ANNUAL MEETING

BOSTON, MA • May 11-15, 2026

ASGCT 2026 ANNUAL MEETING ABSTRACTS

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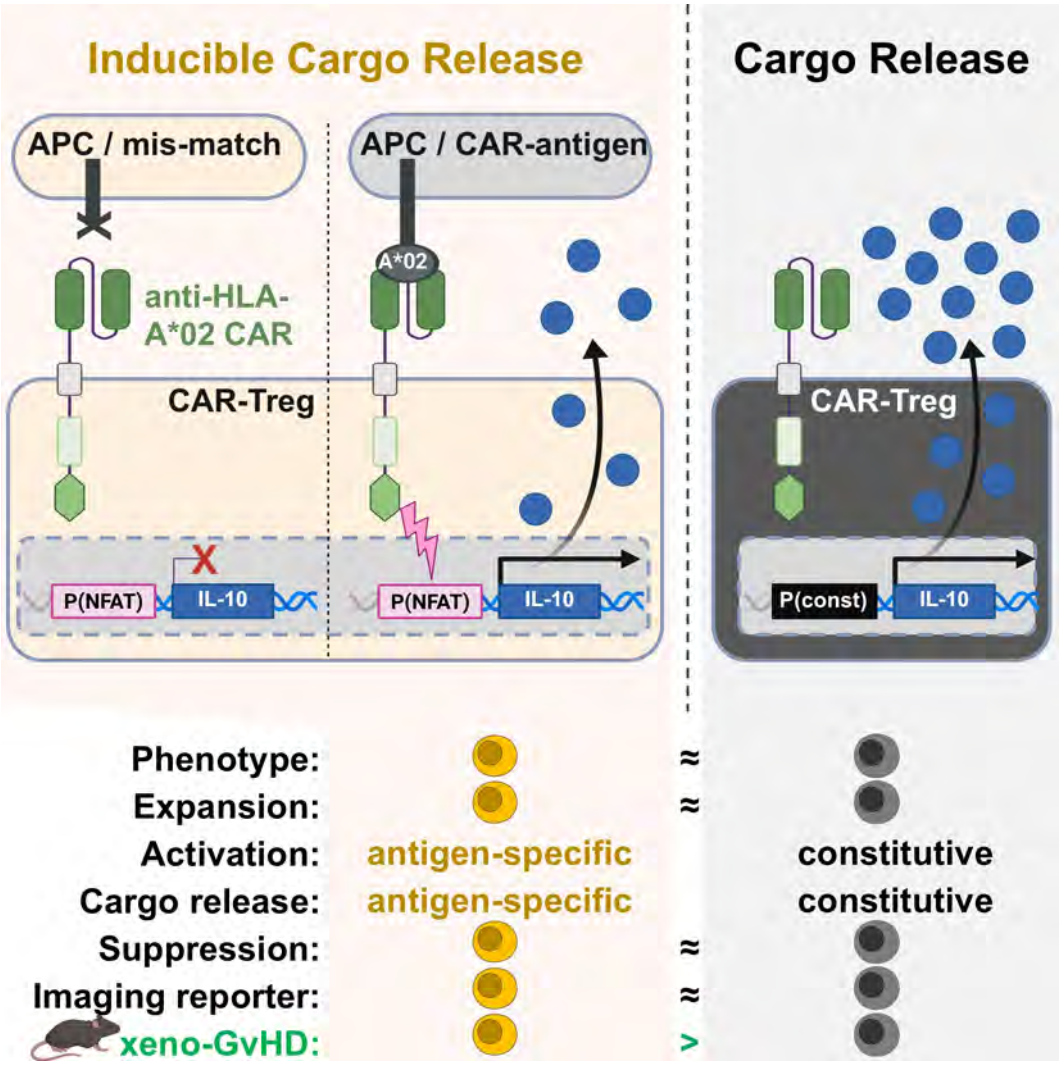


Figure Legend

Cartoon illustrating the concept of CAR-Tregs that release interleukin-10 upon CAR antigen engagement, which were fully characterised in this work including a comparison with CAR-Tregs that constitutively express interleukin-10. For methodological details and results, see abstract.

AAV capsid engineering III

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

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Introduction Adeno-associated virus (AAV) is a widely used gene delivery vector for treating muscle disorders. Effective systemic gene delivery requires both enhanced expression in cardiac and skeletal muscle and minimized liver off-targeting. Although incorporation of the RGD motif is a well-established strategy for enhancing muscle tropism, identifying novel functional motifs is critical for expanding the AAV targeting toolkit. Here, through directed evolution in nonhuman primates (NHPs), we identified a novel motif and developed capsids that achieve potent muscle expression with reduced liver targeting.

Methods We employed the DynEvoLib (Dynamic Evolution Library) platform to perform muscle-targeted evolution of AAV9-derived capsids in NHPs. The platform integrates a diversified, CAG promoter-driven capsid mutagenesis library that includes both random and rationally designed peptide insertions, coupled with RNA-seq-based functional screening of capsid variants. The platform also incorporates a barcoded capsid library with internal calibration for in vivo functional ranking, together with epitope-tagged reporters, enabling parallel assessment of multiple capsids within a single animal.

