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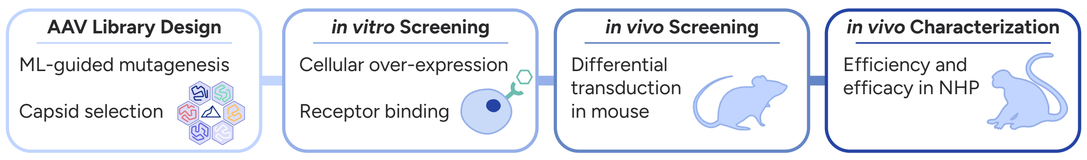
AI-guided engineering of dual-mechanism AAV capsids enhances muscle delivery, reduces CNS tropism, and confers cross-species translation in NHP and mouse

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Introduction

- While AAV capsids remain the most effective modality for achieving systemic therapeutic expression for muscular diseases, **existing muscle capsids in clinical trials lack the efficiency and specificity required without resorting to high doses**, often starting at 1e14 vg/kg.
- High vector doses introduce significant safety risks due to off-target effects in peripheral organs, particularly in the liver, underscoring the need for capsids with improved efficacy.
- **We used AI to engineer novel capsids that combine two mechanisms** – DR1, a newly identified receptor that mediates muscle transduction, and integrin-binding functionality – **achieving improved muscle tropism with liver detargeting in mice and NHPs at a low dose of 5e12 vg/kg.**



Integrin-binding reduces CNS tropism of DR1 capsids

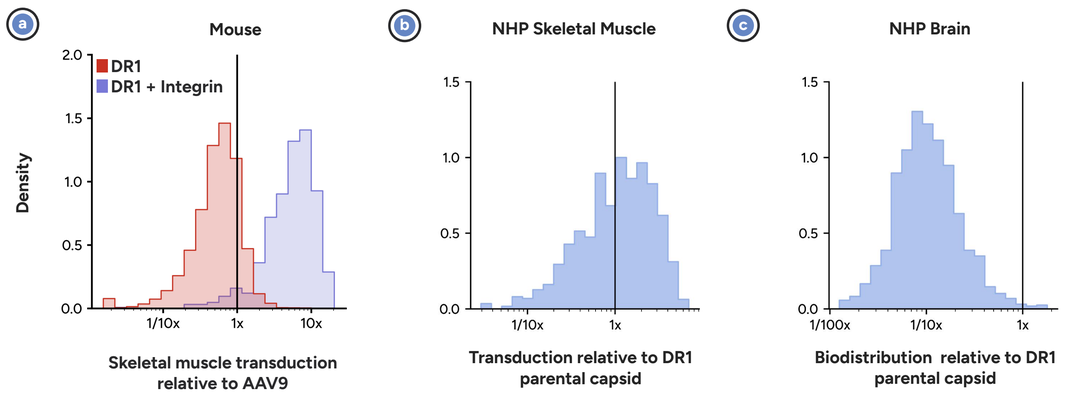


Figure 1. Impact of adding integrin-binding functionality to DR1 capsids in mouse and NHP. When integrin-binding functionality was added to muscle- and CNS-targeting DR1 capsids in the 200k-capsid library screen: **(a)** in mouse, capsids that bind both DR1 and integrin-αVB6 showed increased skeletal muscle transduction compared to DR1-only capsids. In NHP **(b)** muscle transduction was generally maintained or improved, while **(c)** brain biodistribution was reduced relative to the corresponding DR1 parental capsid.

