



Directed Evolution in Nonhuman Primates Identifies AAV Capsids with a Novel Motif Enabling Efficient Muscle Targeting with Reduced Liver Tropism

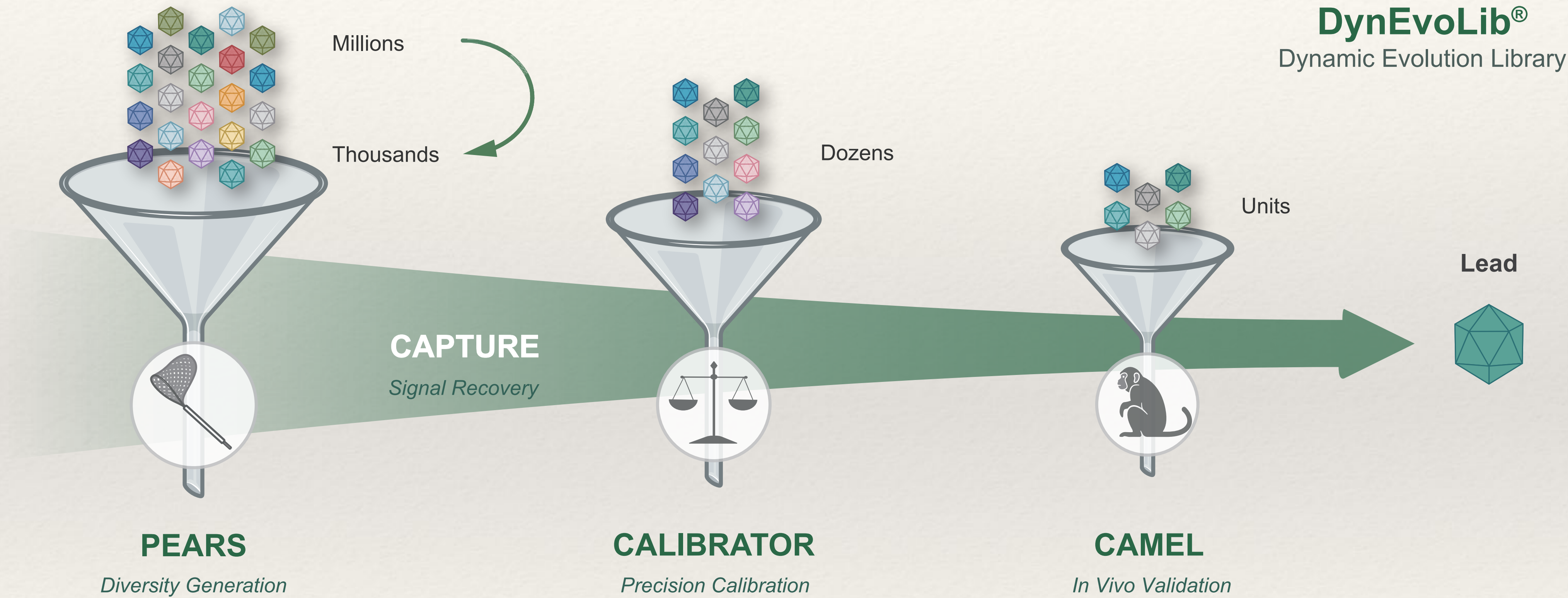
Gang Wang, Ying Bai, Qingyu Deng, Yan Jiao, Ruixue Chen, Yangyang Zhong
Byongen Therapeutics, Beijing, China

ASGCT 29th Annual Meeting | Boston, Massachusetts | May 14, 2026

Disclosures

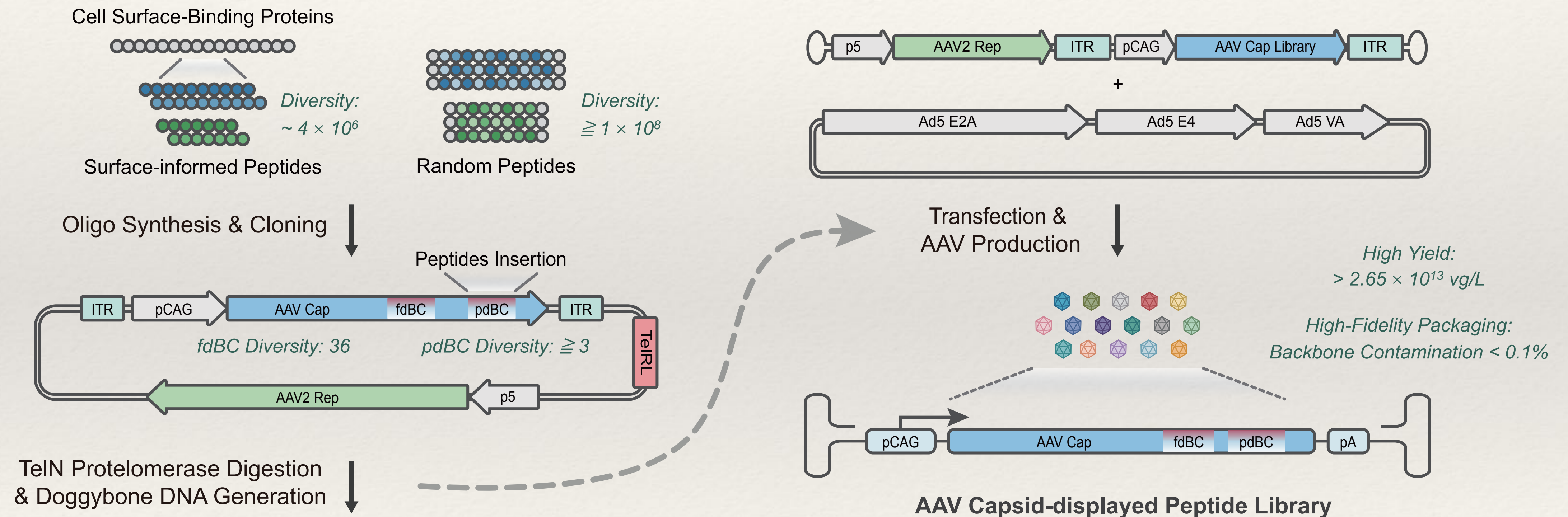
Gang Wang is a full-time employee and shareholder of Byongen Therapeutics and listed as an inventor on patents related to AAV capsid engineering.

The DynEvoLib Platform: A Comprehensive Directed Evolution Pipeline for AAV Capsids



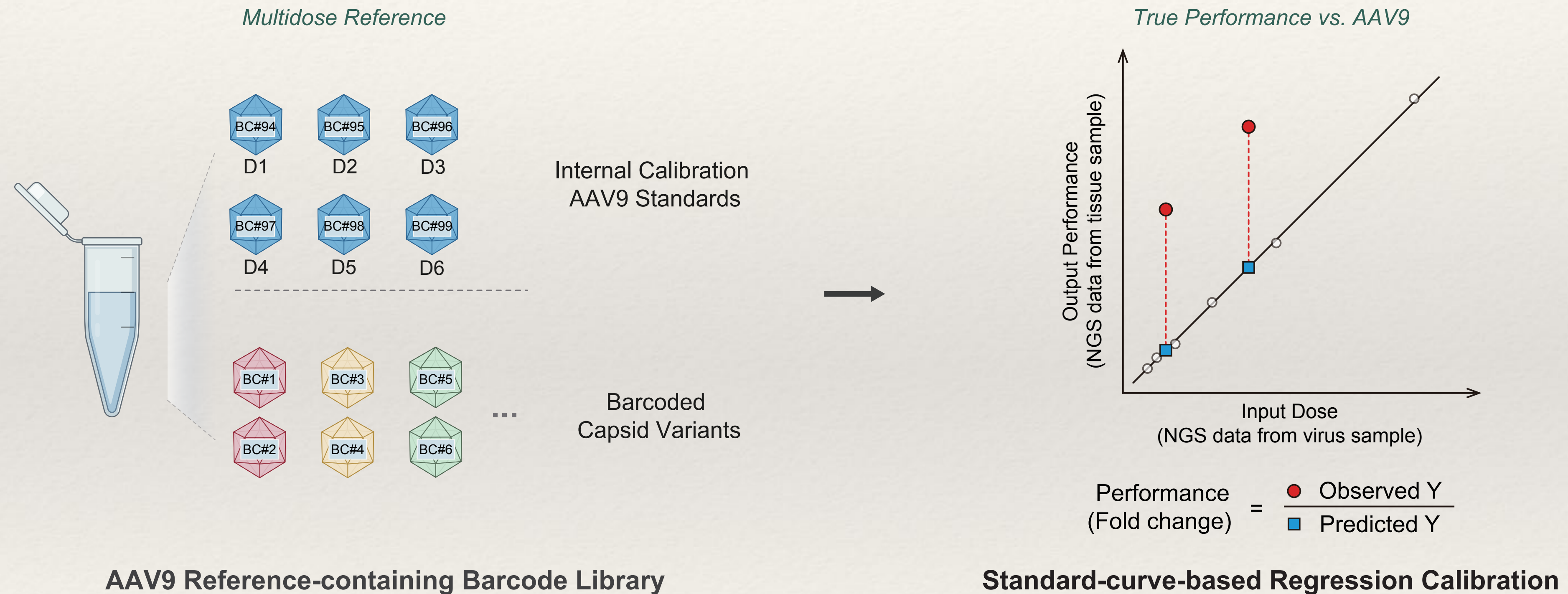
PEARS: Rational Design and Dual-DNA Packaging for High-Diversity AAV Library Construction

Peptide-driven Evolution of AAV via Randomized and Surface-informed libraries



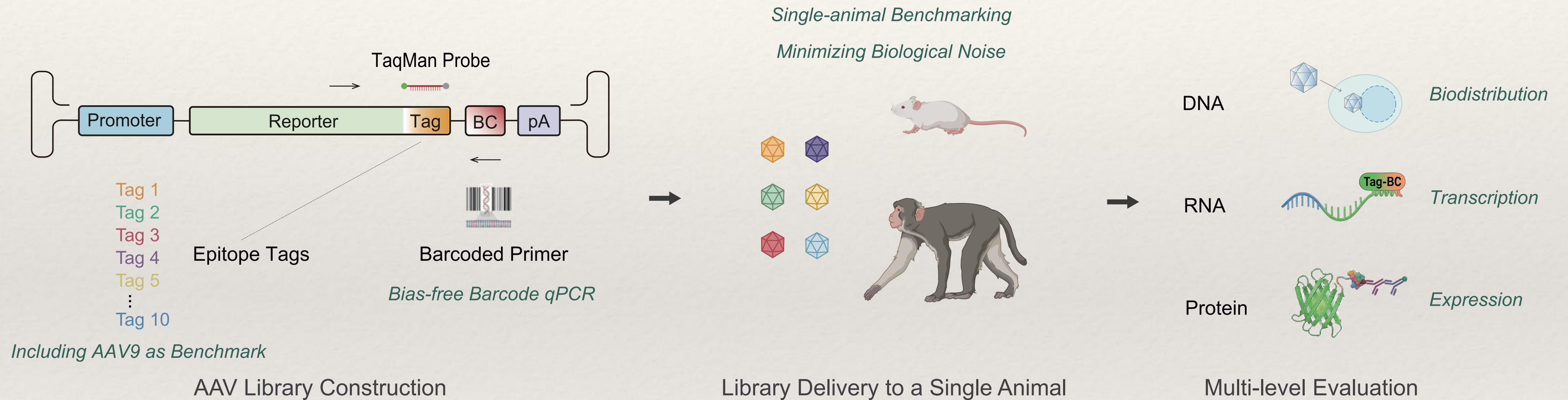
CALIBRATOR: Precision Ranking via Internal Calibration Standards

Calibrated AAV Libraries with Internal Barcode-Referenced Analytes for True Output Ranking



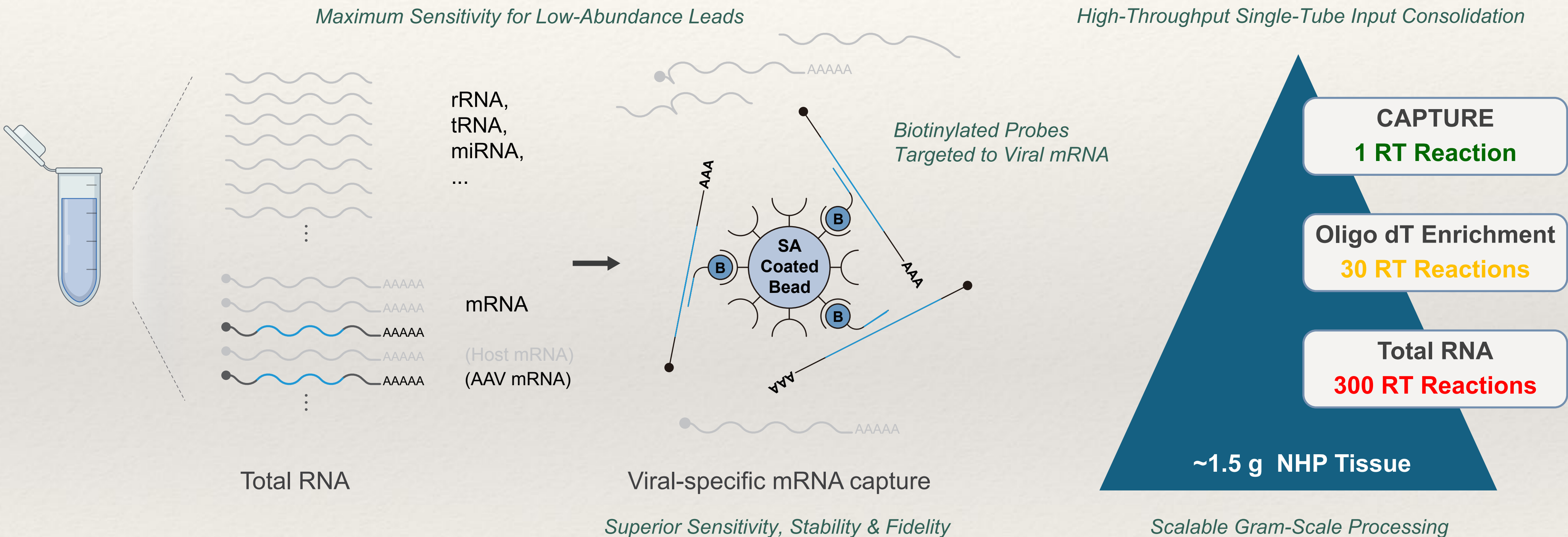
CAMEL : Multi-level Capsid Benchmarking in a Single Animal

