Mechanism of Improved POLARIS-101™ Capsid Transduction

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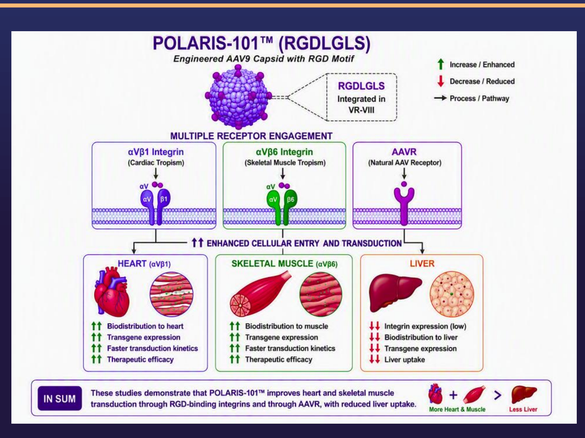
ABSTRACT

Gene therapy with adeno-associated virus (AAV) continues to be a promising therapeutic modality for diseases with high unmet need. However, the high systemic doses required to achieve widespread therapeutic benefits for indications like Duchenne muscular dystrophy (Duchenne) can pose potential safety risks. To develop a more potent vector, we rationally modified AAV9 to contain an integrin-targeting RGDLGLS peptide in variable region VIII resulting in POLARIS-101™ (previously named AAV-SLB101). POLARIS-101™ is currently utilized in Solid Biosciences' next-generation investigational Duchenne gene therapy, SGT-003, which is being evaluated in the Phase 1/2 INSPIRE DUCHENNE (NCT06138639) and Phase 3 IMPACT DUCHENNE (NCT07160634) clinical trials. Here, we demonstrate that POLARIS-101 improves both cardiac and skeletal muscle transduction, with decreased liver uptake in multiple preclinical models. Furthermore, POLARIS-101™ transduces tissues faster than first-generation capsids, potentially contributing to its improved safety profile. We found that POLARIS-101 transduction was significantly improved when compared to AAV9 in HEK293 cell lines engineered to over-express human integrin receptors ITGAV, ITGA8, ITGB1, ITGB3, ITGB6, and ITGB8. Importantly, siRNA knockdown of integrin receptors ITGAV and ITGB1 in human iPSC-derived cardiac myocyte/cardiac fibroblast co-cultures prevented POLARIS101™ transduction. Bioinformatic evaluation of integrin receptor distribution in human organs and cells suggest that the improved cardiac transduction results from avb1 integrin, whereas the improved skeletal muscle transduction results from avb6 and avb8 integrin. The observation of lower liver biodistribution of POLARIS-101 in preclinical models is supported by bioinformatic results demonstrating low levels of integrins in the liver. Interestingly, POLARIS-101™ also showed improved transduction of HEK293T cells that over-express AAVR, when compared to AAV9, providing evidence of more efficient use of the natural AAV receptor by POLARIS-101. Together, these studies demonstrate that POLARIS-101™ improves heart and muscle transduction through RGD-binding integrins and through AAVR.

