

# ASGCT 28th Annual Meeting Abstracts

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Due to an editorial oversight, the ASGCT 28th Annual Meeting Abstracts in the April 28, 2025 special issue of *Molecular Therapy* omitted 30 abstracts that had been intended for publication. These 30 abstracts have now been added to a revised abstracts file, which is now available in Document S1 with this correction online. The editors sincerely apologize to the authors of the previously omitted abstracts for this oversight.

## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.ymthe.2025.08.030>.



performing input variant. Next, we screened these *in silico* designed variants in mice and observed >60 newly designed variants showing higher cardiomyocyte transgene expression than the best-performing input variant. Individual validation of selected variants confirmed the library-based screen results. We further characterized a subset of *in silico* designed variants in cynomolgus monkeys and identified multiple candidates with greater cardiomyocyte transgene expression than AAV9, the best-performing input variant, and an industrial benchmark muscle-tropic capsid. Finally, our novel capsid candidates mediate significantly higher *in vivo* cardiac gene editing efficiency compared to AAV9, highlighting their potential therapeutics advantage. Our state-of-the-art capsid engineering platform that combines high throughput *in vivo* experimental screening and various *in silico* design methods has led to the identification of multiple novel capsid candidates with enhanced cardiomyocyte targeting efficiency following systemic delivery in both murine and non-human primate models, which may enable more efficacious and safer next generation gene therapies for cardiac disorders.

### 1397 An Improved Approach Enables Efficient and Cost-effective Directed Evolution of AAV Capsids in Non-human Primates via Different Delivery Routes

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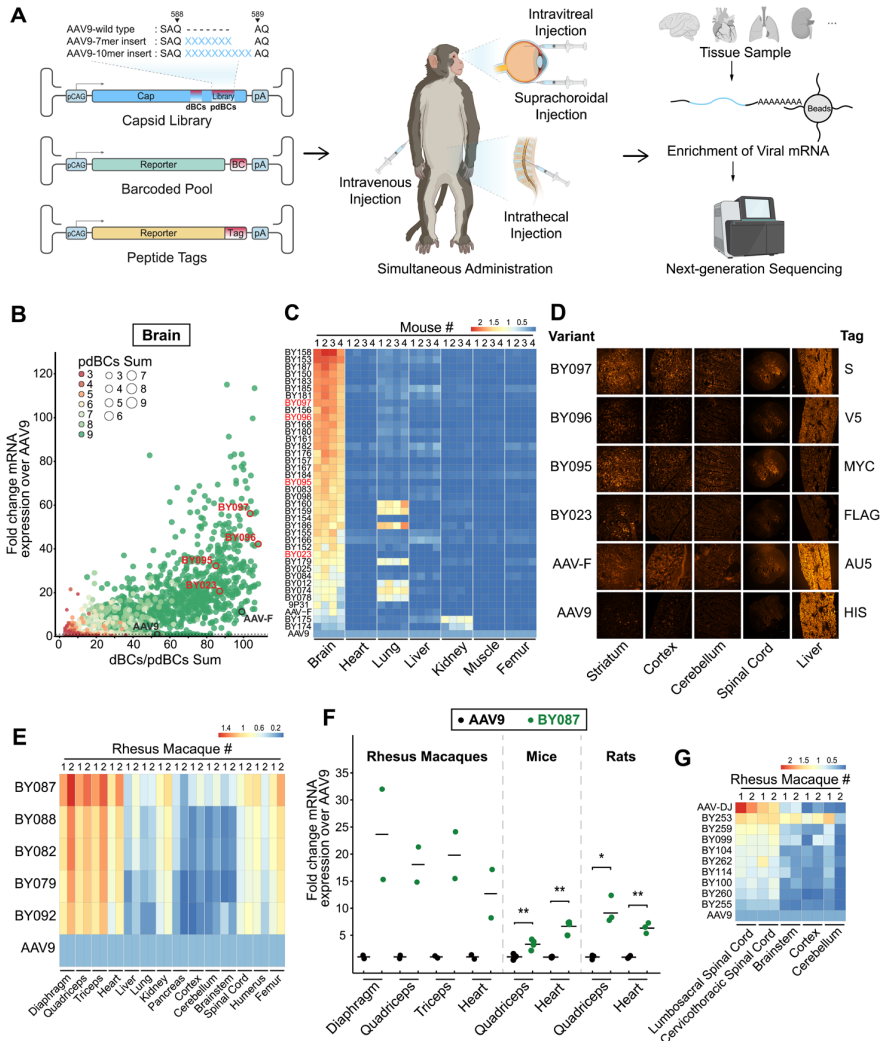
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**Introduction:** Non-human primates (NHPs) are ideal and valuable models for the directed evolution of Adeno-associated virus (AAV) capsids. It is attractive to use fewer NHPs to obtain more experimental data. Here, we developed a modified platform called DynEvoLib (Dynamic Evolution Library) for simultaneous directed evolution of capsids targeted at multiple tissues via different delivery routes in NHPs.