Capsid Assessment via a Multiplexed Epitope-tagging Library



CAPTURE: Highly Efficient Viral Transcript Enrichment

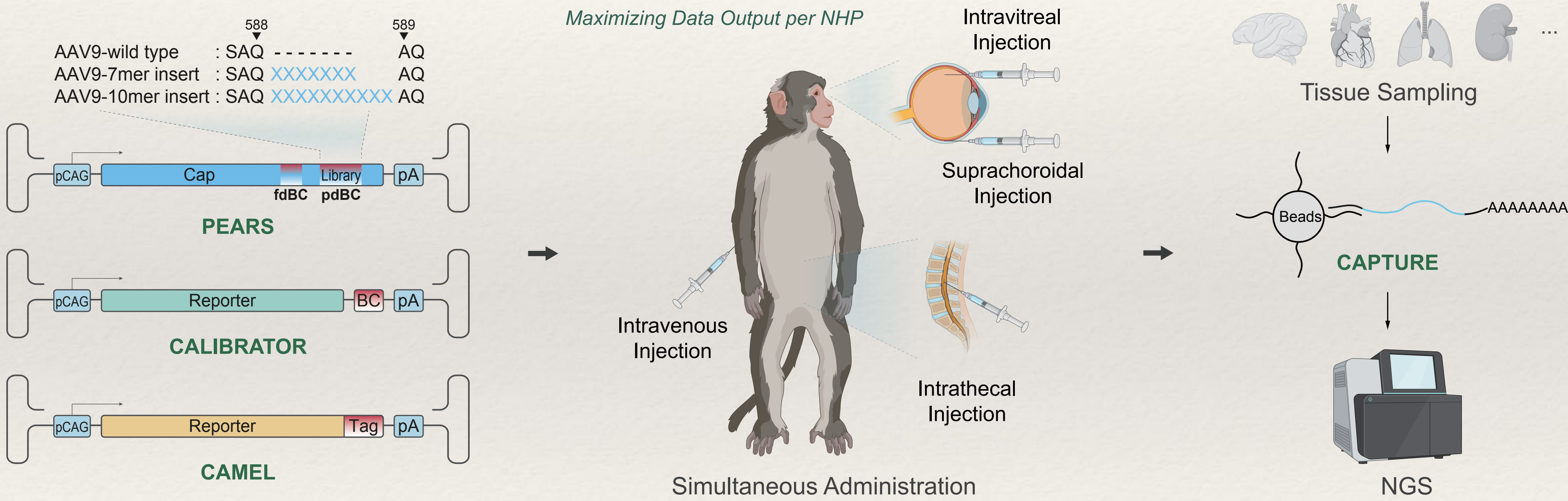
Capsid-Associated Probe-based Transcript Universal Recovery and Enrichment



DynEvoLib: An N-in-One Platform for Next-Generation AAV Evolution

One Model for All

Parallelizing **Multi-library** Screening and **Multi-route** Delivery for **Multi-tissue** Tropism Mapping



Clinical-Lead Portfolio: Tissue-Tropic Capsids Validated via DynEvoLib

Intravenous Delivery



5-10 x Higher Muscle Transduction vs AAV9

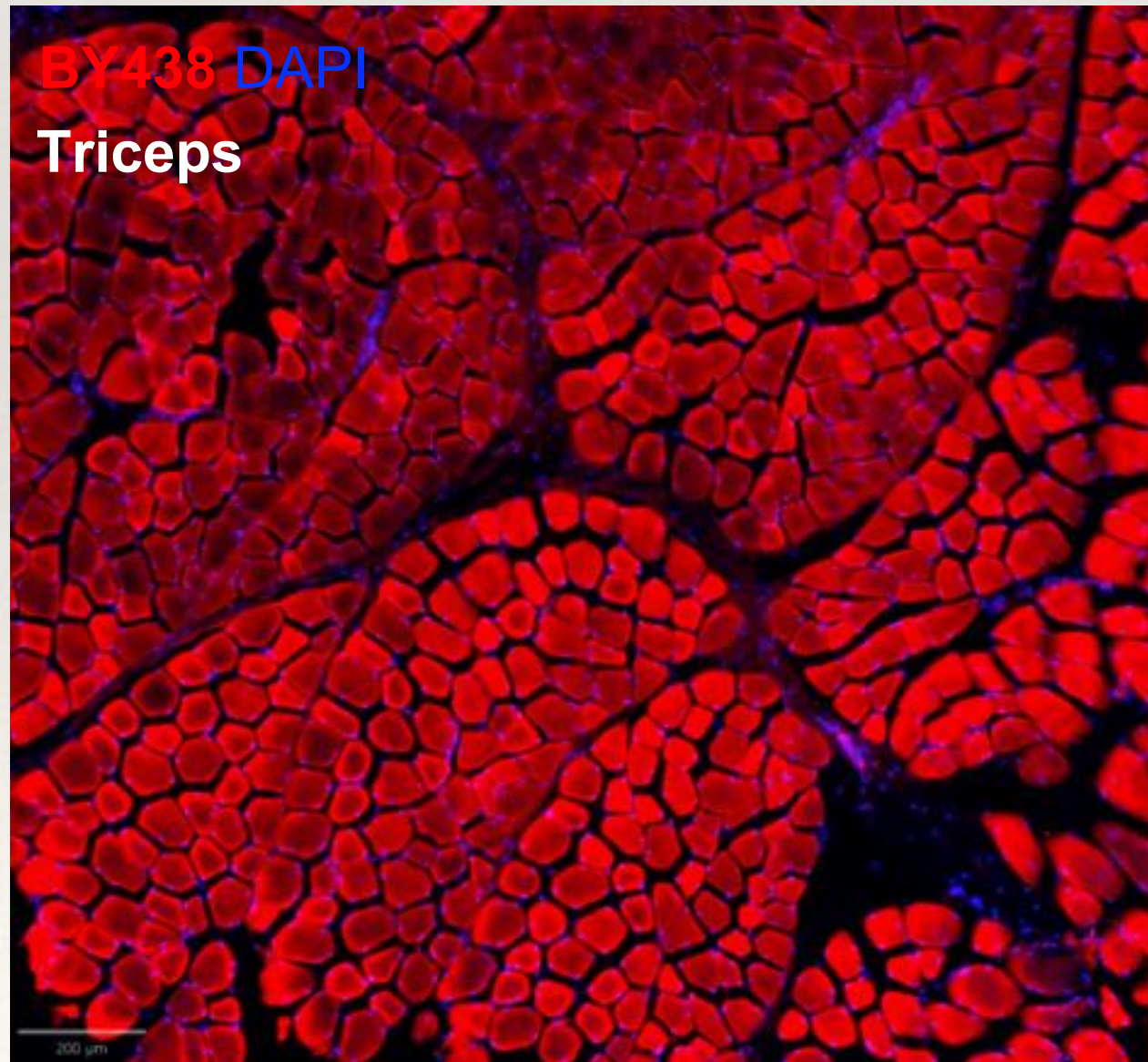
10-20 x Higher Muscle/Liver Ratio vs AAV9



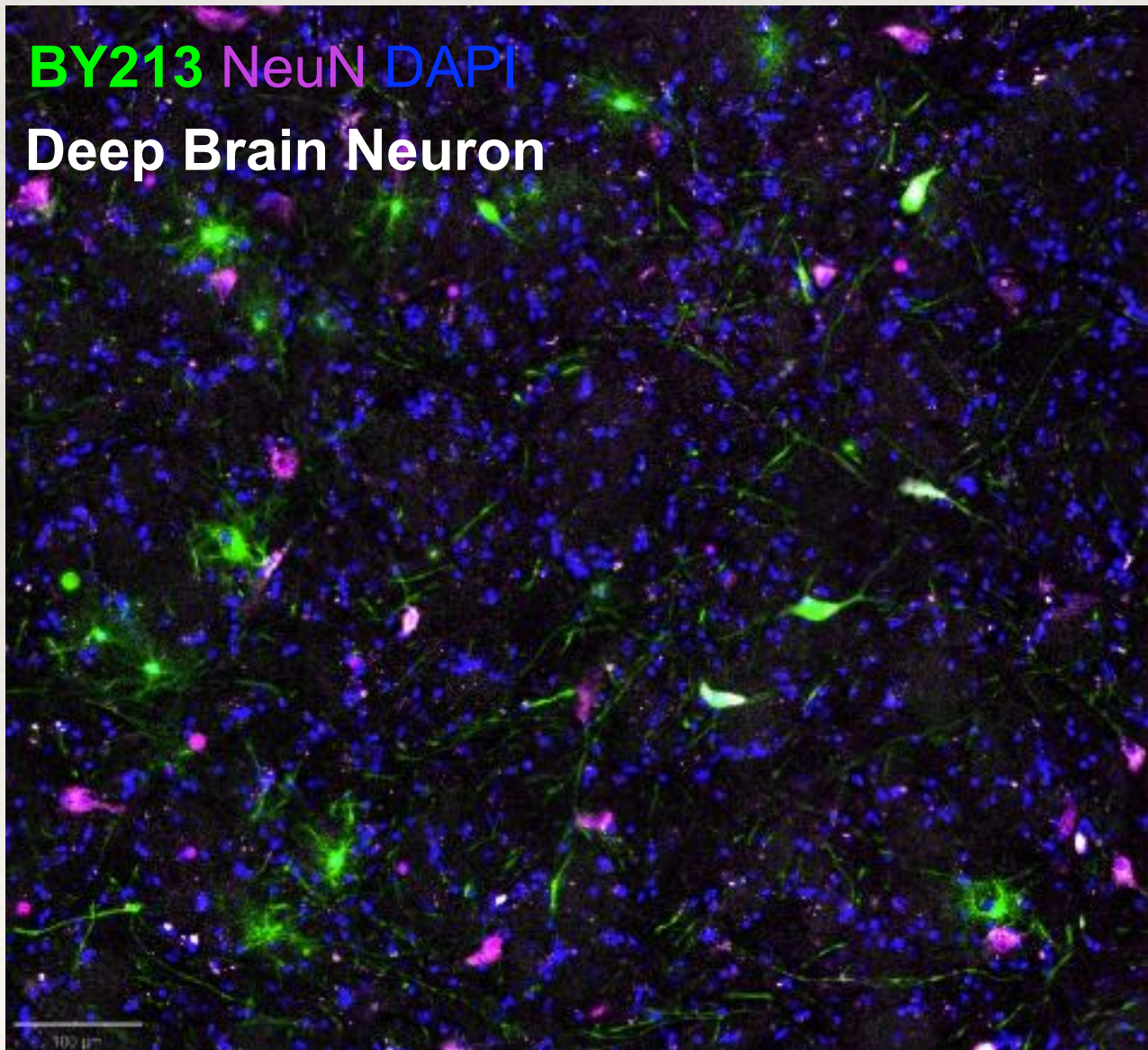
10-30 x Higher Brain Transduction vs AAV9