GRAPHICAL ABSTRACT

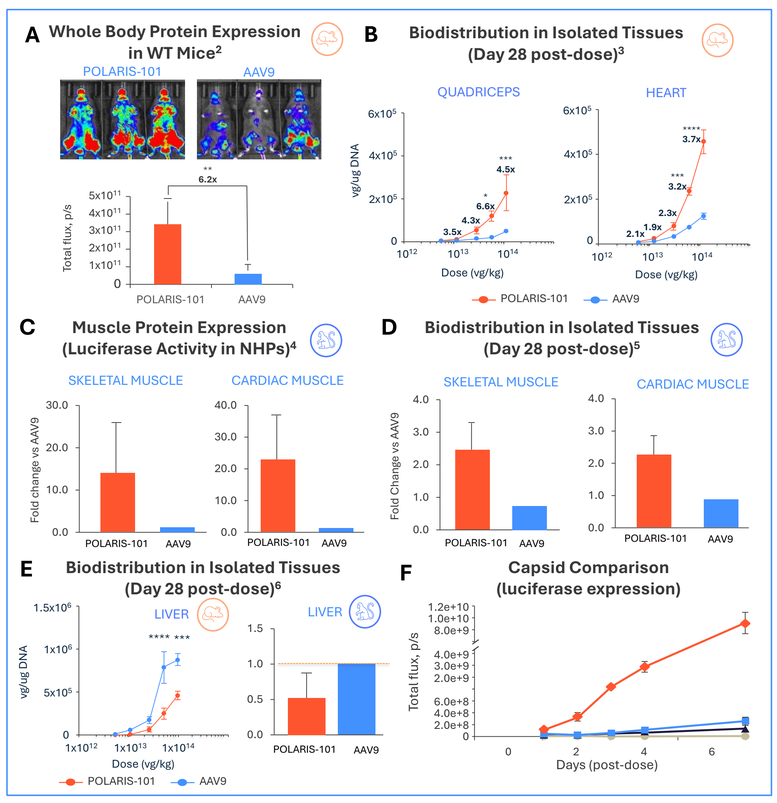


METHODS

- In vitro studies: AAV capsids were evaluated in HEK293T cells and cell lines overexpressing individual integrins (ITGs) or AAVR.
- In vivo studies: AAV capsids were administered to mice and non-human primates (NHPs) at 5 × 10^{13} vg/kg, and tissues were collected 28 days post-dose.
- Cell type-specific integrin expression was characterized by snRNA-seq analysis of mouse heart and gastrocnemius muscle tissues.

RESULTS

Figure 1. Improved transduction of POLARIS-101 compared to AAV9[1]



- **Figure 1:** POLARIS-101 resulted in faster transgene expression and improves both cardiac and skeletal muscle transduction, with decreased liver uptake in preclinical models.

REFERENCES

1. Boehler J, et al. Presented at ASGCT, May 13-17, 2025, New Orleans, LA. **2.** Asterisks indicate statistical significance between groups (**p<0.01). 3E14vg/kg, Day 15. **3.** Asterisks indicate statistical significance (*p<0.05, ***p<0.001, ****p<0.0001) between groups with fold changes depicted. **4.** Average fold differences calculated from the five skeletal muscle tissues sampled and three regions of cardiac tissue sampled. 5E13 vg/kg, Day 29 post-dose. **5.** Average fold differences calculated from the five skeletal muscle tissues sampled, three regions of cardiac tissue sampled, and the single liver sample. Dose 5e13 vg/kg. **6.** Asterisks indicate statistical significance between groups (***p<0.001, ****p<0.0001).

ACKNOWLEDGMENTS

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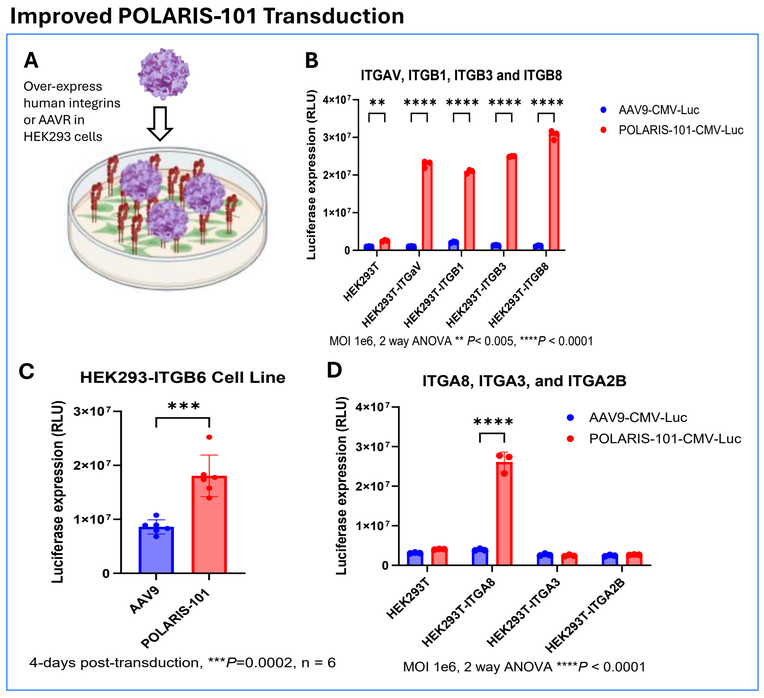
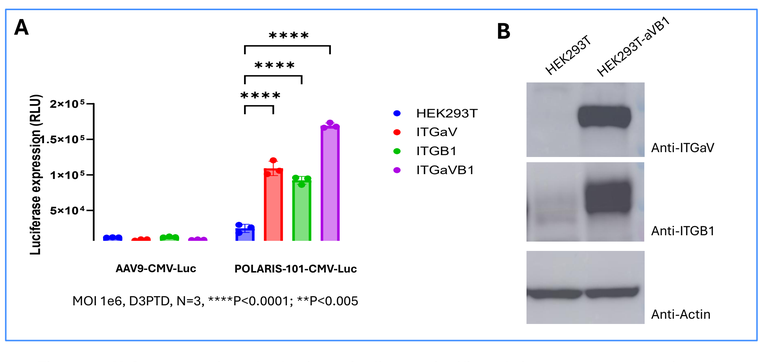
RESULTS

