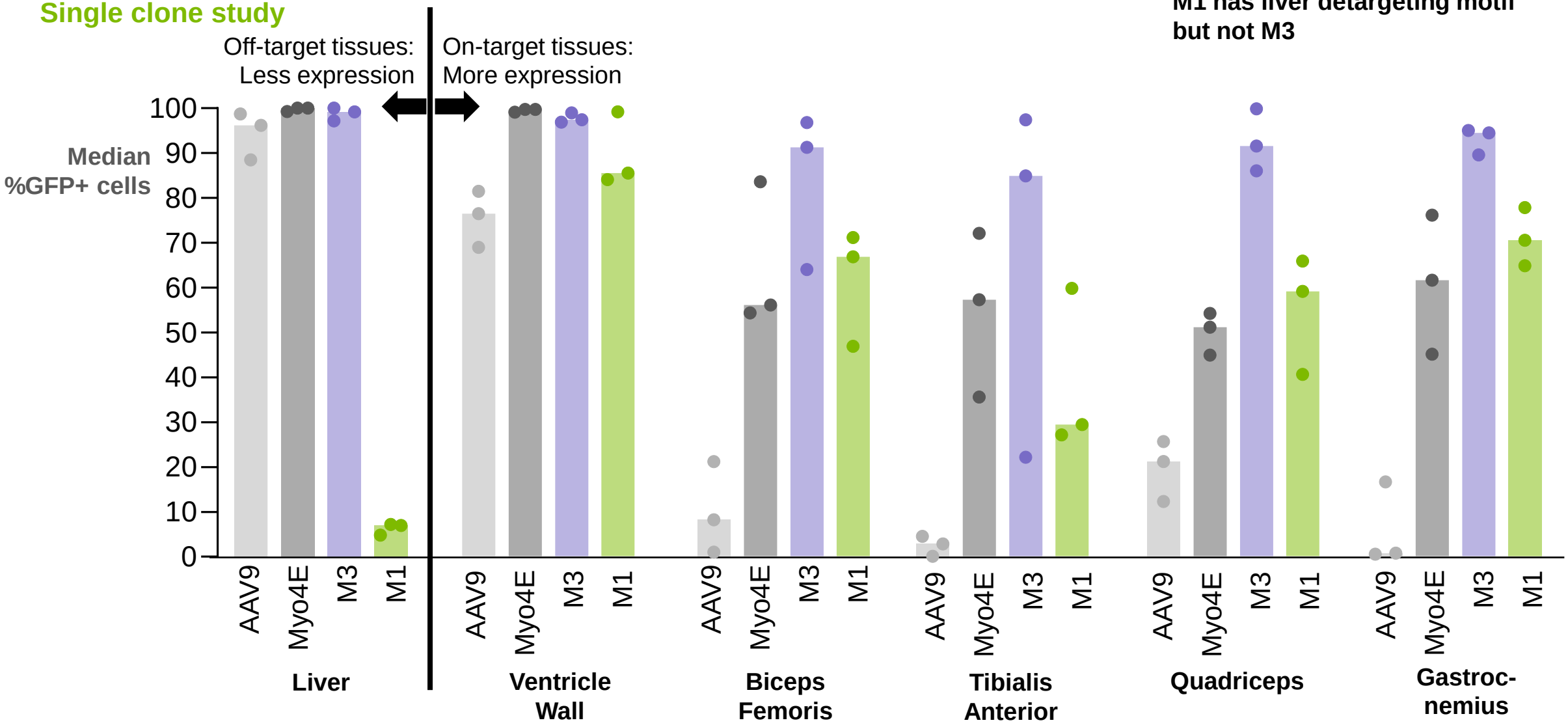


AFT-PR-0014, 3 cynos, IV, 5e13 vg/kg total dose, CMV promoter, day 28

M1 and M3 increased GFP+ cells relative to AAV9 in both skeletal & heart muscle while M1 decreased liver GFP+ cells

Single clone study

M1 has liver detargeting motif but not M3



AFT-PR-0020; Cyno (3 animals per group); dose 1e14vg/kg delivered IV; GFP and CAG promoter; day 28