Transduces 35% Neurons of NHP Deep Brain at 3×10^{13} vg/kg

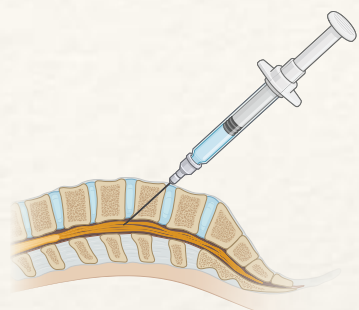
Lead: MyoCAP[®] Series



Lead: BY213



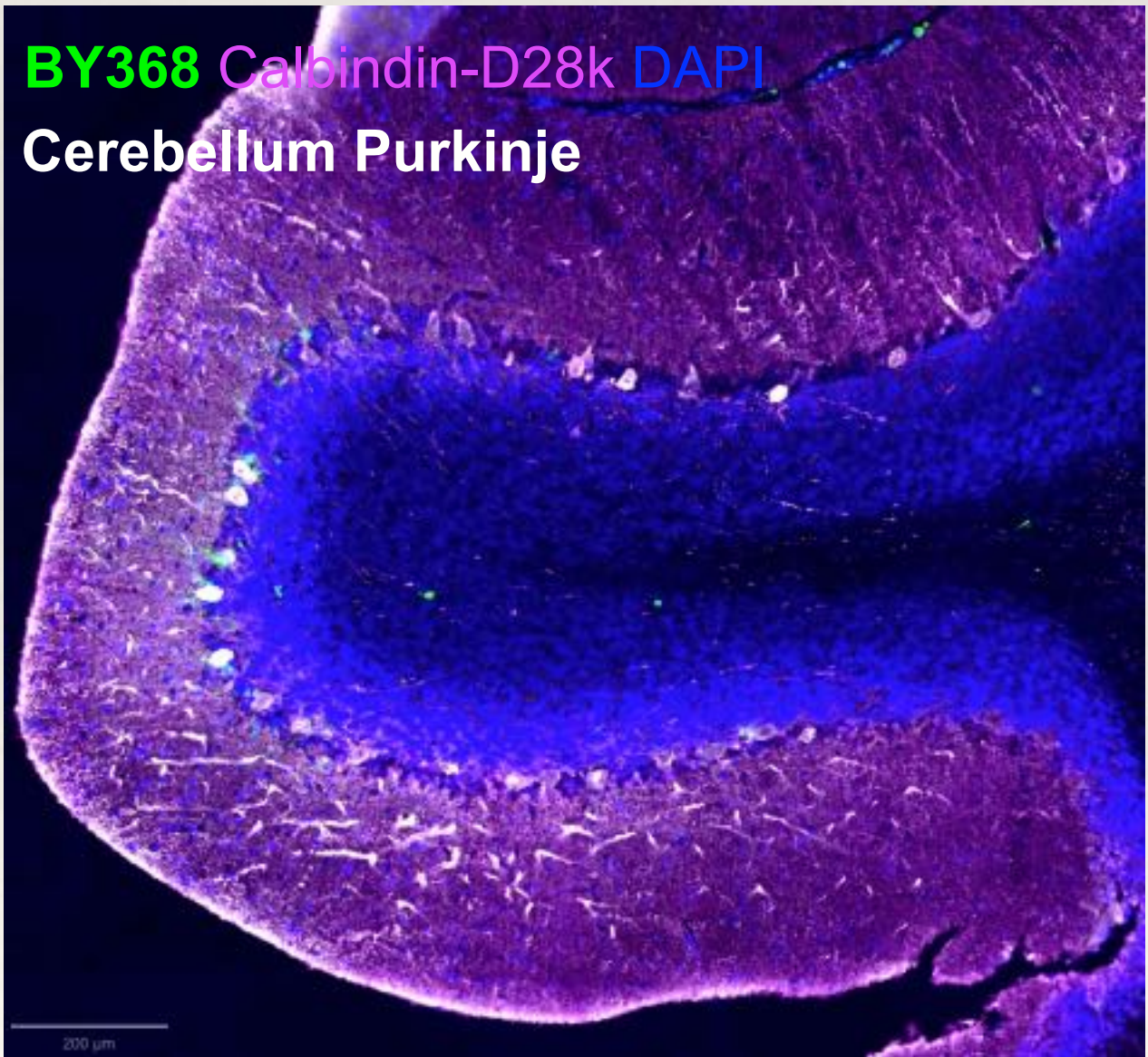
Intrathecal Delivery



> 30 x Higher CNS Transduction vs AAV9 & Pan-CNS Neuro-glial Coverage

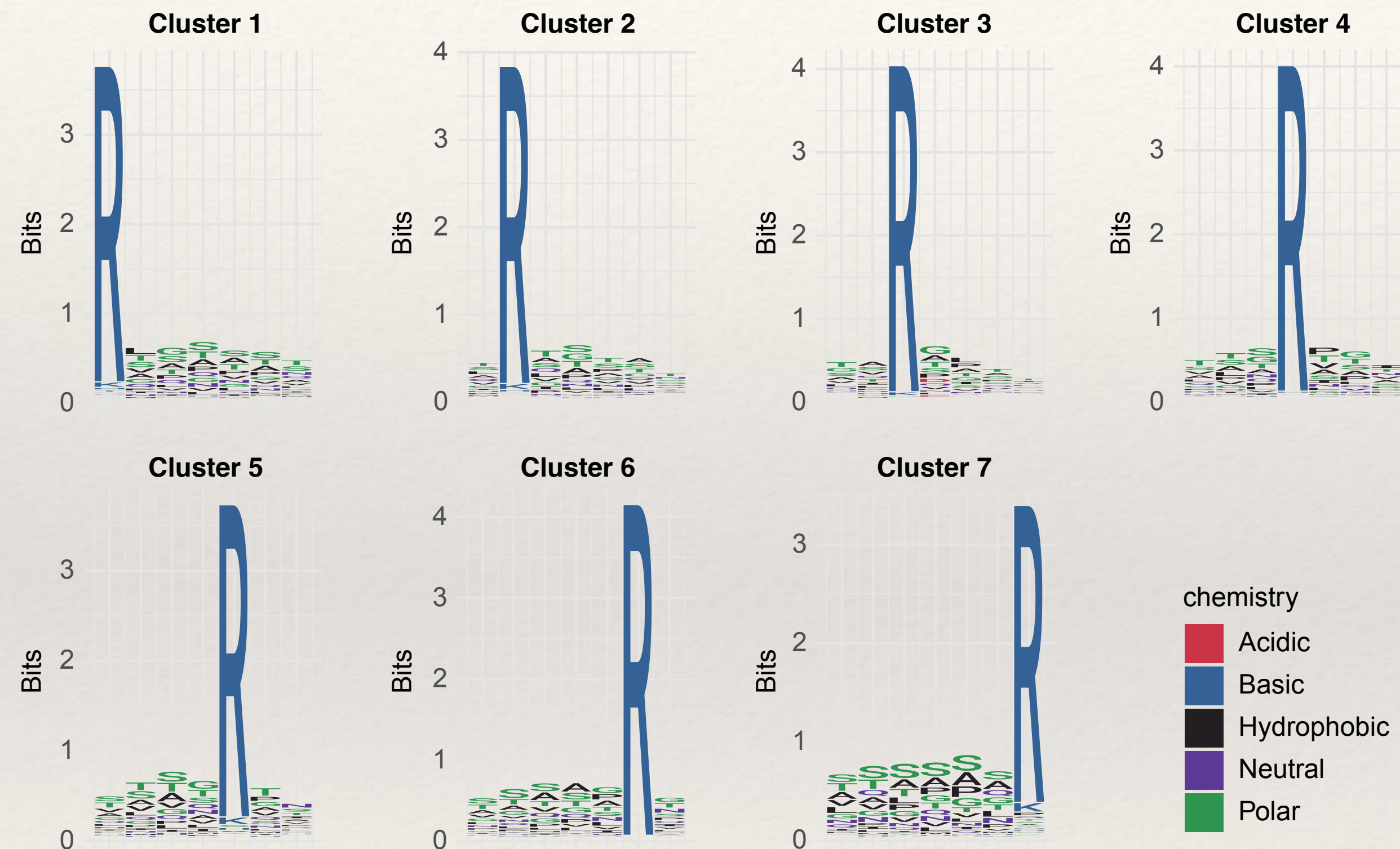
Transduces > 90% NHP Purkinje Cells at 2×10^{13} vg per animal

Lead: SynCAP[™] Series



Immunofluorescence Data derived from NHPs.

First-Round *In Vivo* Screening Reveals Arginine-Rich Motifs as Key Sequence Features for Muscle Tropism



Data derived from adult rhesus macaque screening (n=2).

Conserved Feature:

Arginine (R) is the predominant consensus residue across all 7 clusters.

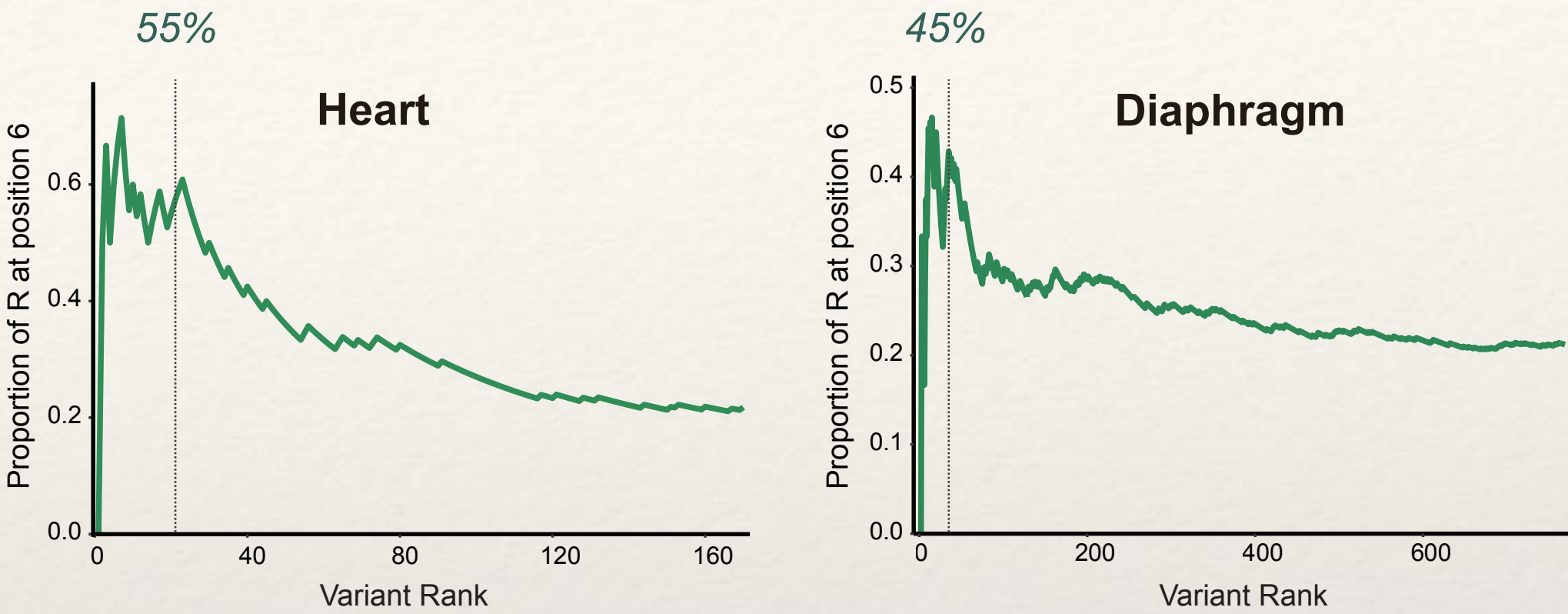
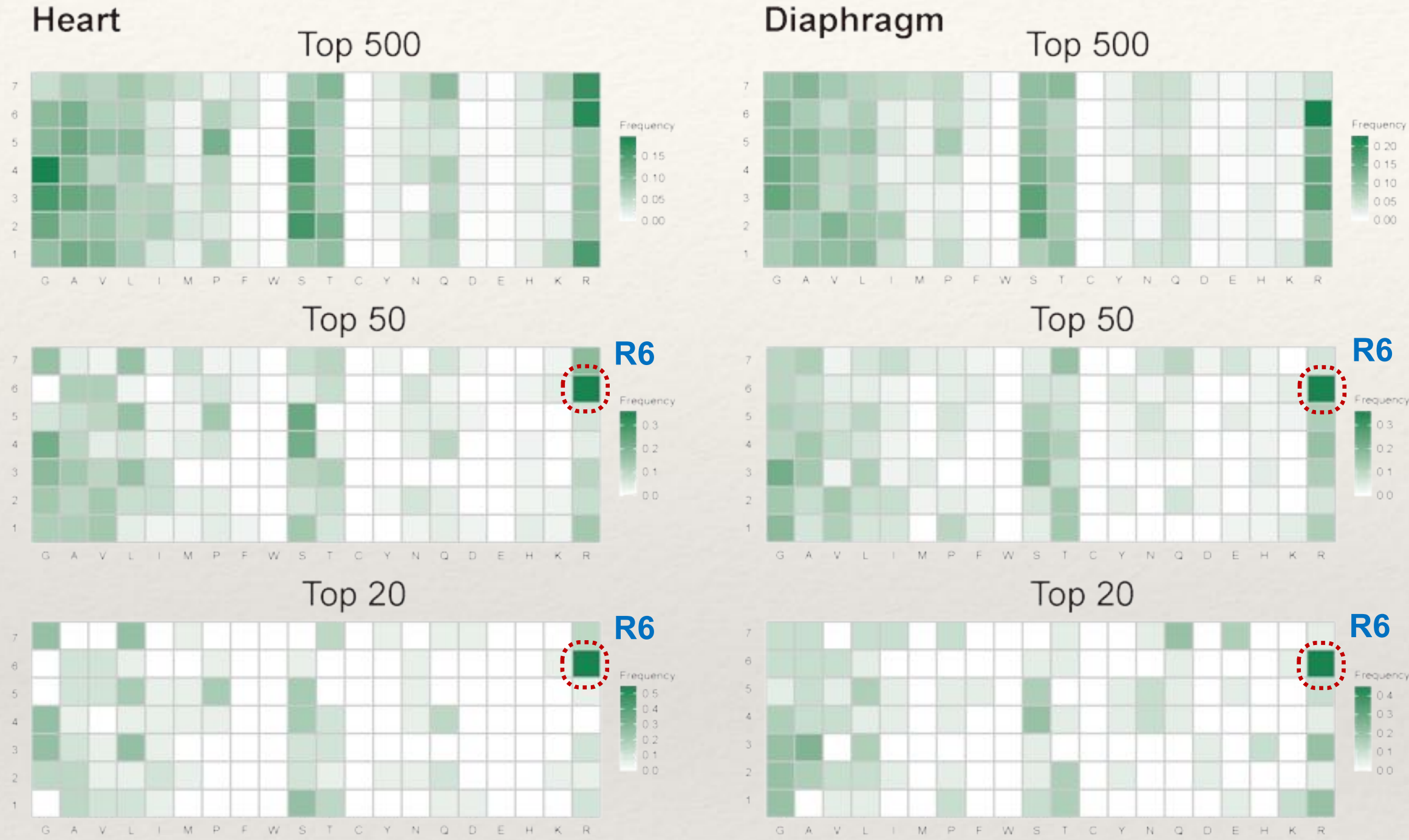
Position Independence:

R residues are distributed globally from N-term to C-term within 7-mer inserts.

Evolutionary Insight:

This distinct **Arg-bias** serves as the starting point for iterative refinement of muscle-targeting capsids.

Second-Round Screening Reveals R6 Occupancy as a Key Sequence Feature for Muscle Tropism



R6 frequency peaks at 45~55% in top 20 variants.

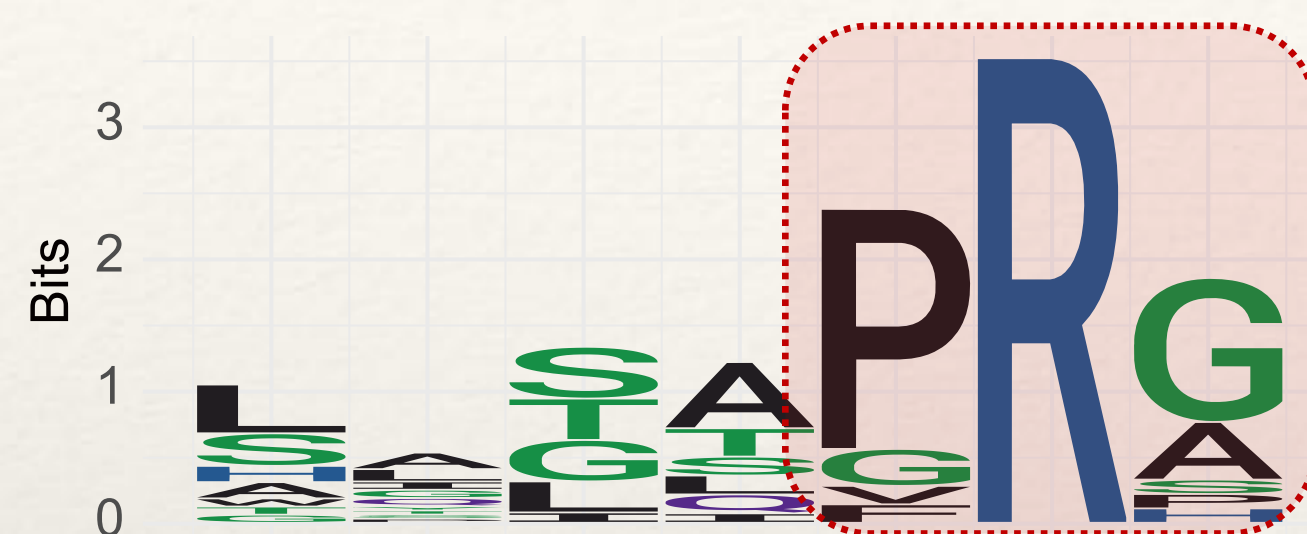
Selection pressure enriches R at position 6.

Conclusion:
High-frequency **R6** occupancy correlates with superior transduction efficiency in multiple muscle tissues.

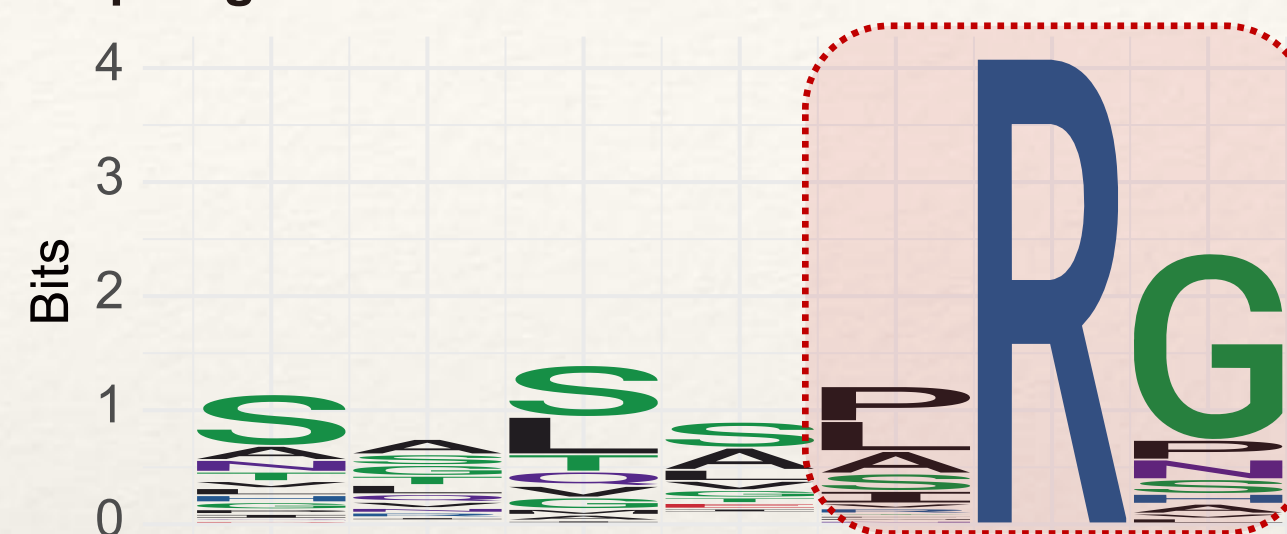
Data derived from adult rhesus macaque screening (n=4).