Top capsids from pooled screen show improved muscle tropism in both NHP and mouse

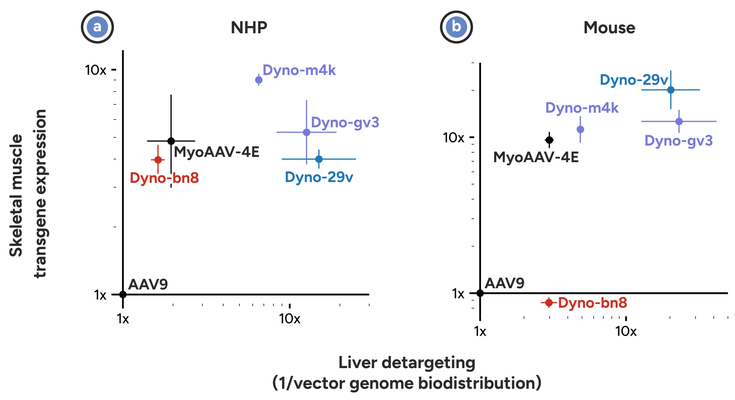


Figure 2. Novel Dyno cross-species capsids improve muscle tropism and liver detargeting in both NHP and mouse. We identified lead candidates from a focused 200k-capsid library screen in mice and NHPs. Transduction rates of lead capsids in skeletal muscle and the inverse of biodistribution rates in liver relative to AAV9 are shown in **(a)** NHP and **(b)** mouse. Dyno capsids are color-coded by receptor family: **integrin-αVB6** (**blue**), **DR1** (**red**), or **DR1 + integrin-αVB6** (**purple**). Reference capsids, such as AAV9 and MyoAAV-4E[1], are shown in **black**. Error bars show SEM. DR1 = Dyno Receptor. (1) Tabebordbar M., *et al.* Cell. 2021;184(19):4919-4938.

Dyno-m4k and Dyno-gv3 engage multiple receptors to outperform AAV9 in both human and mouse cell lines

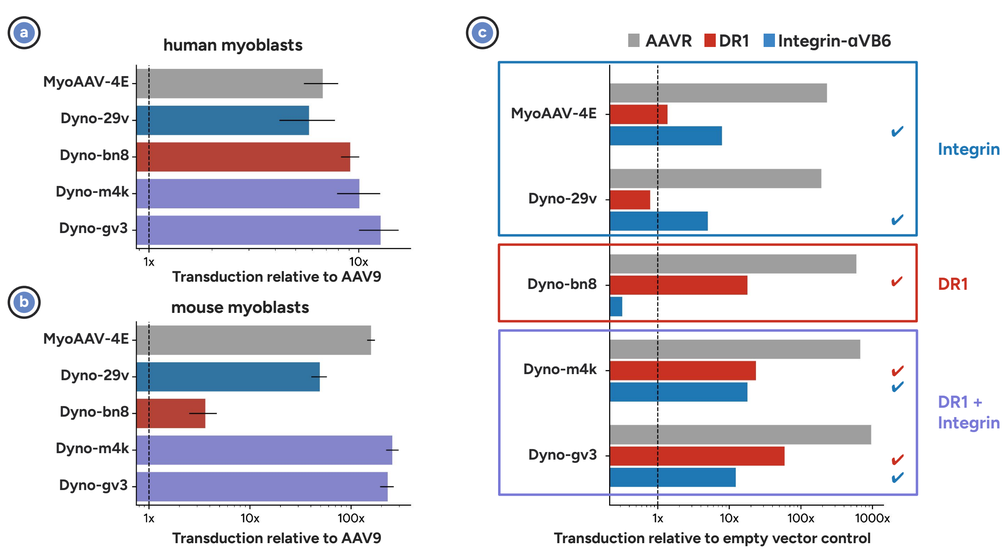


Figure 3. Dyno capsids are functional and transduce human and mouse myoblasts in culture, relying on both DR1 and integrin-αVB6 to transduce cells. Capsids expressing a luciferase reporter were used to transduce **(a)** AB1190 human myoblasts and **(b)** C2C12 mouse myoblasts, and the resulting luminescence over untreated cells was measured, with relative transduction to AAV9 shown. Dyno capsids are color-coded by receptor family: **integrin** (**blue**), **DR1** (**red**), or **DR1 + integrin** (**purple**). MyoAAV-4E reference capsid is in **gray**. Error bars show 95% confidence interval. **(c)** Capsids expressing a luciferase reporter were tested in AAVR-KO HEK293T cells over-expressing receptors AAVR, DR1, or integrin-αVB6, and the resulting luminescence was measured relative to empty-vector control. All capsids showed enhanced transduction with AAVR expression; MyoAAV-4E and Dyno-29v showed elevated transduction with integrin-αVB6, while Dyno-bn8 showed increased transduction with DR1. The multi-mech capsids (Dyno-m4k and Dyno-gv3) showed marked transduction in the presence of either integrin-αVB6 or DR1.