Figure 2. RGD-Integrin Receptor Expression in HEK-293 Cells Improved POLARIS-101 Transduction



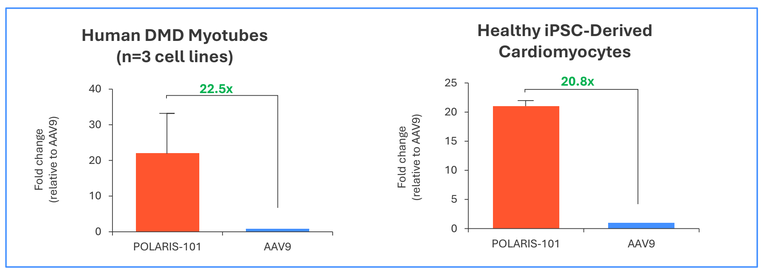
- **Figure 2:** Multiple RGD-binding integrins improve Polaris-101 transduction. **A)** POLARIS-101 or AAV9 was added to HEK-293 cell lines over-expressing various integrin receptors, and the resulting luciferase expression was measured. **B)** RGD integrin receptors significantly improved transduction of Polaris-101. **C)** ITGB6, a skeletal muscle integrin, improved transduction of POLARIS-101. **D)** ITGA8 also improved POLARIS-101 transduction. The non-RGD integrins ITGA3, ITGA2B, ITGA9 did not improve transduction of Polaris-101.

Figure 3. αVβ1 Co-expression Enhances POLARIS-101™ Transduction



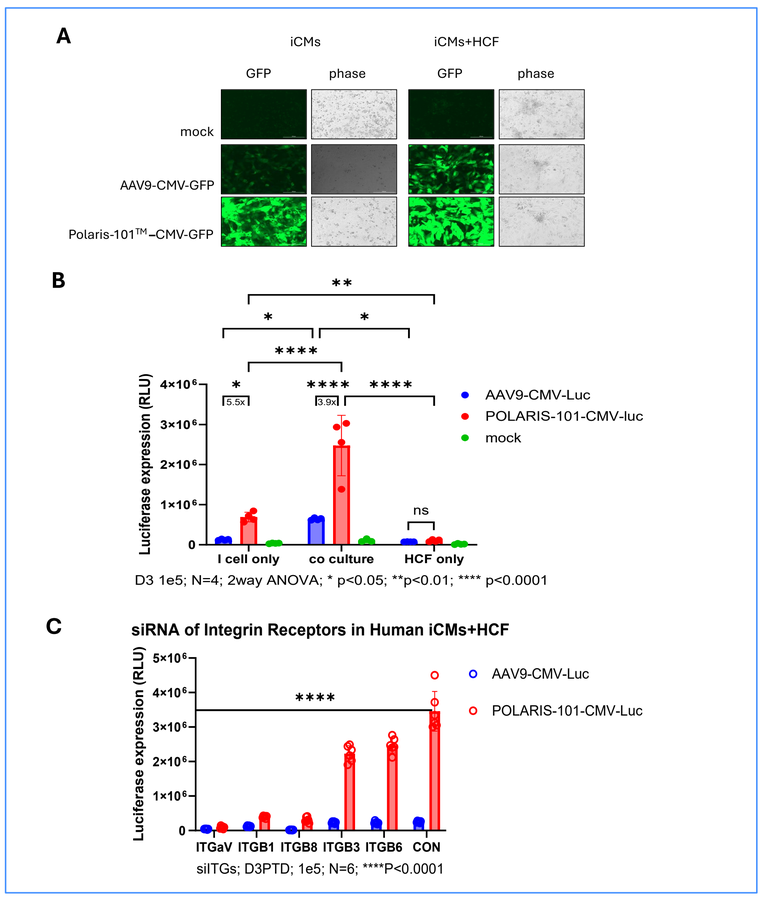
- **Figure 3: A)** Co-expression of avb1-integrin dimers significantly improved transduction of POLARIS-101. **B)** Confirmation of expression of avb1-integrin by western blot.