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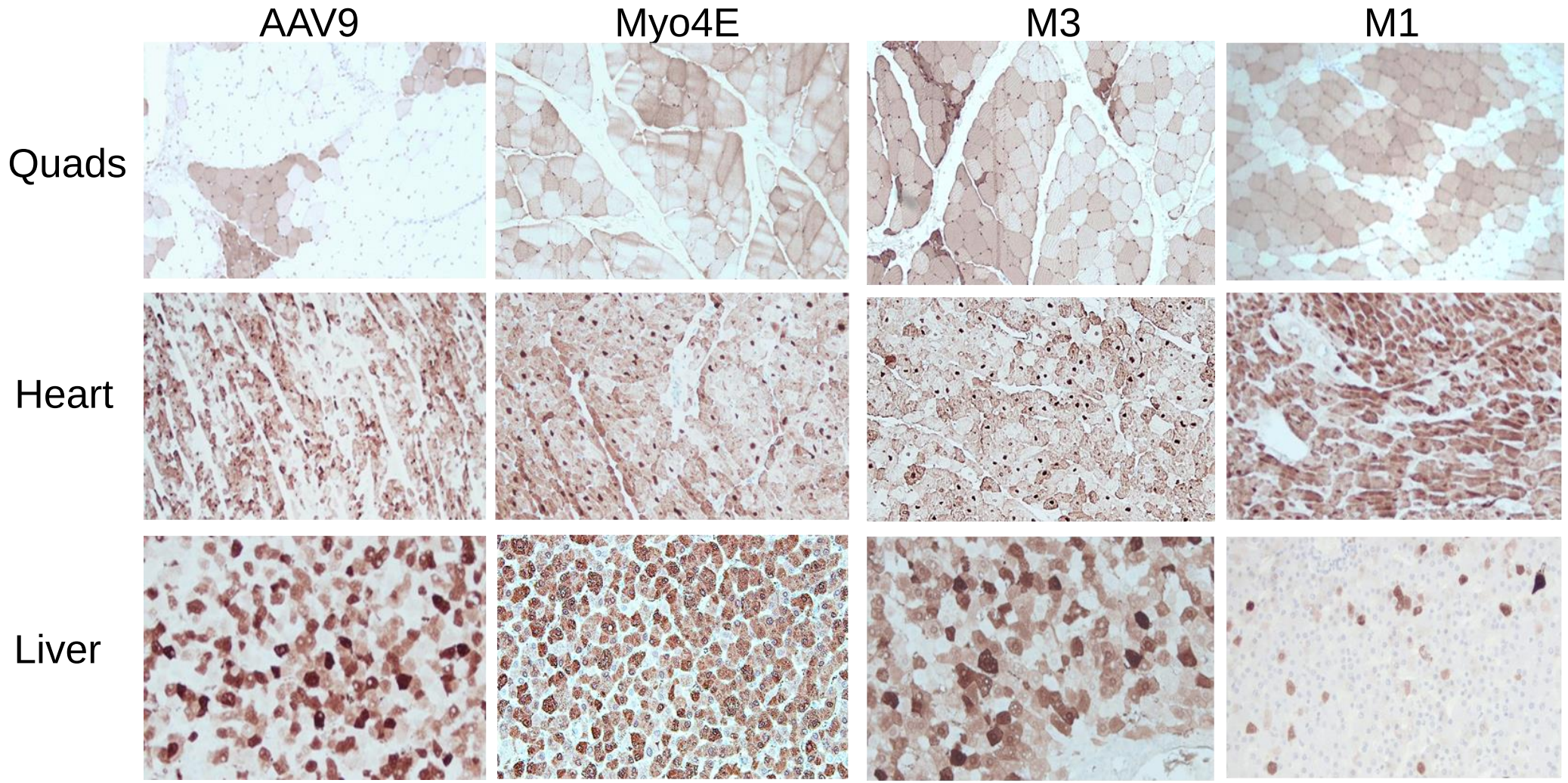
M1 and M3 capsids increased GFP protein in both skeletal & heart muscle while M1 decreased liver GFP relative to AAV9