Dyno capsids target muscle and heart better than MyoAAV-4E, with less expression in liver

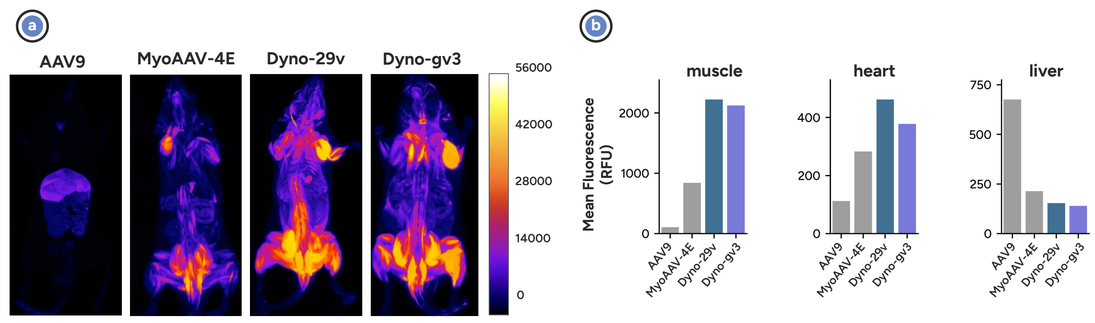


Figure 4. Whole-body imaging in mice: all Dyno capsids demonstrate improvement in on-target muscle expression compared to AAV9 and MyoAAV-4E. C57BL/6J male mice (n=1) were IV injected with a capsid expressing either Cbh-mCherry (AAV9 only) or Cbh-GFP (Dyno-29v, Dyno-gv3, and MyoAAV-4E) at 5e12 vg/kg. After 28 days in life, whole-body cryo-fluorescence tomography (CFT) was performed by EMIT Imaging. **(a)** Maximum intensity projections from 50-ms exposure with scale showing relative fluorescent units (RFU) on right. **(b)** Measurements derived from the 50-ms exposure image values. Dyno capsids are color-coded by receptor family: **integrin** (**blue**) or **DR1 + integrin** (**purple**). AAV9 and MyoAAV-4E reference capsids are shown in **gray**.