Refined Analysis Identifies a Conserved PRG Motif for Muscle Transduction

Heart

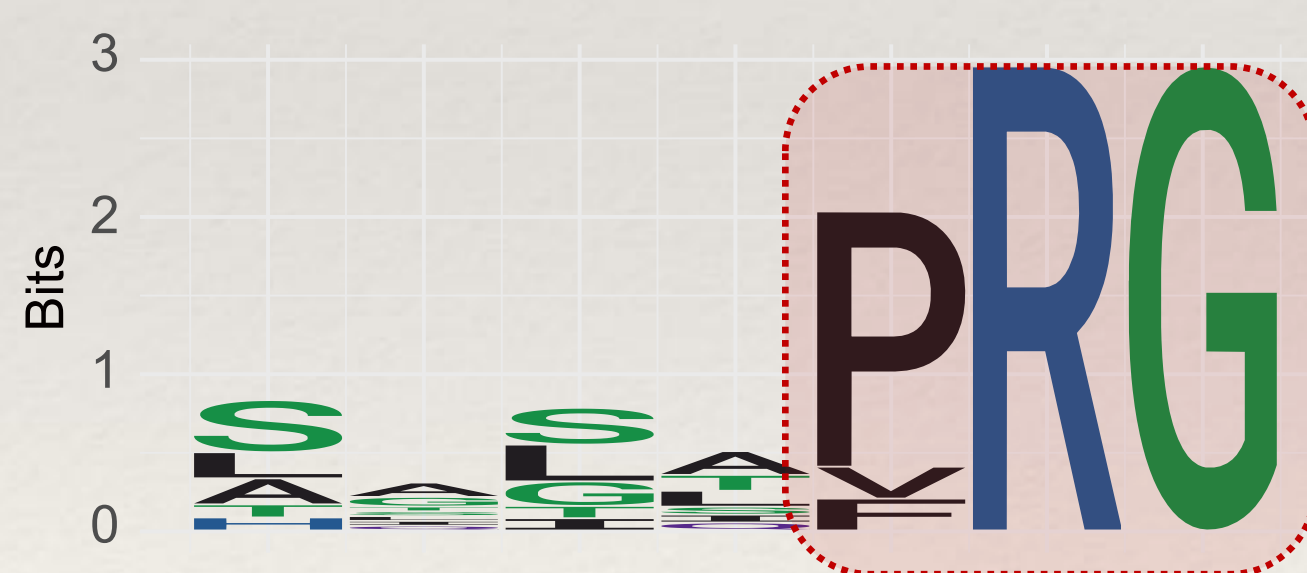


Diaphragm

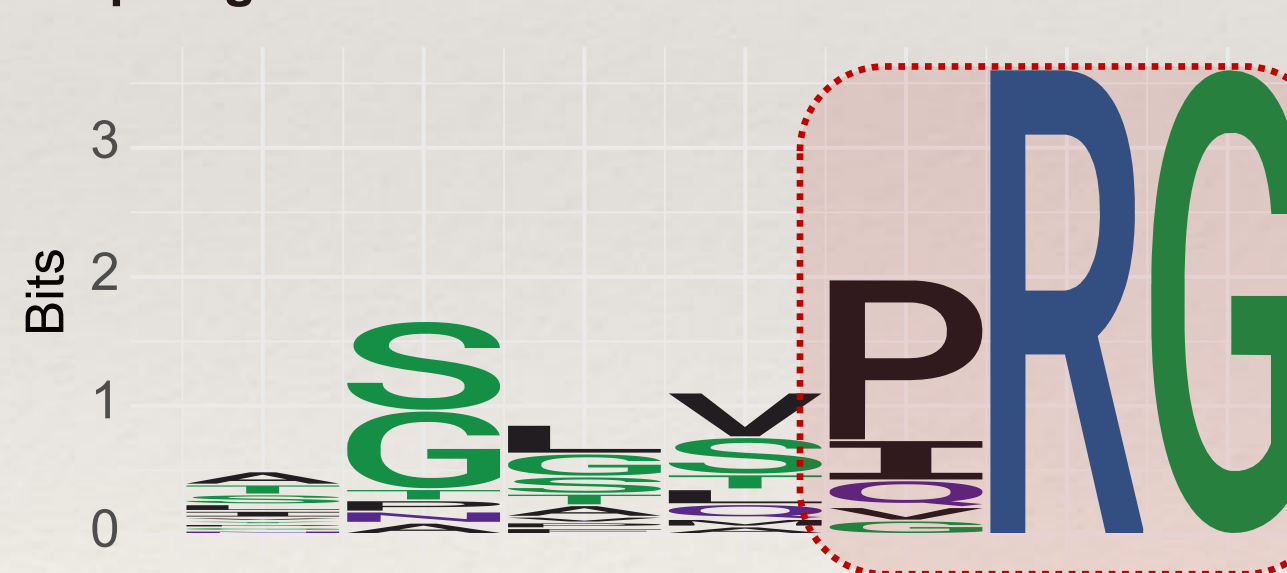


Analysis around the R6 anchor reveals a dominant PRG motif (pos. 5–7) with consistent enrichment across both heart and diaphragm.

Heart



Diaphragm



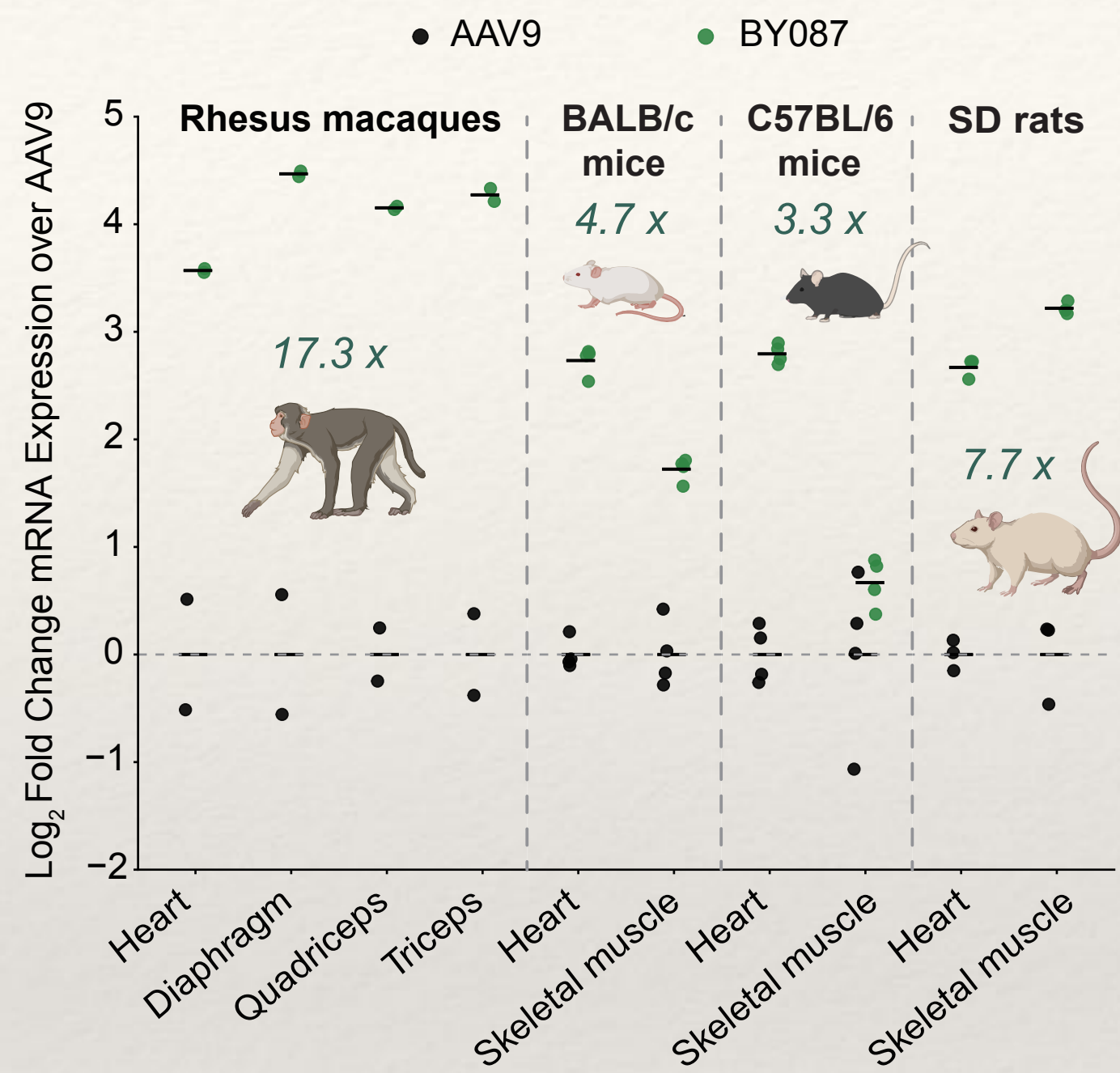
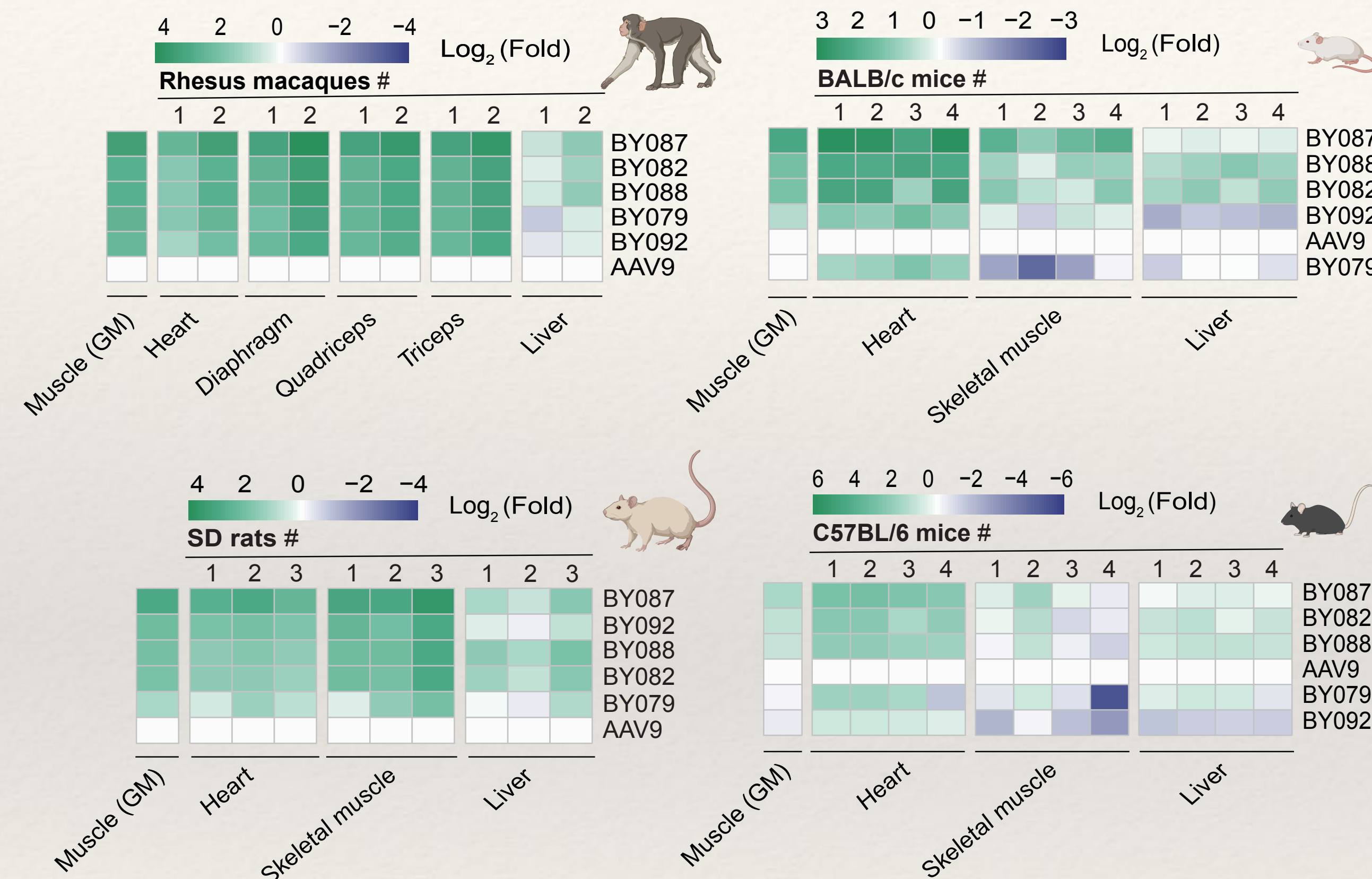
Targeted re-clustering confirms PRG conservation across muscle tissues.

Conclusion:

The tripeptide **PRG** motif emerges as a core sequence determinant for superior myogenic tropism in NHPs.

Data derived from adult rhesus macaque screening (n=4).

Cross-Species Validation Confirms the PRG Motif as a Foundational Determinant for Muscle Transduction



Animal Study:
IV co-injection
($3.1 \sim 8.8 \times 10^{12}$ vg/kg
per vector with unique
epitope tags) in NHPs
(n=2), Rats (n=3), and
Mice (n=8); mRNA
quantified at 3 weeks
post-injection.

PRG Candidates
BY079: HSREPRG
BY082: SLLSPRG
BY087: SALTPRG
BY088: SGGLPRG
BY092: non-PRG

Functional Conservation: Robust cross-species translatability across **NHPs** and **rodents** validates the PRG motif as a foundational determinant for next-generation muscle-tropic gene therapies.

Superior Potency: PRG variants demonstrate significantly higher mRNA expression across all tested muscle tissues compared to AAV9.

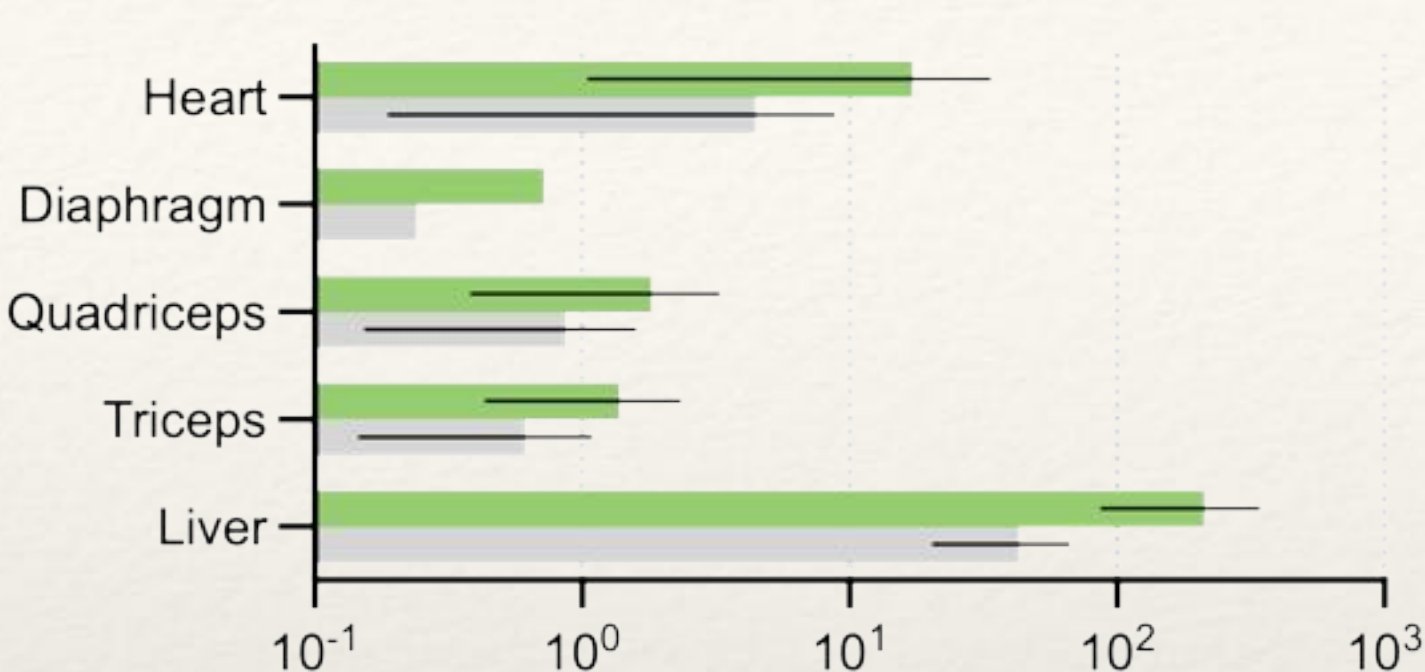
BY087: A highly translatable lead capsid with robust, multi-species muscle tropism.