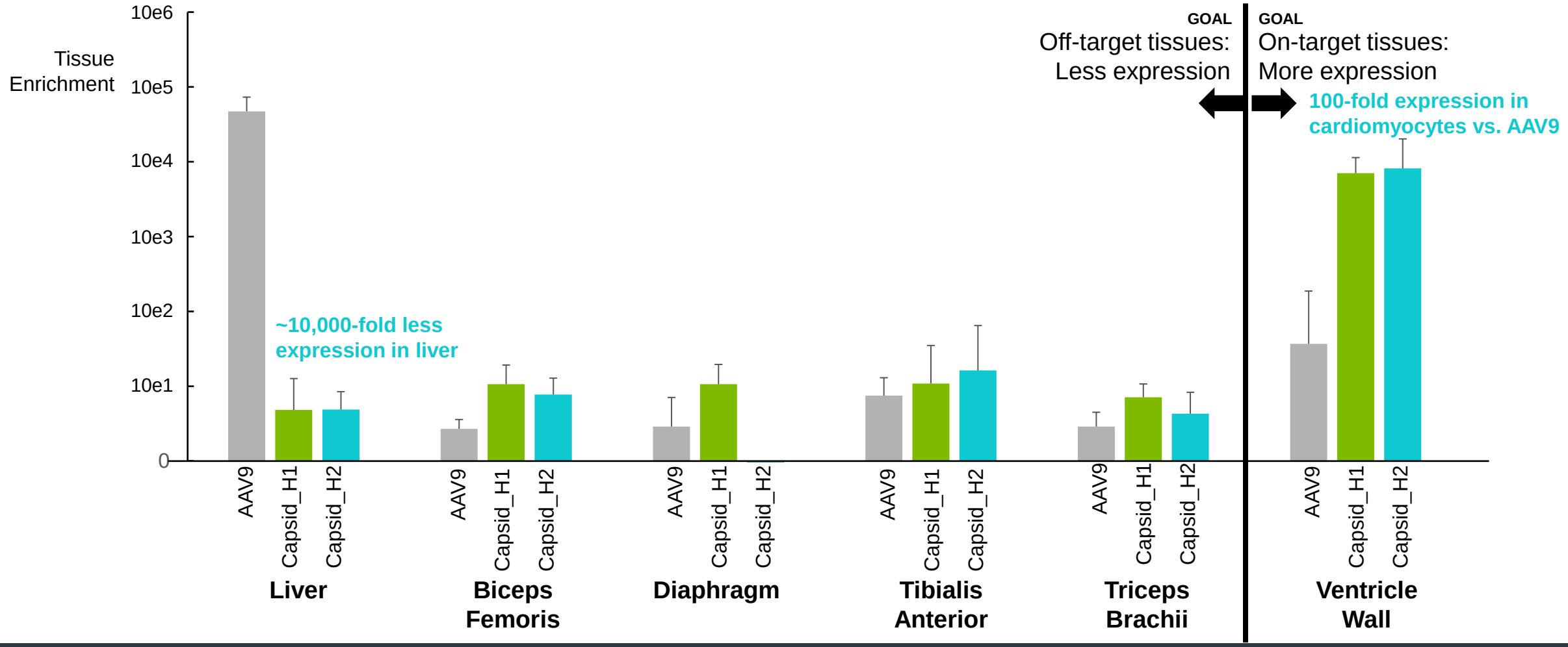
Single clone study, IHC, 10x magnification



AFT-PR-0020; Cyno (3 animals per group); dose 1e14vg/kg delivered IV; GFP and CAG promoter; day 28