Dyno dual-mech capsids potently target mouse muscle at 5e12 vg/kg

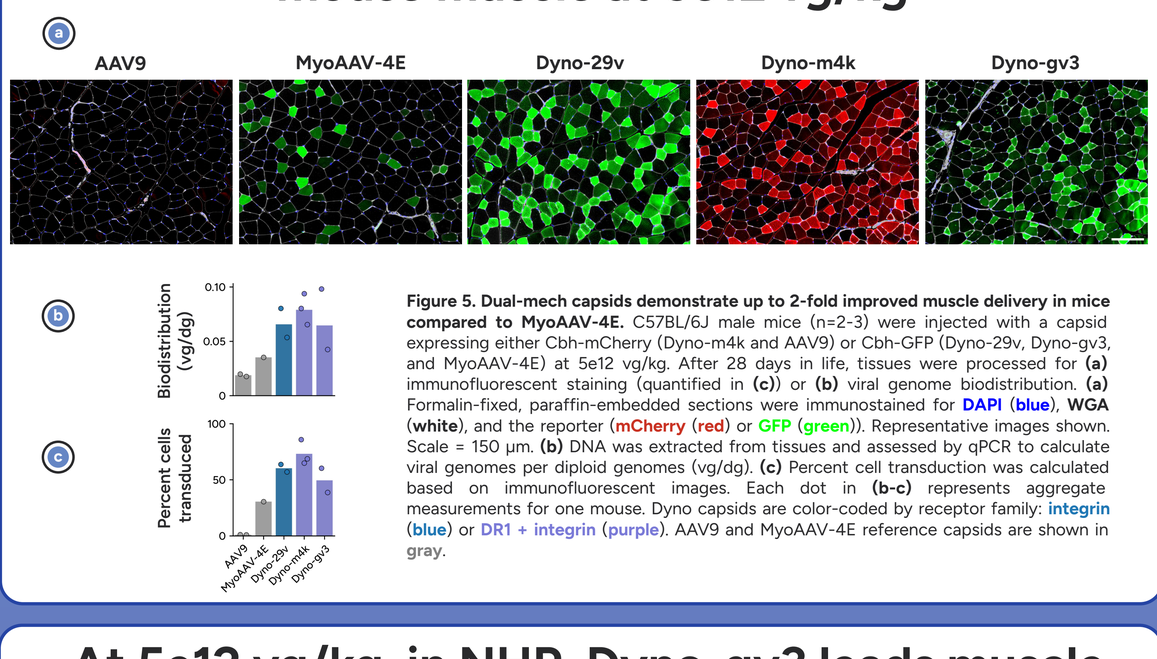


Figure 5. Dual-mech capsids demonstrate up to 2-fold improved muscle delivery in mice compared to MyoAAV-4E. C57BL/6J male mice (n=2-3) were injected with a capsid expressing either Cbh-mCherry (Dyno-m4k and AAV9) or Cbh-GFP (Dyno-29v, Dyno-gv3, and MyoAAV-4E) at 5e12 vg/kg. After 28 days in life, tissues were processed for **(a)** immunofluorescent staining (quantified in **(c)**) or **(b)** viral genome biodistribution. **(a)** Formalin-fixed, paraffin-embedded sections were immunostained for **DAPI** (**blue**), **WGA** (**white**), and the reporter (**mCherry** (**red**) or **GFP** (**green**)). Representative images shown. Scale = 150 µm. **(b)** DNA was extracted from tissues and assessed by qPCR to calculate viral genomes per diploid genomes (vg/dg). **(c)** Percent cell transduction was calculated based on immunofluorescent images. Each dot in **(b-c)** represents aggregate measurements for one mouse. Dyno capsids are color-coded by receptor family: **integrin** (**blue**) or **DR1 + integrin** (**purple**). AAV9 and MyoAAV-4E reference capsids are shown in **gray**.