Further NHP Validation: BY087 Outperforms AAV9 Across DNA, RNA, and Protein Levels

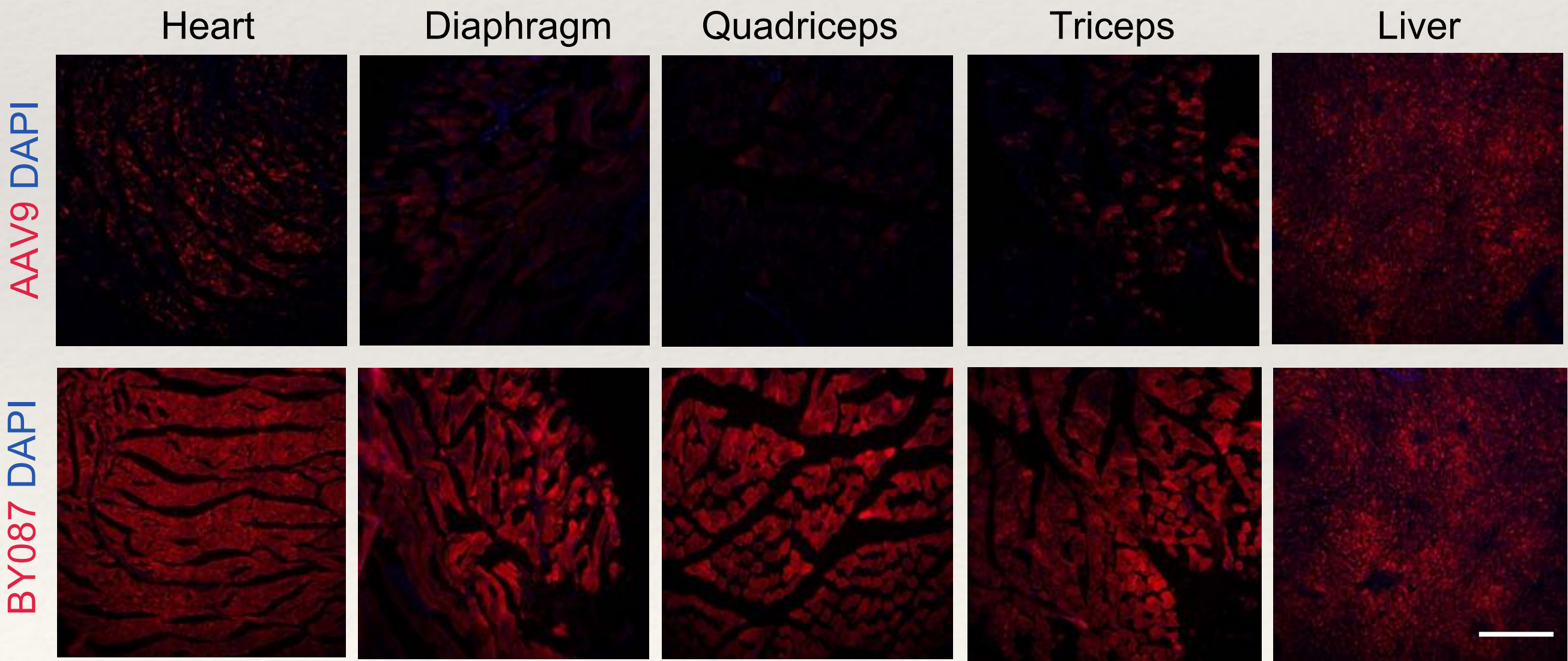
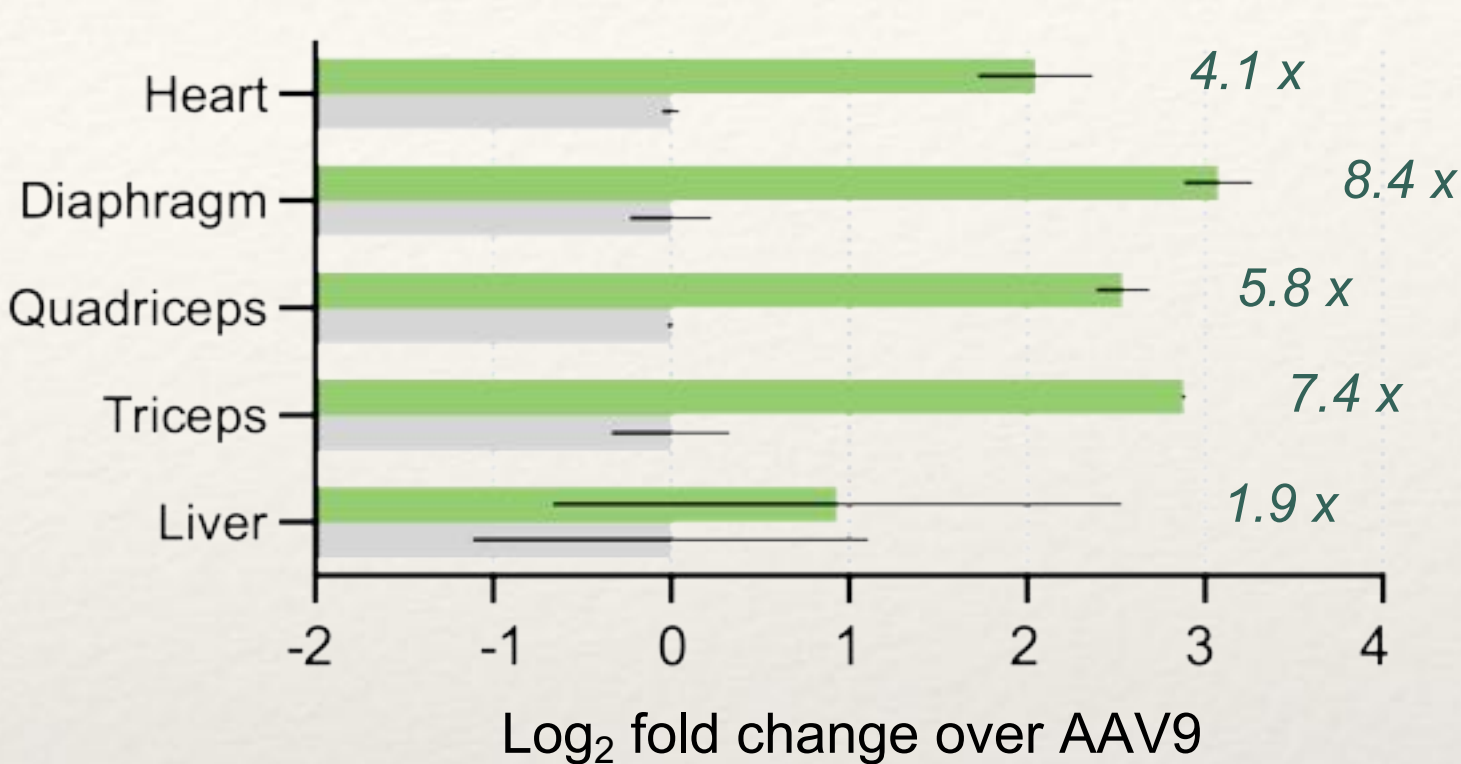
NHP Study:

Adult rhesus macaques (n=2);
IV co-injection of BY087 (CAG-HA-tag)
and AAV9 (CAG-His-tag);
 5×10^{12} vg/kg per vector each;
3-week study duration;
Transgene expression

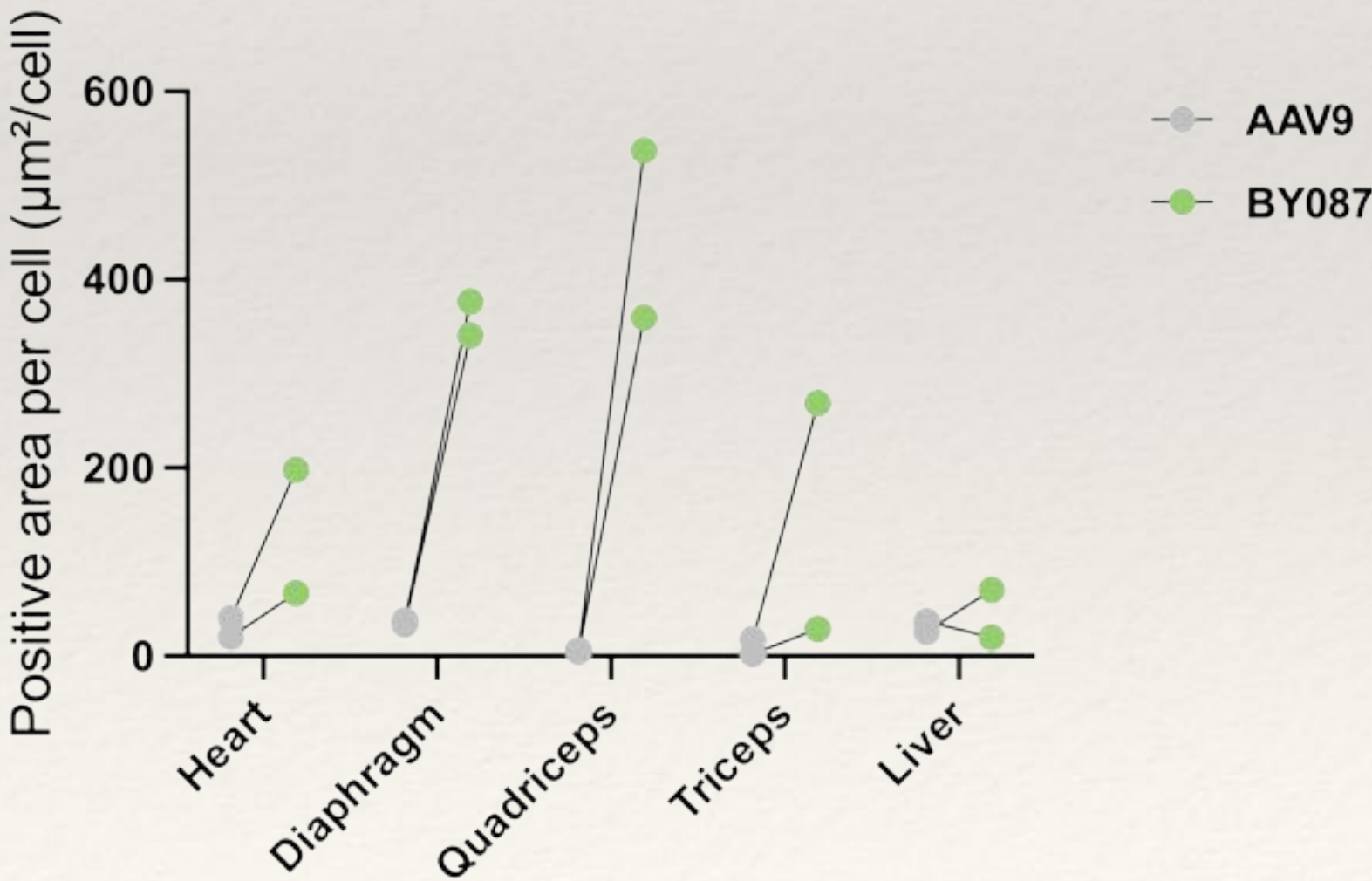
Biodistribution (vg/dg)



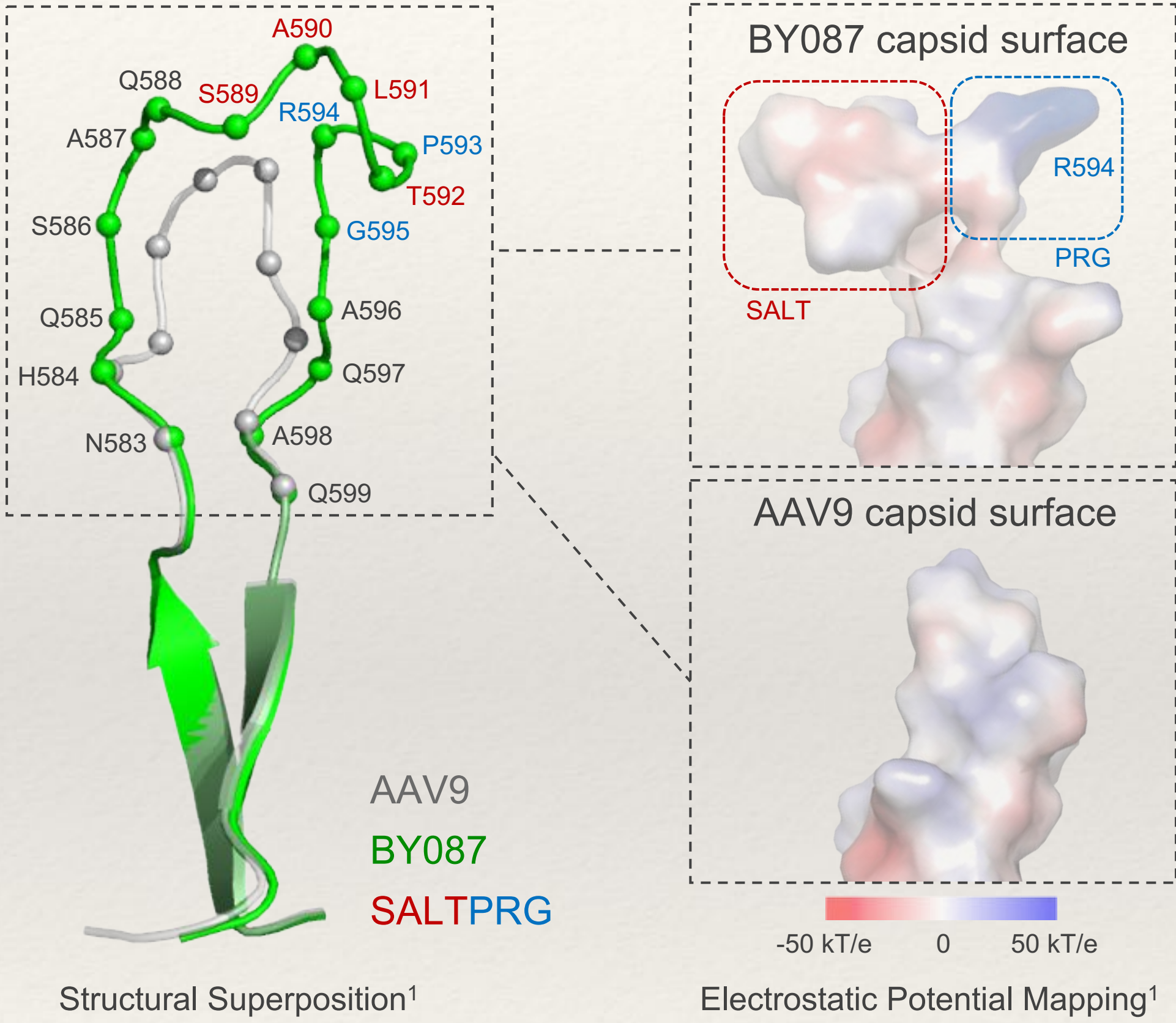
Normalized mRNA expression



Scale: 200 μ m



Structure-Informed Optimization: Refining the PRG Motif via N-terminal Flanking Evolution