**Methods:** DynEvoLib contained three types of libraries and pools (Fig. 1A). The first-round screening was conducted using high-throughput capsid mutation libraries, in which random peptides or peptides derived from known proteins were inserted into the AAV9 capsid. Degenerate code mutations were introduced to flank the insertion site and used as degenerate barcodes (dBCs) to distinguish different libraries. Degenerate oligonucleotides generated peptide inserts (peptide-derived degenerate barcodes, pdBCs) for capsid libraries of subsequent screening rounds. mRNA expression of variants was then quantified by deep sequencing of barcoded AAV pools. Finally, AAVs containing multiple peptide tags were used to characterize variants at DNA, RNA, and protein levels. Libraries containing different dBCs/pdBCs, barcoded pools, and/or pools of peptide tags were administered to a single animal simultaneously via multiple delivery routes (Fig. 1A). Specific oligo-based magnetic beads were used for the enrichment of viral mRNA from total RNA to improve the acquisition of viral transcripts (Fig. 1A).

**Results:** DynEvoLib was optimized in a mouse model. It showed that dBCs/pdBCs combinations not only facilitated distinguishing between libraries used for multiple delivery routes but also improved the accuracy of peptide transcription analysis based on unique barcode counts compared with the analysis based on mRNA expression levels alone. Up to 576 variants showed more efficiency in brain transduction compared to AAV-F (Fig. 1B). There was good consistency between capsid libraries, barcoded pools, and peptide tags, including enhanced brain transduction and liver de-targeting (Fig. 1B, C, D). DynEvoLib was applied to rhesus macaques via multiple delivery routes. It showed that the mRNA enrichment strategy processed up to 10g of single tissue sample, resulting in a 151-fold increase in the collection of viral transcripts over the regular single-tube RT-PCR strategy. Enrichments of variants were first seen in the heart, muscle, kidney, and bone by intravenous administration, and in the central nervous system (CNS) by intrathecal administration. Characterizations using peptide tags identified several myotropic variants in rhesus macaques via intravenous injection (Fig. 1E). The optimal variant BY087 transduced skeletal muscle 21-fold and cardiac muscle 13-fold more effectively than AAV9 and performed cross-species muscle tropisms in NHPs, mice and rats (Fig. 1F). CNS tropic variants were characterized using the barcoded pool in rhesus macaques via intrathecal injection. BY253 showed 30-fold higher transduction in the spinal cord than AAV9 and transported more efficiently than AAV-DJ in the spinal cord, brainstem, cortex, and cerebellum (Fig. 1G).

**Conclusions:** DynEvoLib enables efficient and cost-effective simultaneous directed evolution of AAV capsids, simultaneously targeting multiple tissues through different delivery routes in NHPs.



### 1398 *In Silico* and *In Vitro* Analysis Suggests Translational Potential of the Smooth Muscle-Tropic Engineered Capsid, AAV-PR

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