At 5e12 vg/kg in NHP, Dyno-gv3 leads muscle biodistribution and transduction

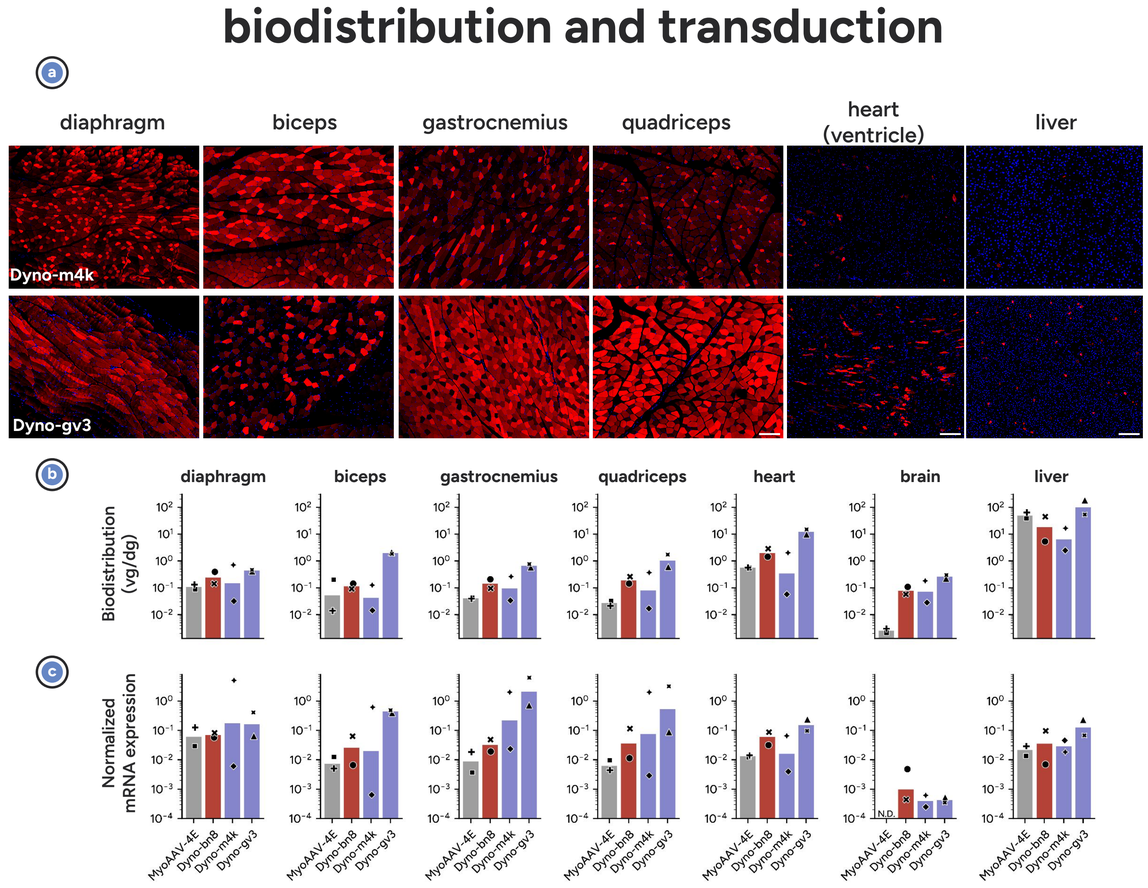


Figure 6. Multi-mech capsids Dyno-m4k and Dyno-gv3 demonstrate high muscle potency compared to single-mechanism capsids, with low CNS and liver biodistribution in NHP. NHPs (n=2) were injected with a capsid expressing Cbh-mCherry (MyoAAV-4E, Dyno-bn8, Dyno-m4k, or Dyno-gv3) at 5e12 vg/kg. After 28 days in life, tissues were processed for **(a)** immunofluorescent staining, **(b)** viral genome biodistribution, or **(c)** mRNA expression. **(a)** Formalin-fixed, paraffin-embedded sections were immunostained for **DAPI** (**blue**) and the **mCherry reporter** (**red**). Representative images shown. Scale = 200 µm. **(b)** DNA was extracted from tissues and assessed by qPCR to calculate viral genomes per diploid genomes (vg/dg). **(c)** RNA was extracted from tissues, reverse-transcribed, and cDNA was quantified via qPCR and normalized to expression by housekeeping genes. Each marker in **(b-c)** represents aggregate measurements for one NHP. Dyno capsids are color-coded by receptor family: **DR1** (**red**) or **DR1 + integrin** (**purple**). MyoAAV-4E reference capsid is in **gray**.

Conclusions

- **AI-guided capsid design targeting a combination of receptors produces capsids with enhanced muscle tropism, reduced off-target biodistribution, and improved delivery at low doses.**
- **Cross-species capsids accelerate preclinical development** by enabling disease model testing in mice as well as translational efficacy and safety assessments in NHPs.
- The **high muscle potency of these multi-mech capsids lowers liver exposure** at clinically relevant doses, **enabling safer advancement of more gene therapies for muscular diseases.**

Contact partnerwith@dynotx.com to learn how Dyno capsids can level up your gene therapy programs.