Figure 4. Human iPSC-derived iCells and Myotubes can increase AAV9 and SLB101 transduction[1]



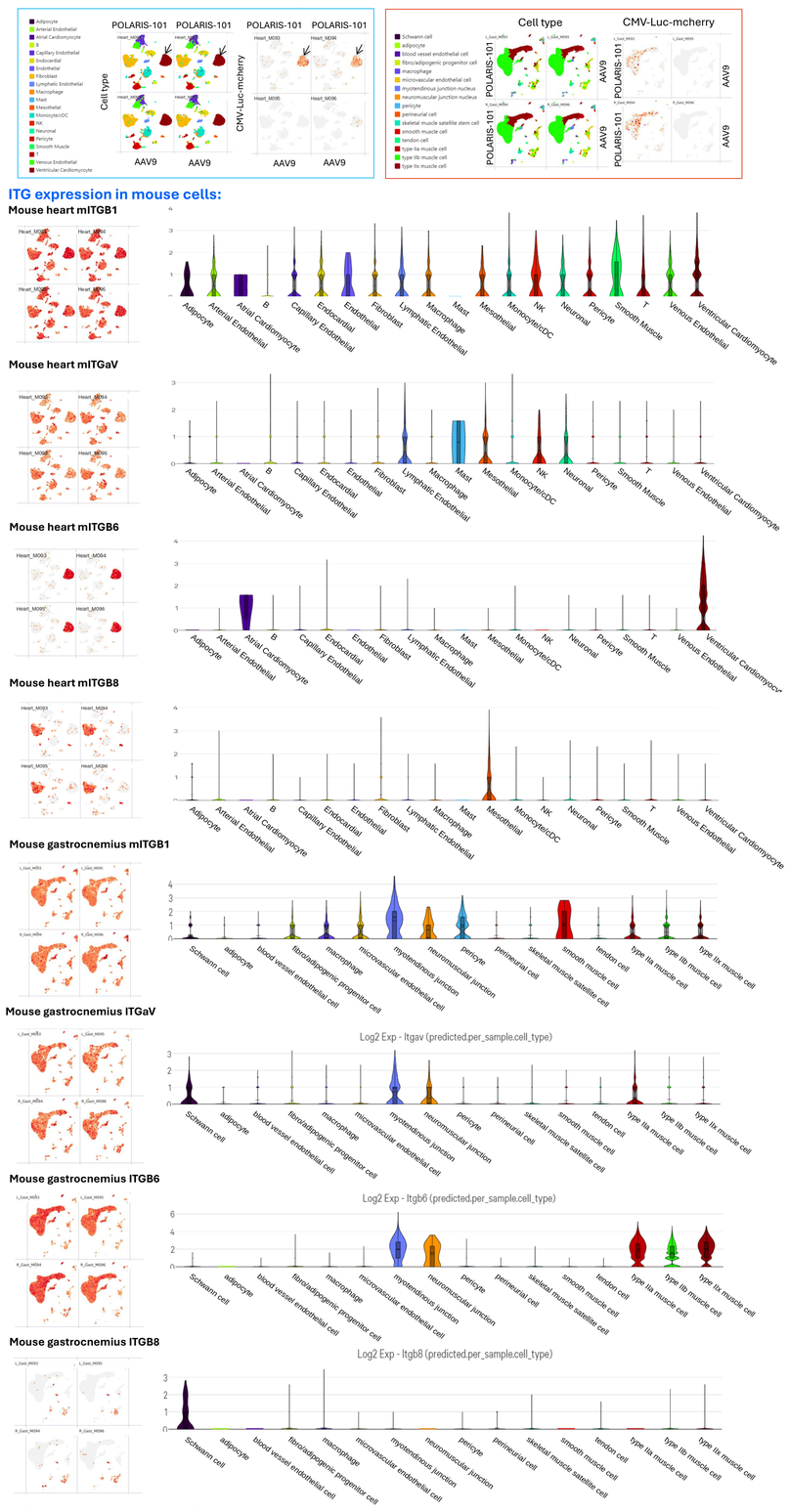
- **Figure 4:** Polaris-101 has improved transduction of A) iPSC cardiac myocytes and B) myotubes when compared to AAV9