Results Two rounds of systemic capsid library screening via intravenous administration in rhesus macaques identified a significantly enriched non-RGD motif associated with enhanced muscle expression. Using a barcoded capsid library, four variants harboring this motif showed substantially higher transgene expression in skeletal and cardiac muscles compared to AAV9. Compared with RGD-derived capsids (MyoAAV), these variants achieved comparable skeletal muscle expression but exhibited stronger cardiac tropism. The lead variant, BY087, was further evaluated in vivo via epitope-tagged reporters. It showed markedly increased transgene expression in skeletal and cardiac muscles relative to AAV9, although with modestly increased liver expression. To further enhance muscle and heart expression while reducing liver tropism, we performed NNK saturation mutagenesis of multiple residues flanking the motif. Substitutions at residues immediately adjacent to the motif had the strongest effects. In particular, histidine (H) reduced liver tropism, whereas tyrosine (Y) increased expression in both liver and muscle. These findings guided the design and barcoded screening of next-generation variants. The screening results revealed two distinct expression profiles: one group of variants (BY438, BY466, BY470) retained strong muscle expression comparable to BY087 while reducing liver tropism; the other group (BY427, BY472, BY478, BY479) showed broad enhancement in both skeletal and cardiac muscles relative to BY087, albeit with a modest increase in liver expression (Fig. 1A). These optimized capsids matched or exceeded MyoAAV 4E/4C in muscle expression while achieving comparable or improved reduction of liver off-targeting. Based on the screening results, BY438 was selected for in vivo evaluation at therapeutic doses. Using epitope-tagged reporters, the expression of co-injected BY438 and AAV9 was assessed in the same animals. BY438 showed markedly higher mRNA expression than AAV9 in skeletal and cardiac muscles, while liver expression was reduced (Fig. 1B). Immunofluorescence confirmed these findings, showing stronger BY438 signal in muscle tissues and lower signal in the liver (Fig. 1C).

Conclusion Directed evolution using DynEvoLib in NHPs identified a novel motif that enabled the development of optimized capsids with robust skeletal and cardiac muscle expression and reduced liver tropism. These optimized capsids achieve skeletal muscle expression comparable to the RGD-based benchmark vector (MyoAAV) and superior cardiac targeting, offering a promising strategy for muscle-directed gene therapy.

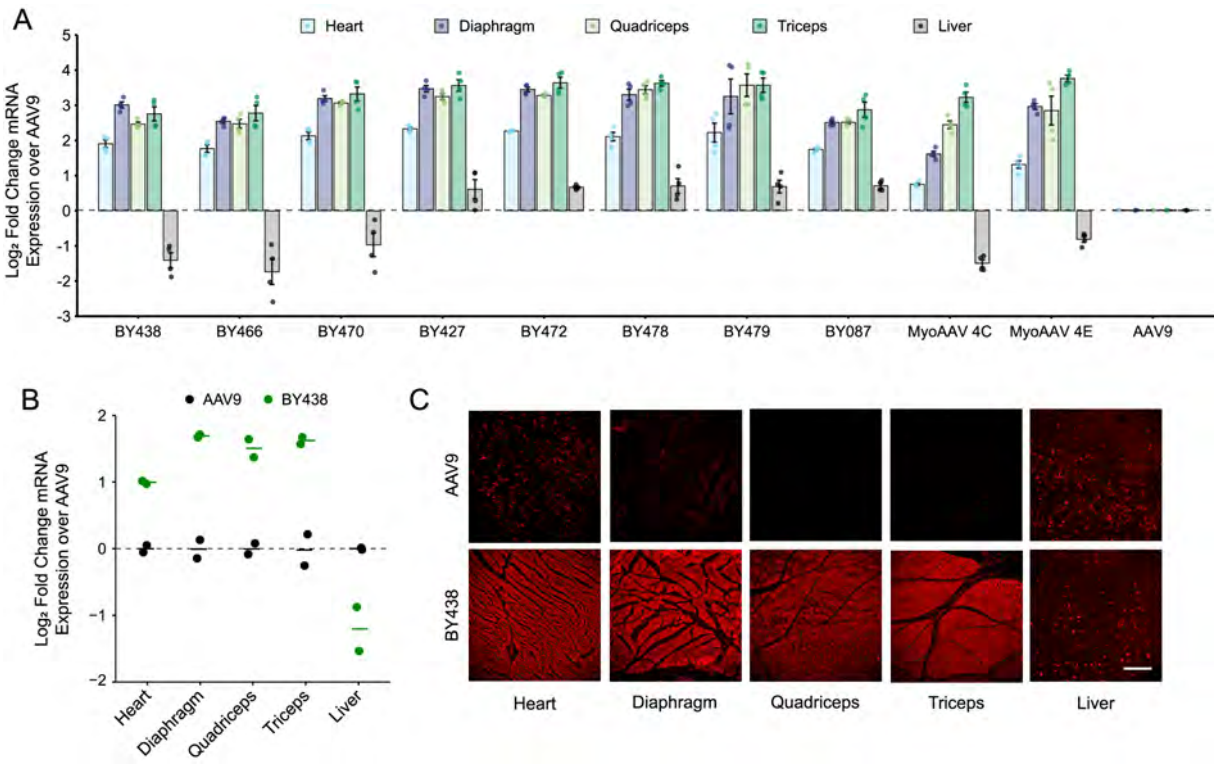


Figure Legend

Figure 1. In vivo validation of engineered AAV capsid variants in rhesus macaques. (A) Relative mRNA expression of capsid variants in selected muscle and liver tissues from a barcoded variant library screening (n = 2), shown as log2 fold change relative to AAV9, with two barcodes per variant. (B, C) Co-administration of epitope-tagged BY438 (S-tag) and AAV9 (His-tag) (n = 2; 1 × 1013 vg/kg per vector). (B) Relative mRNA expression of BY438 compared with AAV9 in selected muscle and liver tissues, quantified by NGS of epitope-tagged transcripts. (C) Immuno-fluorescence detection of epitope tags in tissue cryosections. Scale bar, 400 um.

313 Post-manufacture conjugation of AAV vectors with human transferrin receptor-binding groups demonstrates strong brain transduction and reduced liver targeting.