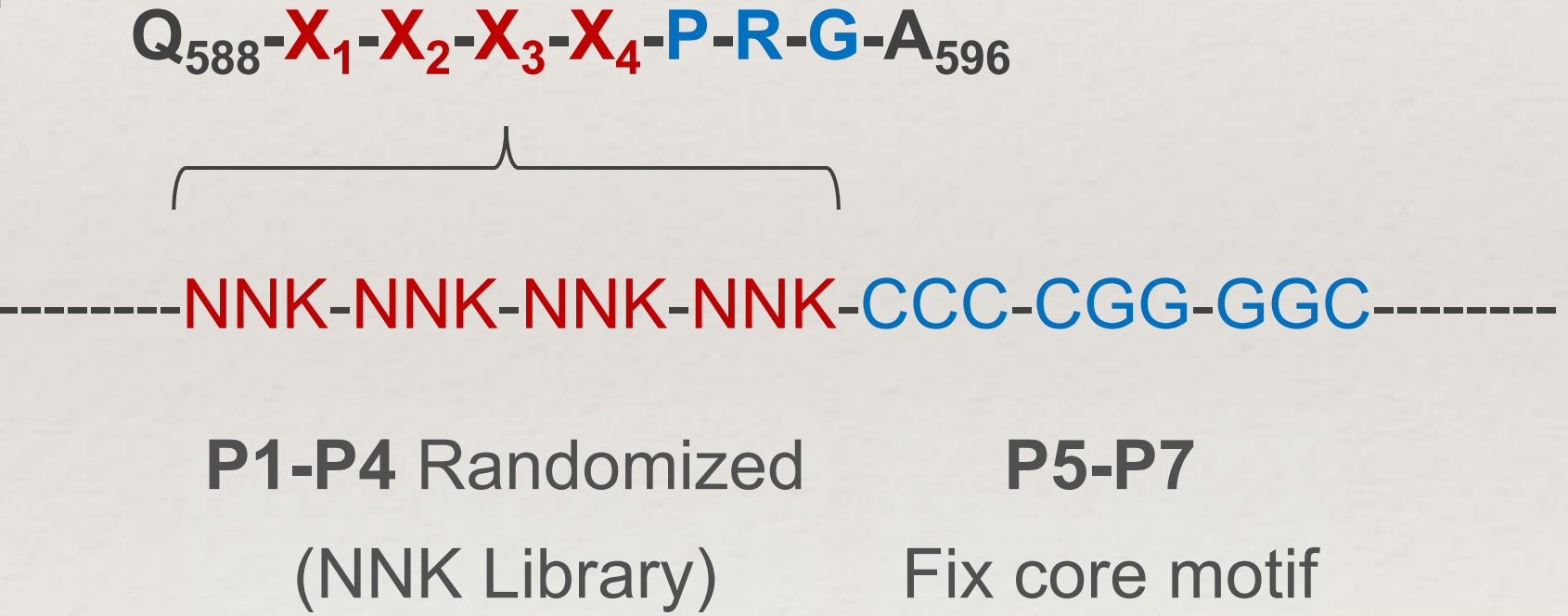
¹ Structural modeling performed via AlphaFold2; visualization and electrostatic mapping generated with PyMOL.

Structural modeling reveals a distinct **conformational protrusion** in the VR-VIII loop induced by the **SALTPRG** insertion.

The **PRG** motif introduces a robust **positive hotspot**, significantly enhancing the local electrostatic surface potential.

Hypothesis: Optimizing the N-terminal context (P1-P4) will maximize the muscle-directed potency of the PRG core.

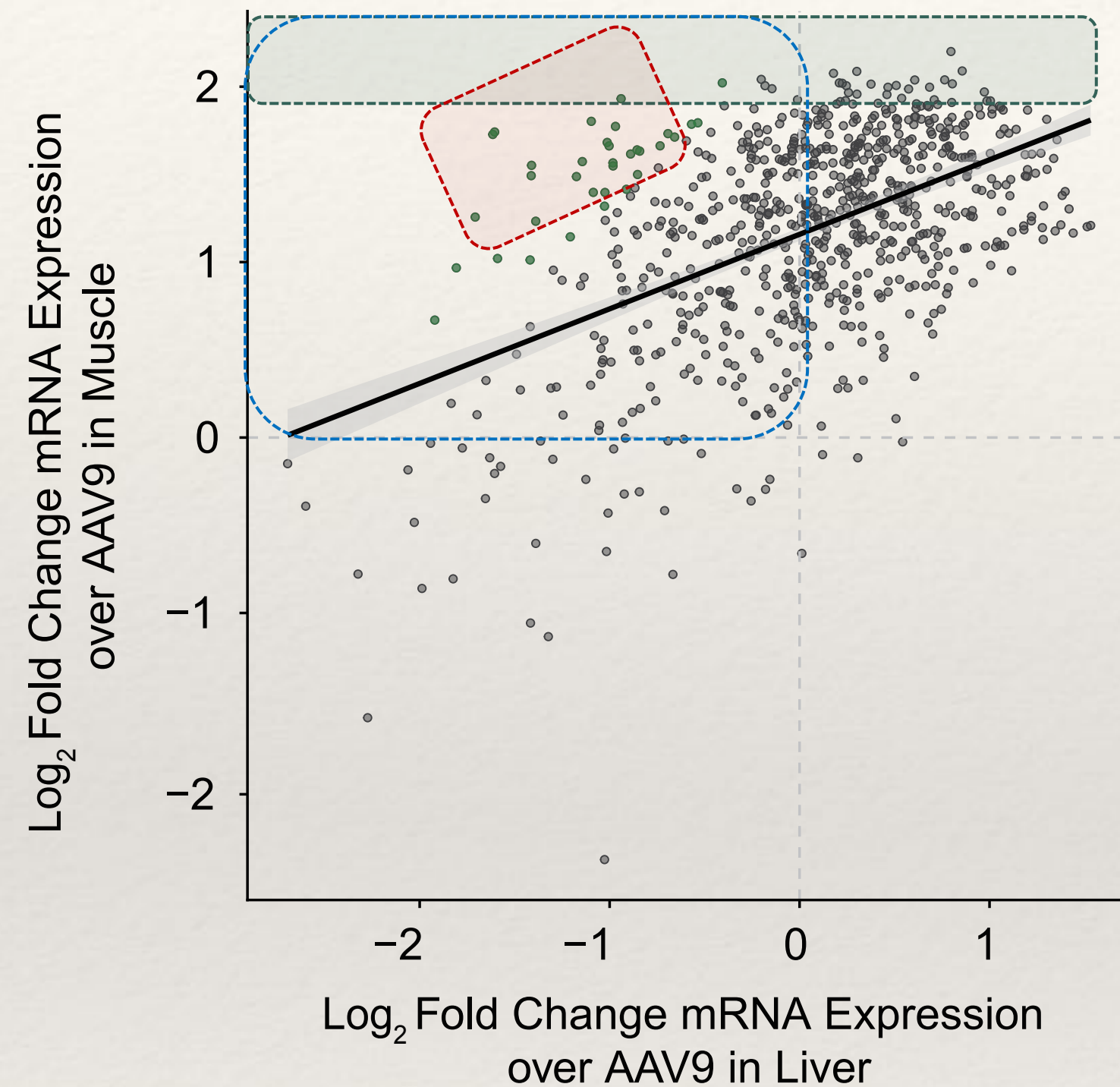
NNK library:



NHP Study:

Adult rhesus macaques (n=2); IV injection of NNK library (7.6×10^{12} vg/kg) ; 3-week study duration; Transgene mRNA expression

NNK Library Screening Reveals Key Motifs for Enhanced Muscle Transduction and Liver Escape

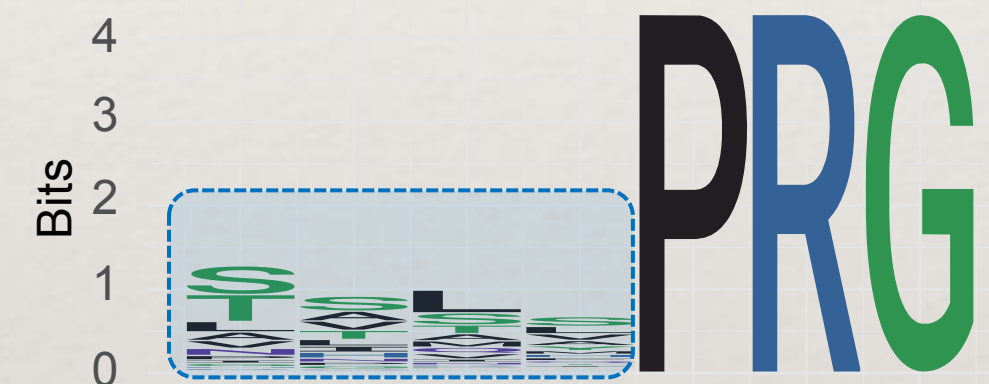


Correlation Analysis: A NHP-based NNK library screen identified variants with divergent profiles in muscle and liver.



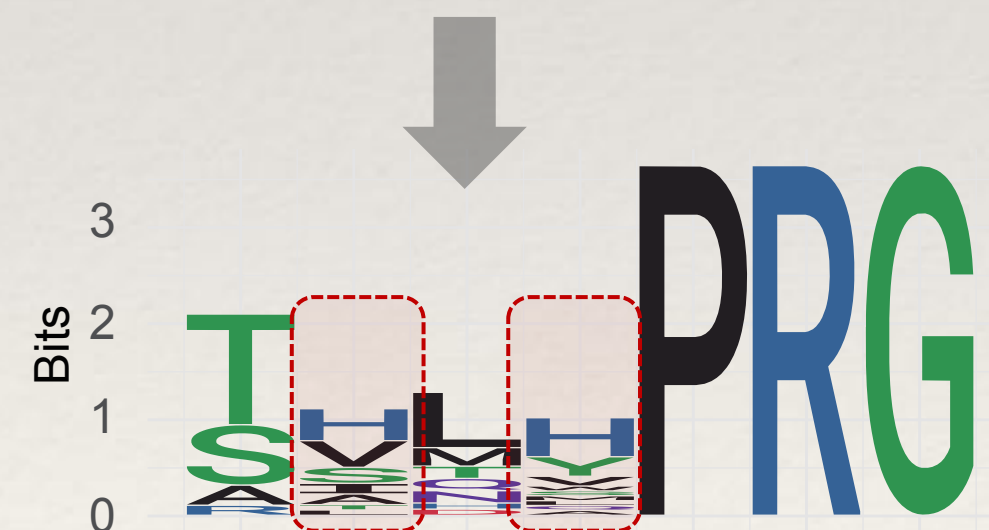
Foundation of Muscle Potency

Specific Consensus:
[S/T]-[A/V/S]-[X]-[Y]-PRG



Keys to Hepatic De-targeting

Specific Consensus:
[T/S]-[H/X]-[L/X]-[H/X]-PRG



The "H-Switch": The site-specific incorporation of Histidine (H) acts as a molecular switch to significantly attenuate hepatic sequestration while maintaining myogenic potency.

Systematic Discovery and Design of Next-Generation MyoCAP Leads



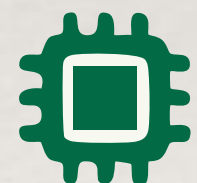
Wet-lab Directed Evolution

Top-performing candidates identified via high-diversity NNK library NHP screening.



Structure-Informed Refinement

Precision engineering of PRG orientation and flanking H-residues to optimize the H-switch logic.

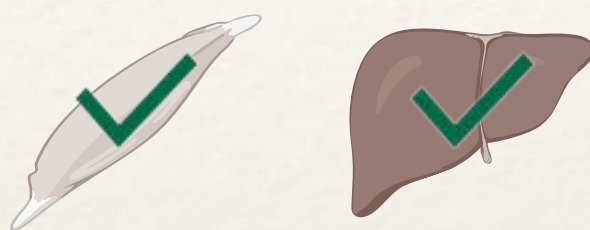


AI-Guided Discovery (xTrimo¹)

Large-scale sequence optimization and fitness prediction leveraging protein foundation models

¹ xTrimo is a life-science foundation model family by BioMap.

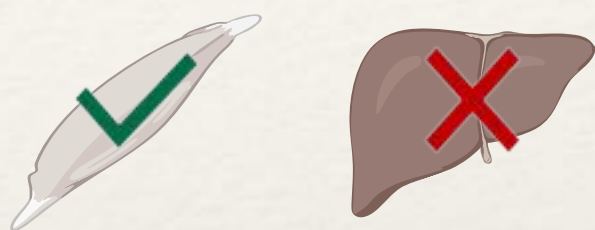
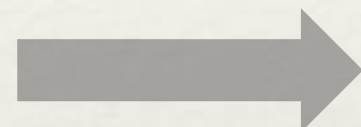
Predictive sequence expansion



Foundation of Muscle
Potency

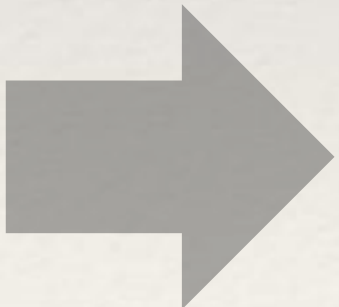
[S/T]-X-X- [Y/X]-PRG

"H-Switch"



Liver De-targeting

[S/T]-[H/X]-[L/X]-[H/X]-PRG

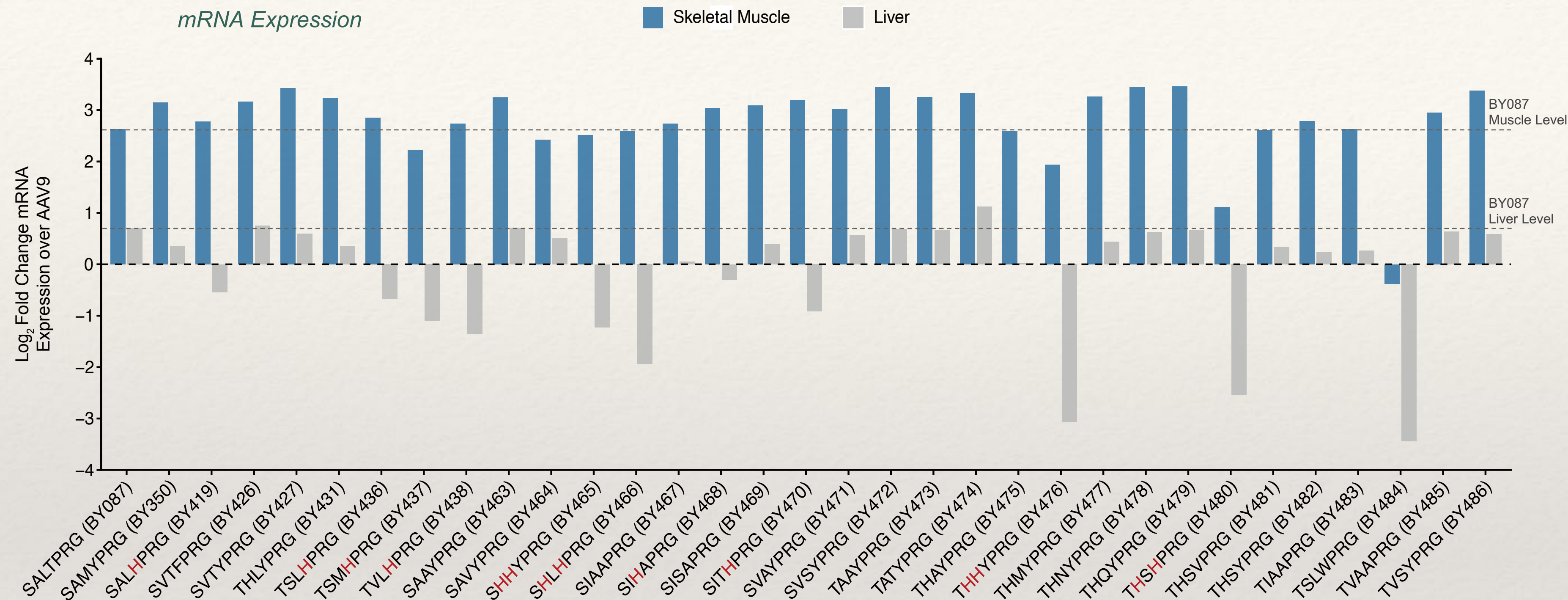


MyoCAP[®] Candidate Library

32 Unique Next-Gen Variants

Integrated with Barcodes (64 BCs) into **CALIBRATOR** for high-resolution NHP validation.