Using SAR to differentiate muscle types: Capsids for heart

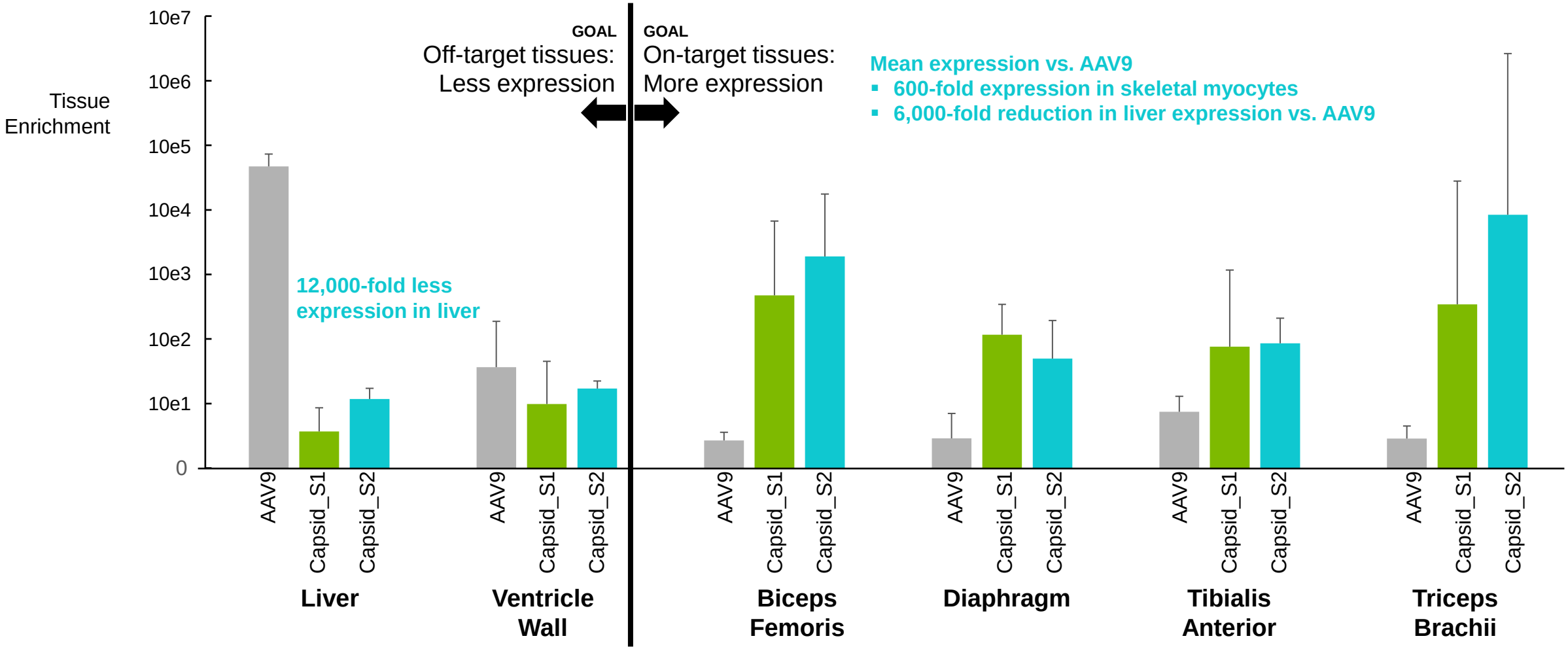
Pooled capsid study



AFT-PR-0014, 3 cynos, IV, 5e13 vg/kg total dose, CMV promoter, day 28

Using SAR to differentiate muscle types: Capsids for skeletal muscle

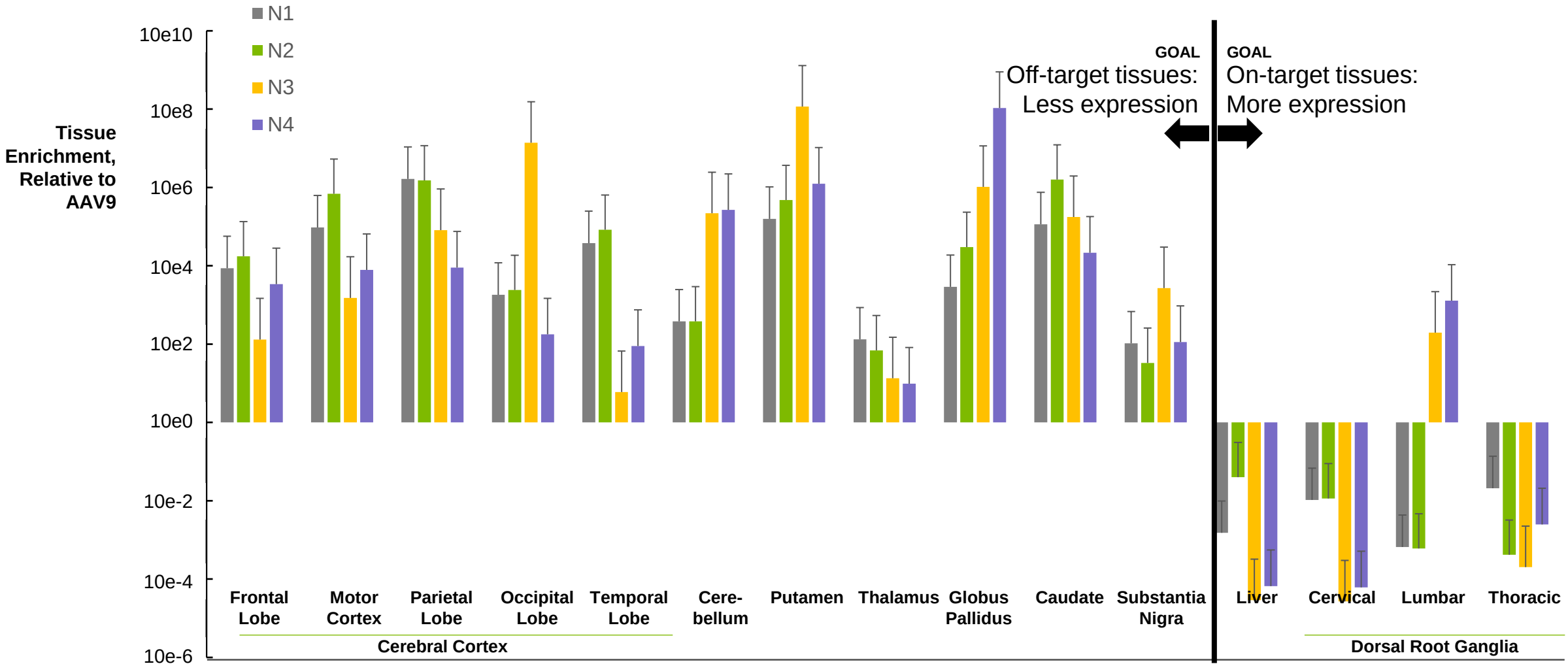
Pooled capsid study



AFT-PR-0014, 3 cynos, IV, 5e13 vg/kg total dose, CMV promoter, day 28

Additional capsids optimized for CNS (Normalized to AAV9, IV delivery)

Pooled capsid study



AFT-PR-0021: 55K capsid pooled study, CMV promoter, total dose 2e13 vg/kg delivered IV (3.6e8 per capsid), 3 cynos, day 28; AFT-PR-0019, 3 cynos, IV, 2e13vg/kg total dose, undisclosed therapeutic transgene, UBC promoter, day 28