Figure 5. Improved Polaris-101 Transduction of Human iPSC Cardiac Myocytes Results From RGD-Integrin Receptors



- **Figure 5:** POLARIS-101 leads to improved transduction of iPSC **A)** Human primary fibroblasts improve Polaris-101 transduction of human iCells with CMV-GFP. **B)** Shown is the mean +/- S.D. of CMV-luciferase expression in human iCMs alone, human primary fibroblasts alone, or co-cultures between both cell types. **C)** siRNA of ITGav, ITGB1 and ITGB8 significantly reduced transduction of POLARIS-101 in human co-cultures. ****P < 0.0001 compared to AAV9.

Figure 6. Cell Type-Specific Transgene and ITG Expression in Mouse Heart and Skeletal Muscle by snRNA-seq



- **Figure 6:** Single nuclei RNAseq of transgene and integrin receptor biodistribution in the mouse heart and gastrocnemius muscle

Figure 7. POLARIS-101 More Efficiently utilizes AAVR as a Natural Receptor for Cellular Transduction Than AAV9

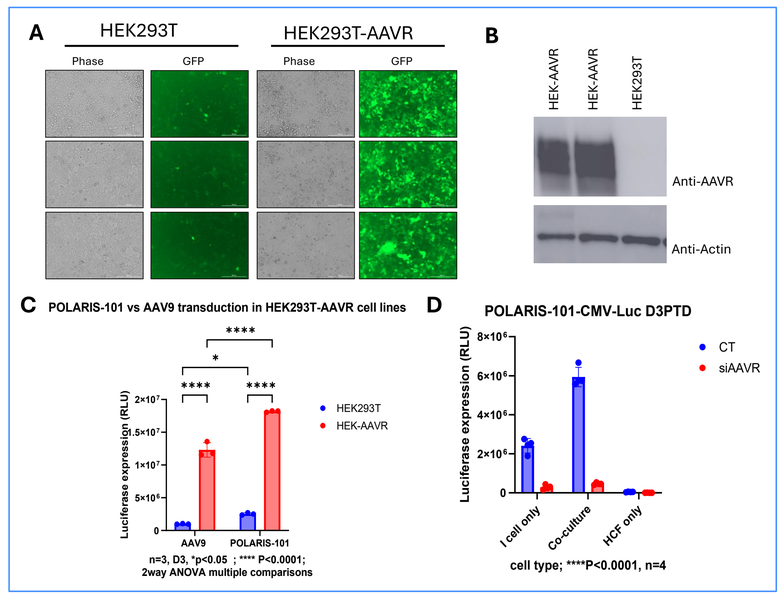


Figure 7: (A-C) POLARIS-101™ demonstrated enhanced transduction in AAVR-overexpressing HEK293T cells compared with AAV9. **D)** AAVR knockdown reduced POLARIS-101 transduction in cardiomyocytes, supporting AAVR as a functional receptor for POLARIS-101™

CONCLUSIONS

- POLARIS-101 demonstrates enhanced heart and skeletal muscle transduction with reduced liver uptake through multiple RGD-binding integrins.
- αVβ1 is a key receptor for Polaris-101 transduction to the heart.
- Human and mouse skeletal muscle is enriched for β6 integrins, suggesting αVβ6 contributes to improved skeletal muscle transduction.
- POLARIS-101 also has enhanced transduction through AAVR when compared to AAV9.
- Collectively, these studies support a dual-receptor mechanism in which POLARIS-101™ achieves enhanced heart and skeletal muscle transduction through both RGD-binding integrins and AAVR, while maintaining reduced liver uptake.

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