Tailored Control of Tissue Tropism via Novel Motif Engineering



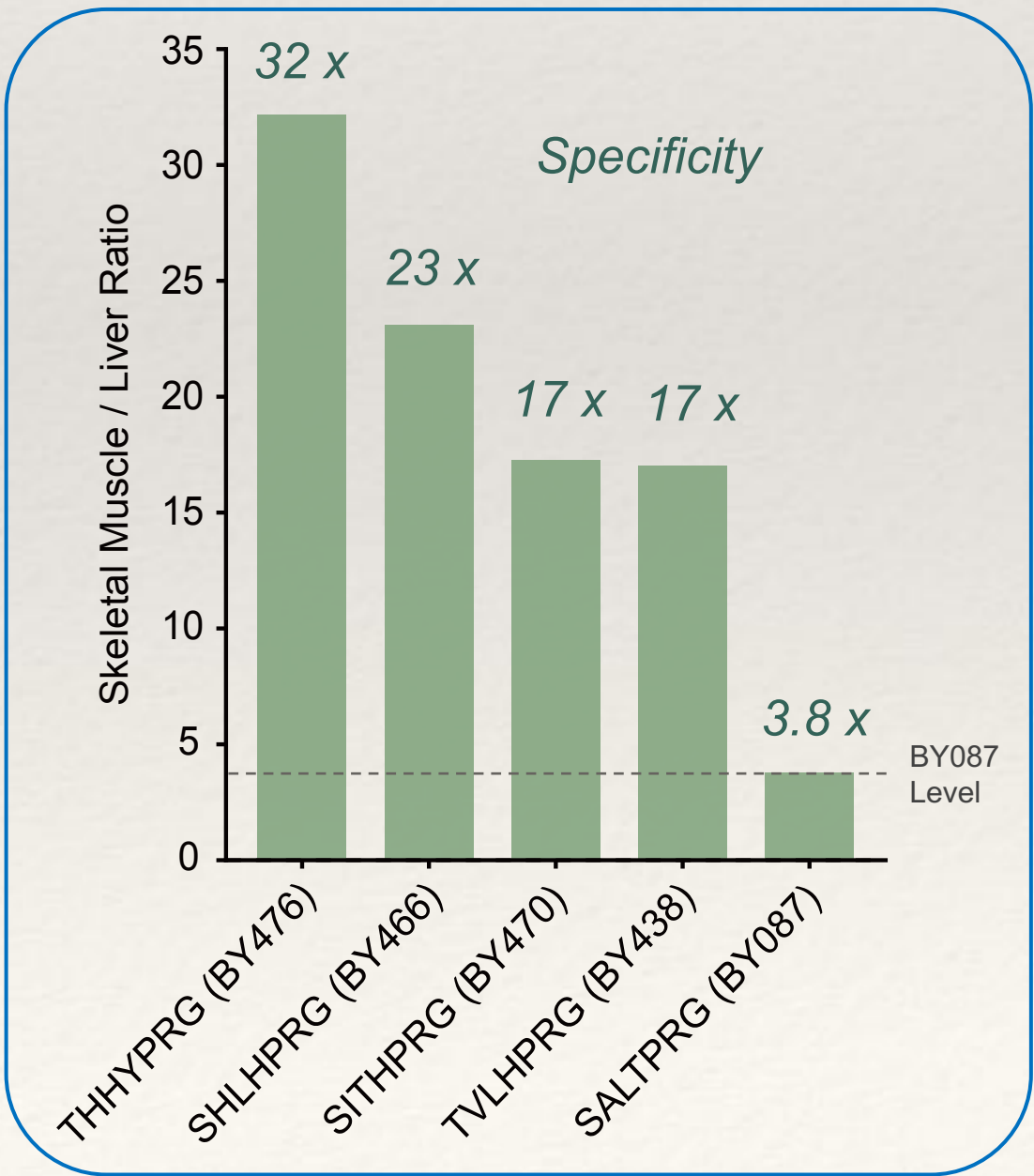
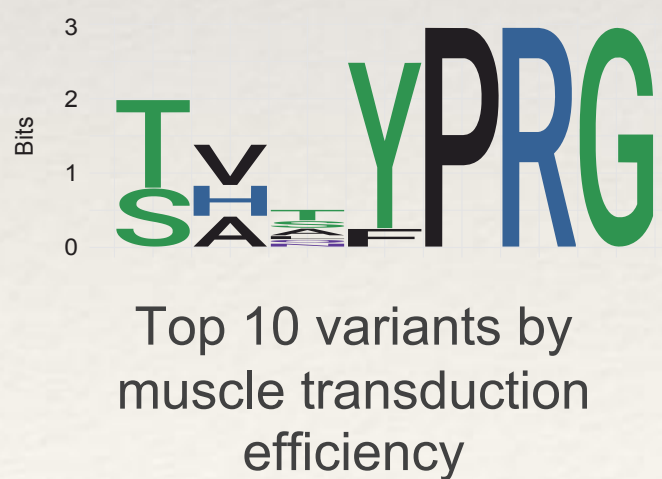
NHP Study:

Adult rhesus macaques (n=2);
 2.3×10^{13} vg/kg barcode library;
 3-week study duration;
 Transgene mRNA expression
CALIBRATOR:
 36 variants, 2 BCs/variants &
 AAV9 with 6 gradients

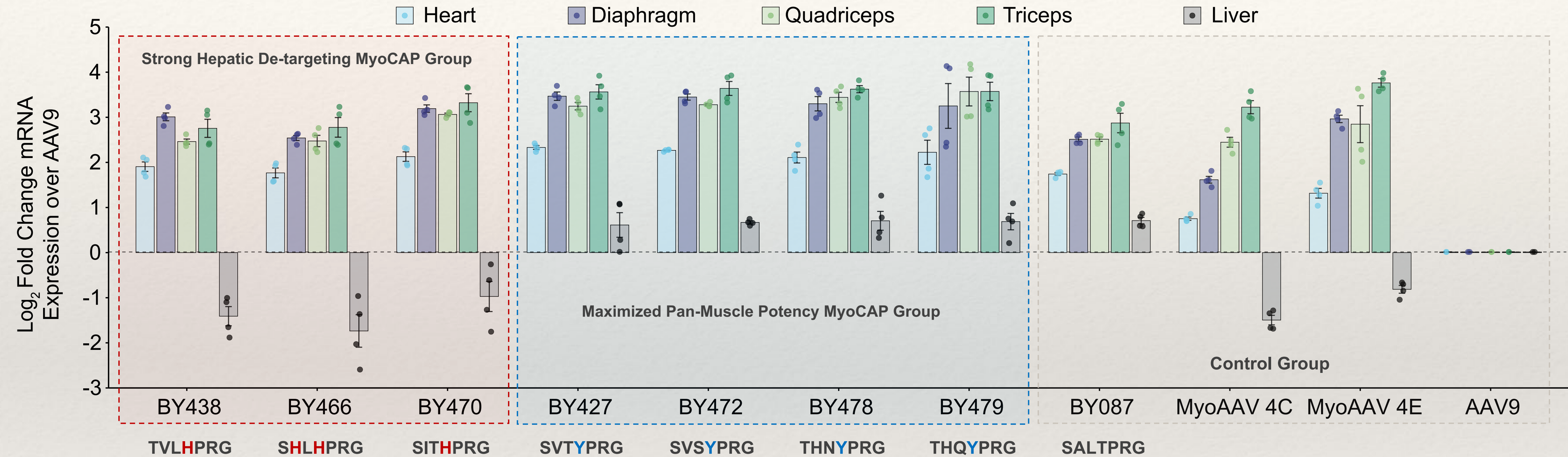
Most candidates **outperform BY087** in muscle mRNA expression.

PRG drives muscle potency, while **strategic H-insertion** maximizes hepatic escape.

MyoCAPs achieve superior **muscle-to-liver ratios**, widening the therapeutic window.



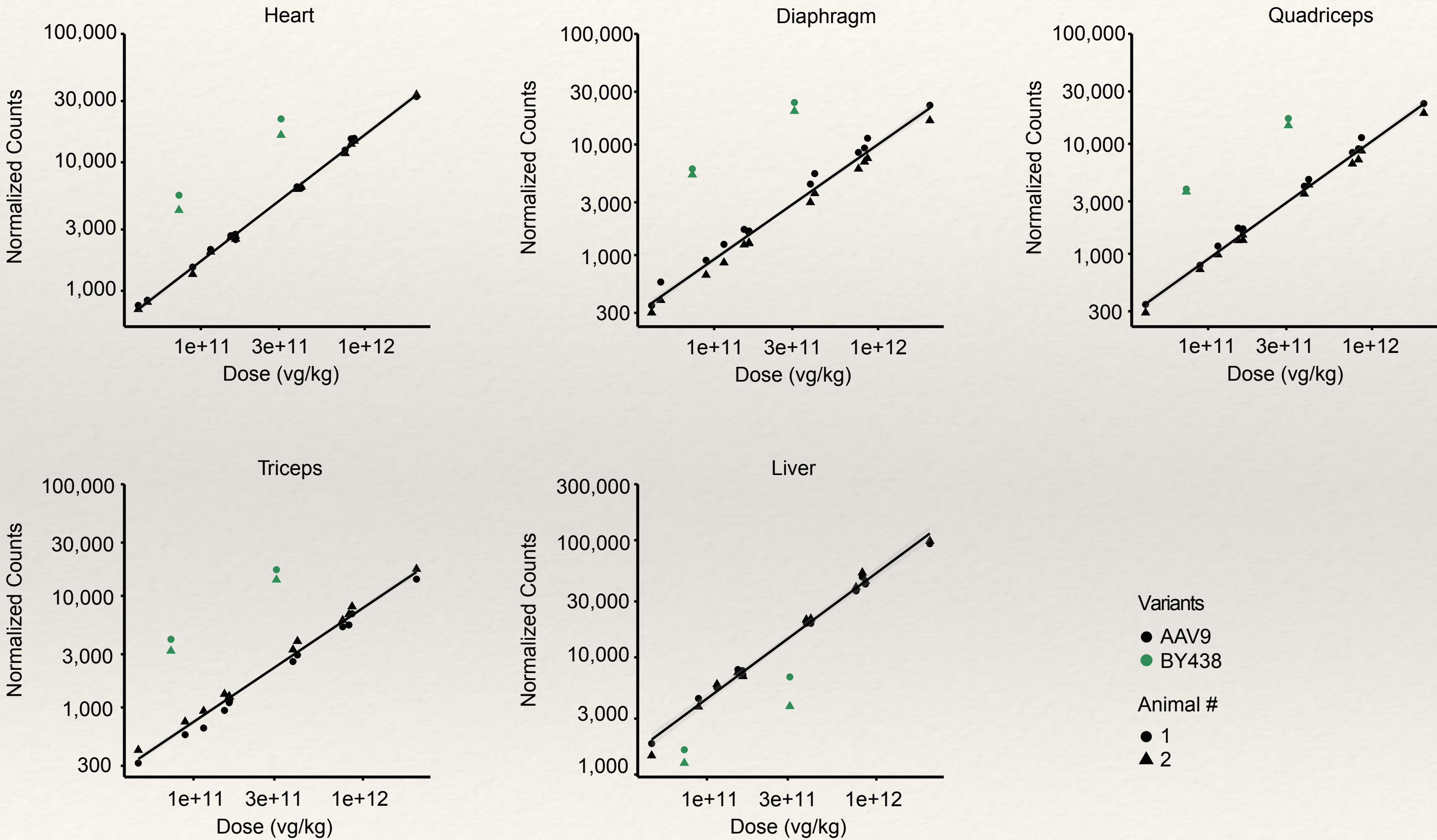
MyoCAP Variants Demonstrate Enhanced Muscle Potency and Hepatic Escape in NHPs



Strong De-targeting (Red Group):
Variants like **BY438** leverage the **HPRG** motif to achieve profound hepatic de-targeting while preserving high-level myogenic expression.

Maximized Potency (Blue Group):
Variants like **BY472** provide the highest pan-muscle transduction levels across all tested tissues.

Lead Variant BY438 Exhibits Superior Muscle Transduction and Efficient Hepatic De-targeting in NHPs



Precision Standard: Linear regression of AAV9 standards in **CLIBRATOR** ensures a mathematical baseline.