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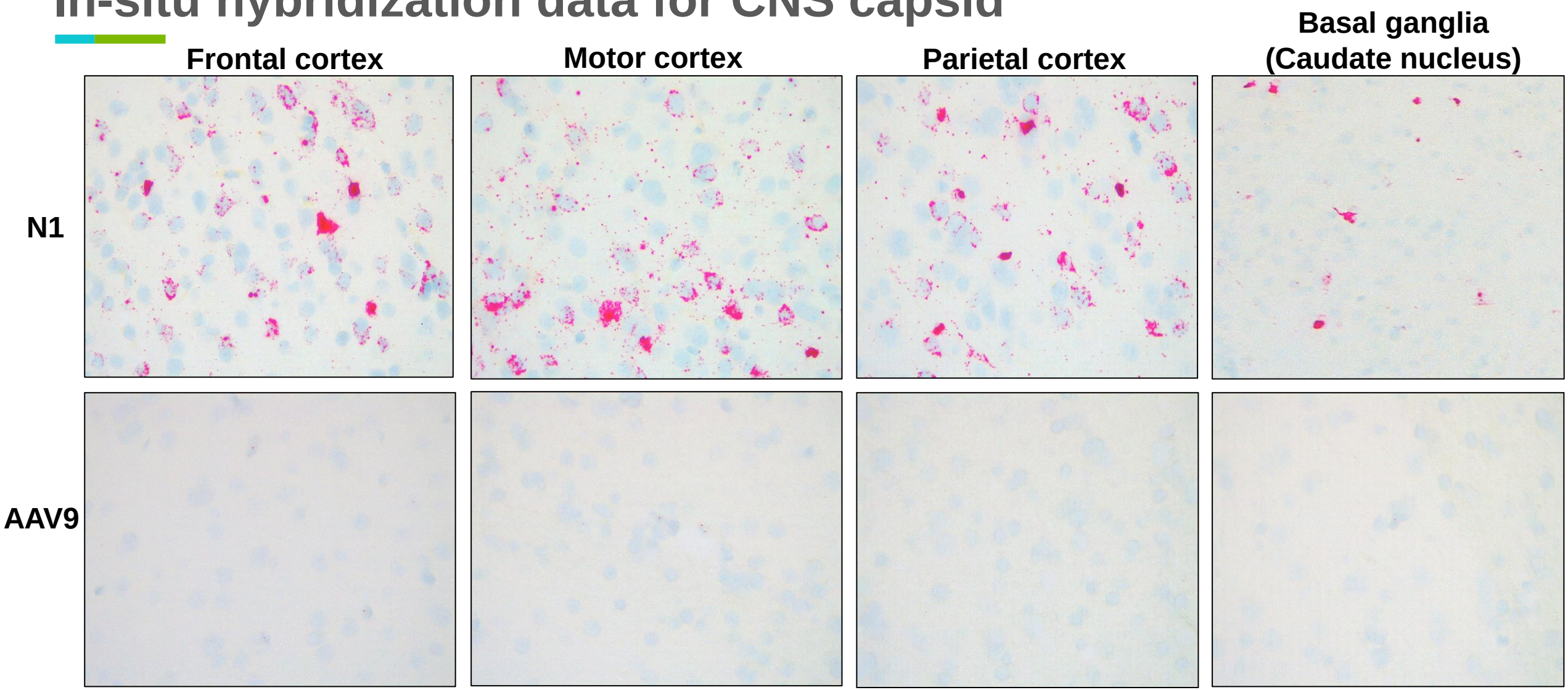
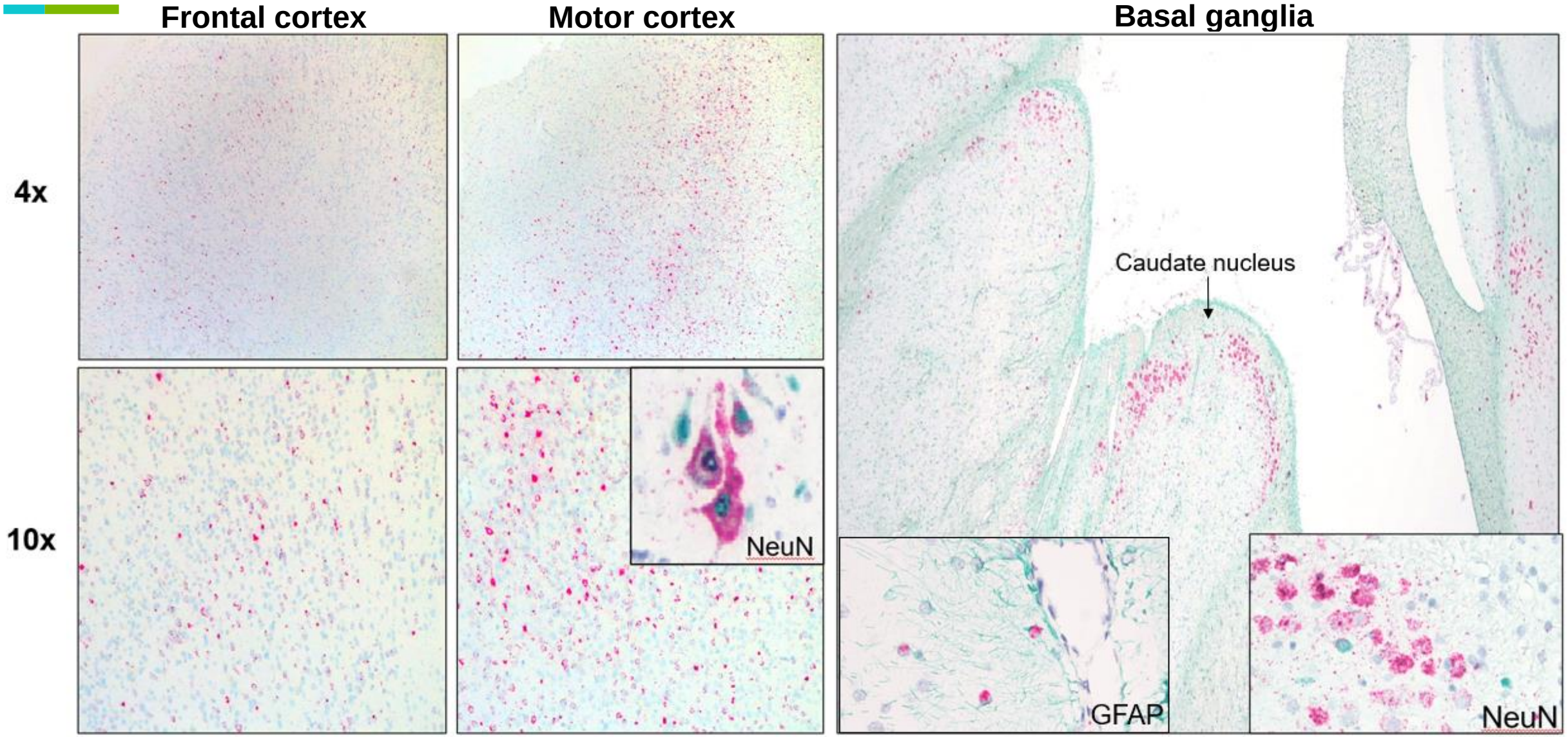
In-situ hybridization data for CNS capsid