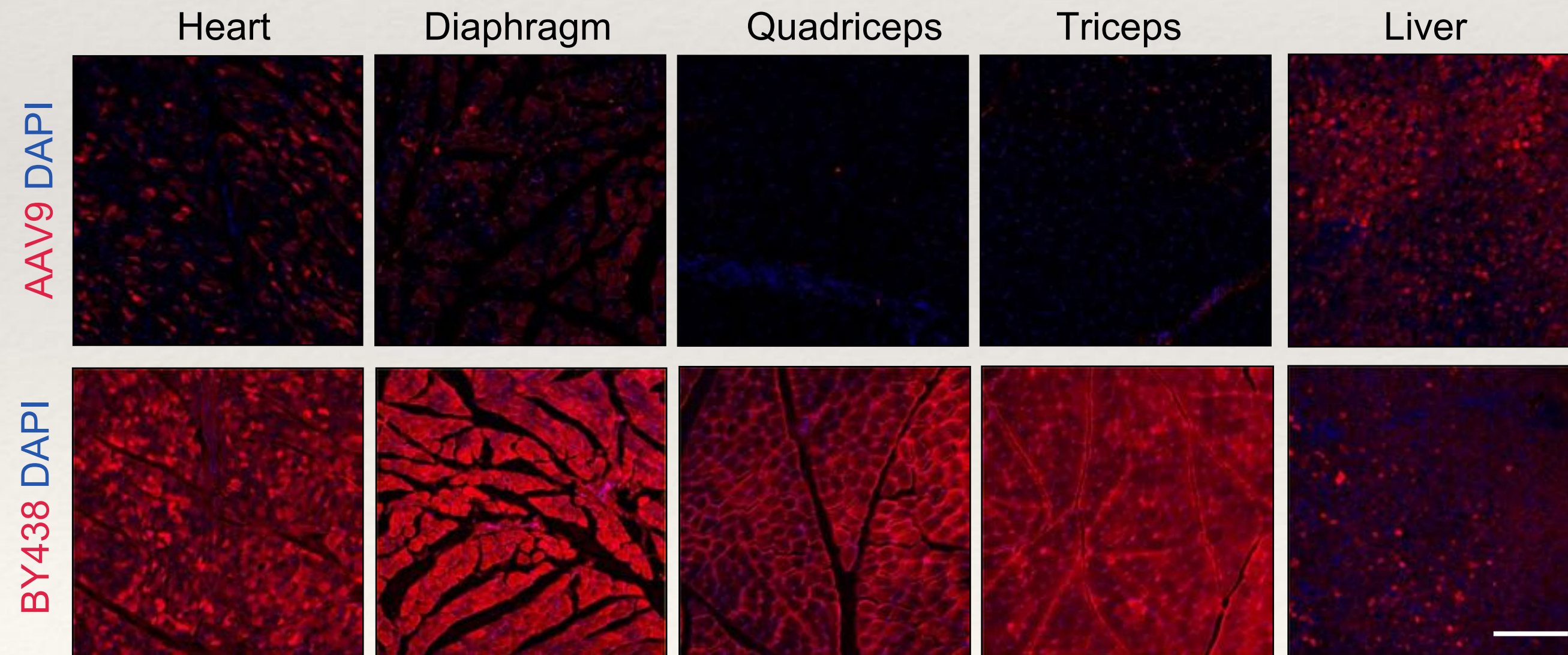
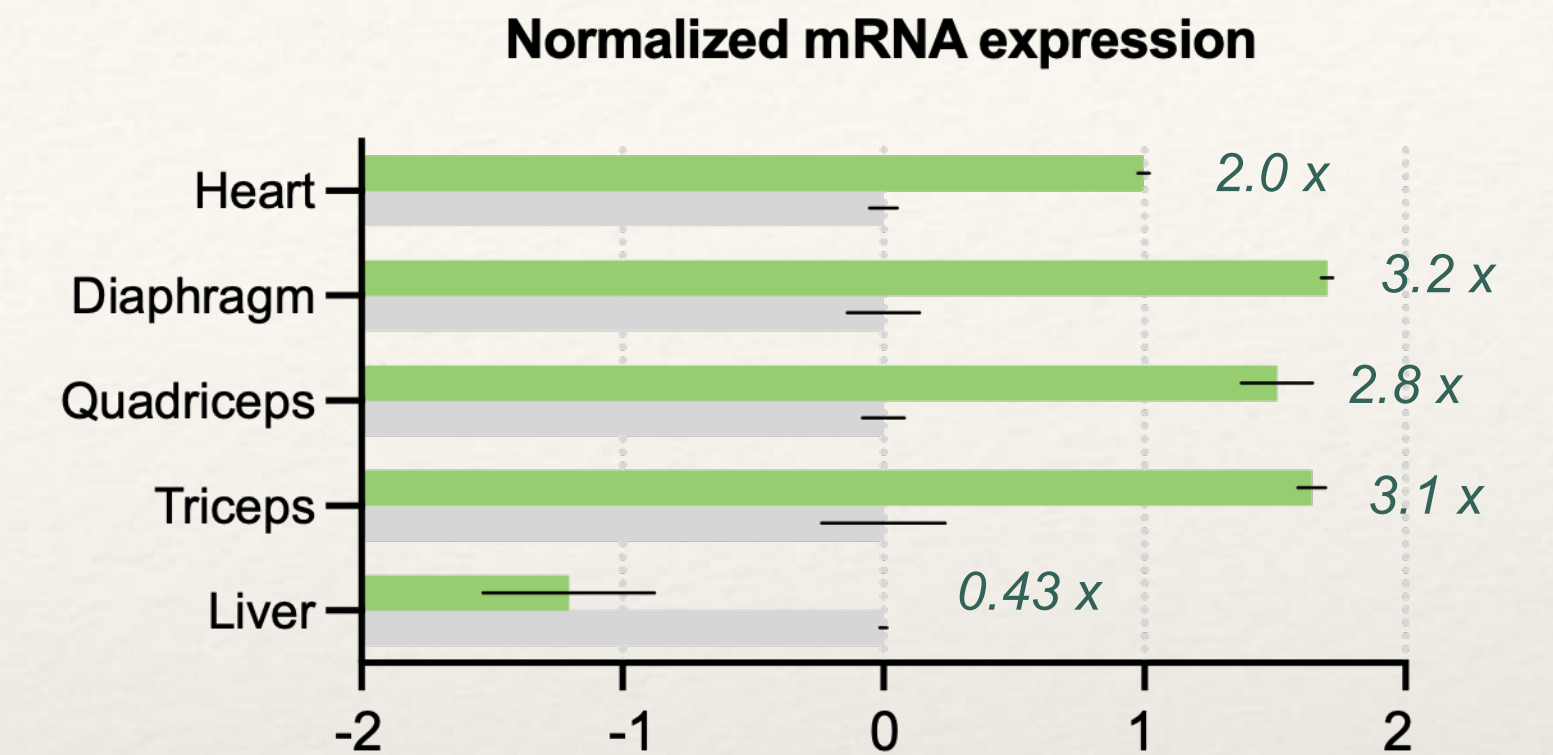
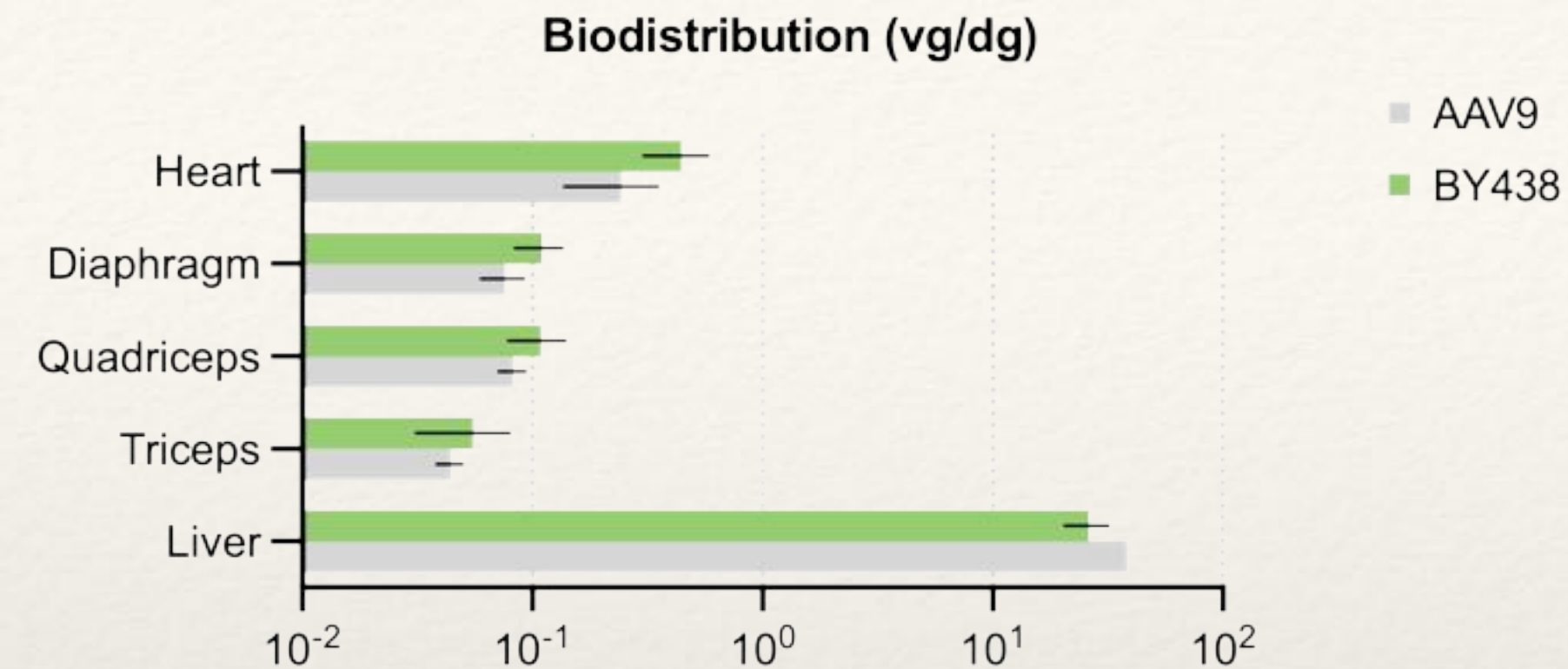
Biological Robustness: Consistent distribution patterns validated across dual-dose barcode gradients.

BY438 Specificity: **3.7x** (heart) and **6.7x** (skeletal muscle) higher mRNA expression vs AAV9; liver uptake reduced to **39%**.

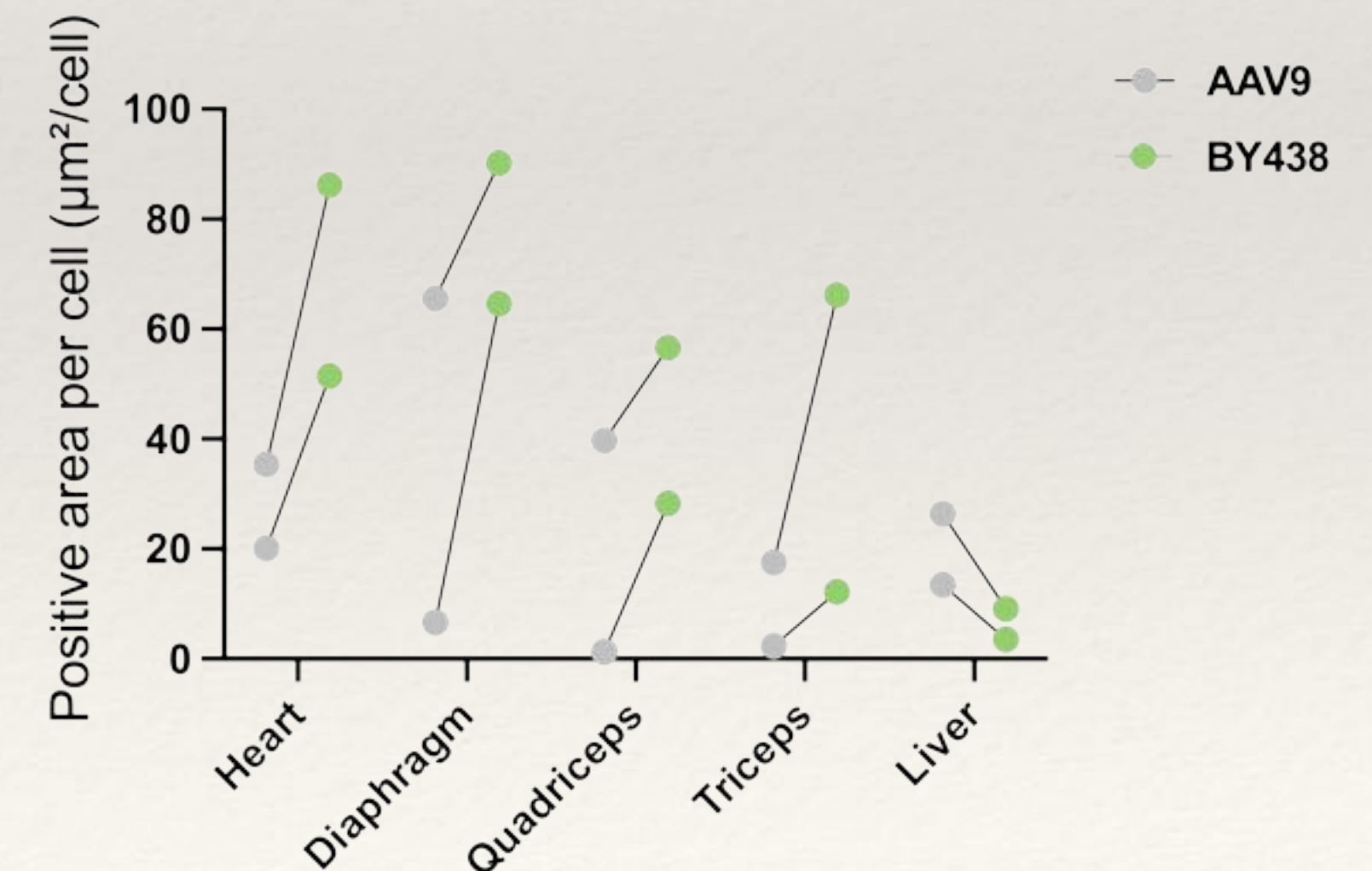
Comprehensive Characterization of BY438 Validates Robust Muscle Expression and Liver Escape in NHPs

NHP Study:

Adult rhesus macaques (n=2);
IV co-injection of BY438 (CAG-S-tag) and AAV9 (CAG-His-tag);
 1×10^{13} vg/kg per vector each;
3-week study duration;
Transgene expression



Scale: 200 μ m



Cross-Species Validation: MyoCAP Variants Achieve Superior Heart & Muscle Potency with Enhanced Hepatic Escape

NHP Study:

Adult cynomolgus macaques (n=2);

1.5×10^{13} vg/kg barcode library; 3 weeks in-life;

Transgene mRNA expression

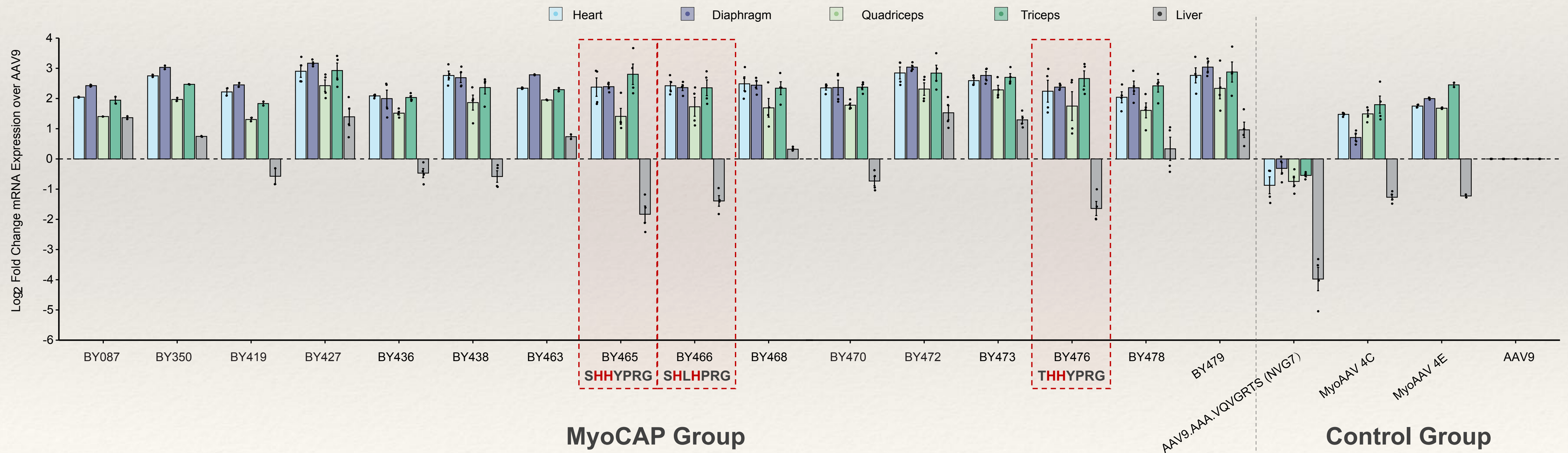
CALIBRATOR:

20 variants, 2 BCs/variants & AAV9 with 6 gradients

Superior Heart & Muscle Potency: MyoCAP leads exceed mRNA expression of published benchmarks in heart and skeletal muscle.

Precise “H-Switch” De-targeting: Strategic H-insertion decouples muscle potency from liver tropism, ensuring robust hepatic escape (e.g., BY465, BY466, BY476).

Cross-Species Robustness: Consistent efficiency and specificity in Rhesus & Cynomolgus macaques validate high clinical translatability.



Note: n=1 for MyoAAV 4E, BY087, BY350, BY419, and BY463 (due to low signal in one animal); all other variants are mean of n=2.

Conclusion

DynEvoLib provides a robust, end-to-end pipeline that translates decoded evolutionary logic into **precision design** and comprehensive validation to expedite next-generation AAV vector discovery.

The **novel PRG motif** serves as the **core engine** for muscle potency, while **flanking H-residues** act as a precise '**switch**' to abolish liver off-targeting.

The **MyoCAP** series, represented by **BY438**, demonstrates exceptional muscle transduction and hepatic escape in NHPs, providing a **validated and highly competitive** toolkit for systemic gene therapy.



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BYONGEN

THANK YOU

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