AFT-PR-0019, 3 cynos, IV, 2e13vg/kg total dose, undisclosed therapeutic transgene, UBC promoter, day 28

ISH/NeuN IHC double staining demonstrates N1 neuronal tropism



Pink=transgene ISH
Green=cell marker IHC (NeuN=neurons; GFAP=astrocytes)

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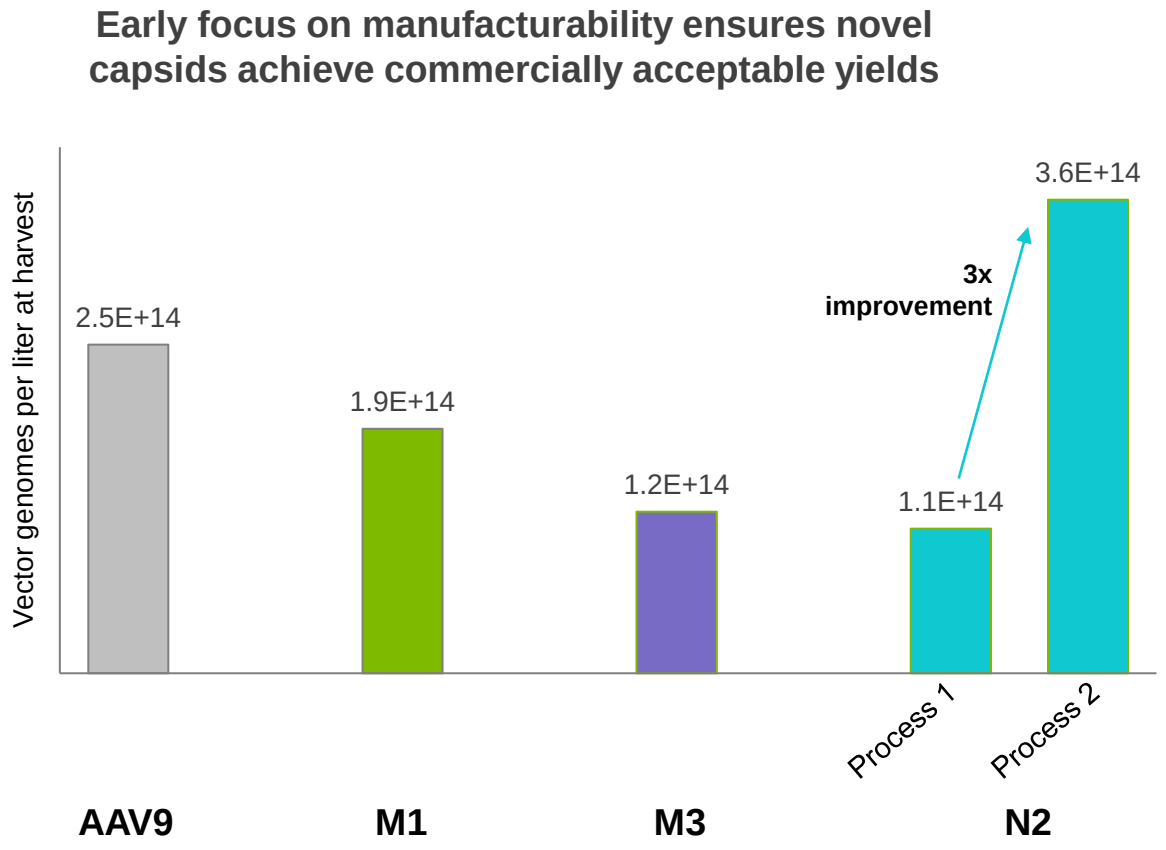
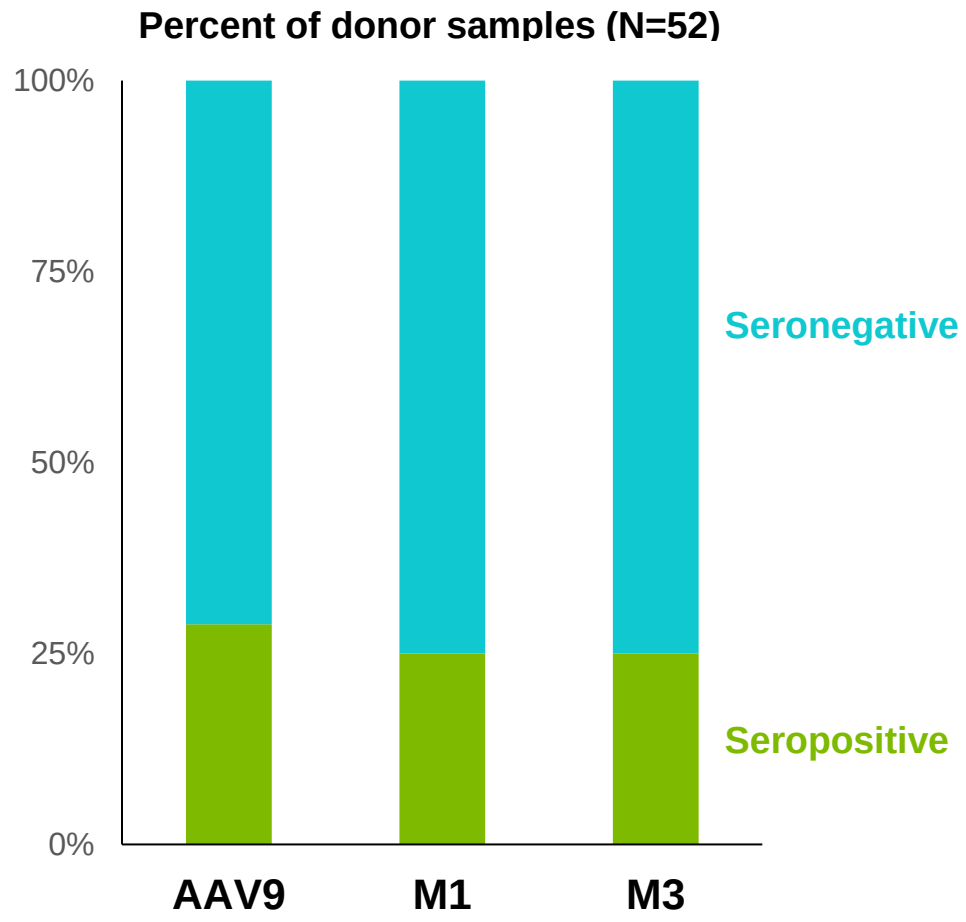
Novel capsids have commercially acceptable manufacturing yields and seroprevalence



All values are with GFP payload except N2 which is with an miRNA payload.



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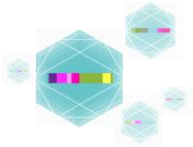

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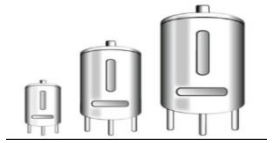

- Capsids with increased expression and fraction of cells expressing
  - Skeletal and Cardiac muscle (M1, M3)
  - Skeletal muscle (S1, S2)
  - Cardiac muscle (H1, H2)
  - CNS (N1, N2, N3, N4)
- Capsids that detarget the liver and DRG
- Capsids with acceptable manufacturing yields and seroprevalence

Acknowledgements

- Affinia Therapeutics
  - Joe Collins
  - Anthony DeMarco
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  - Cara West
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