P0089

# Novel AAV capsids for intravitreal delivery developed by directed evolution in non-human primate eyes and validated in human retinal organoids

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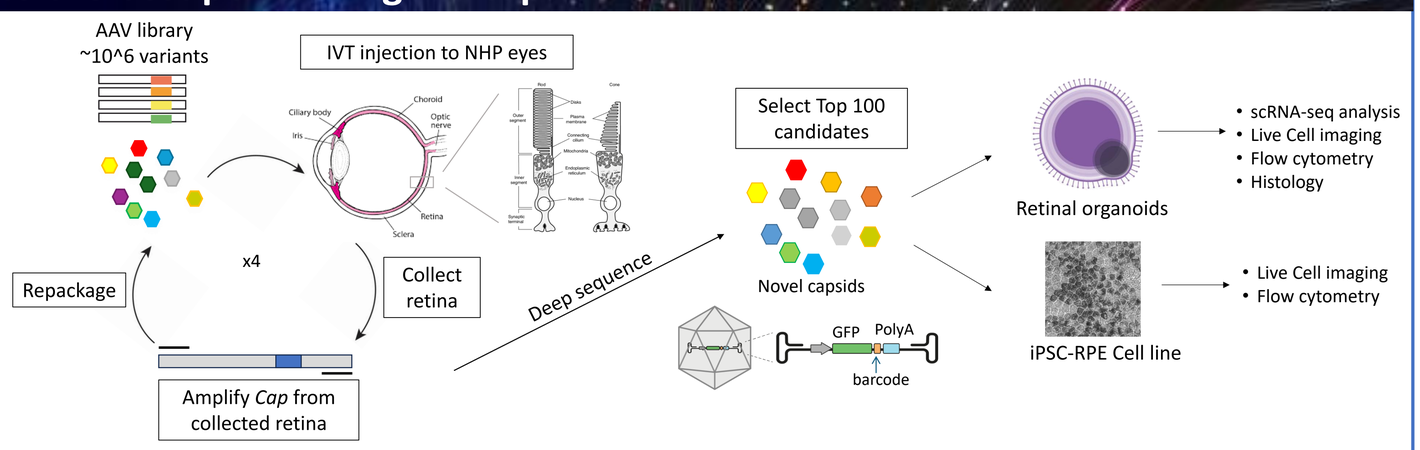
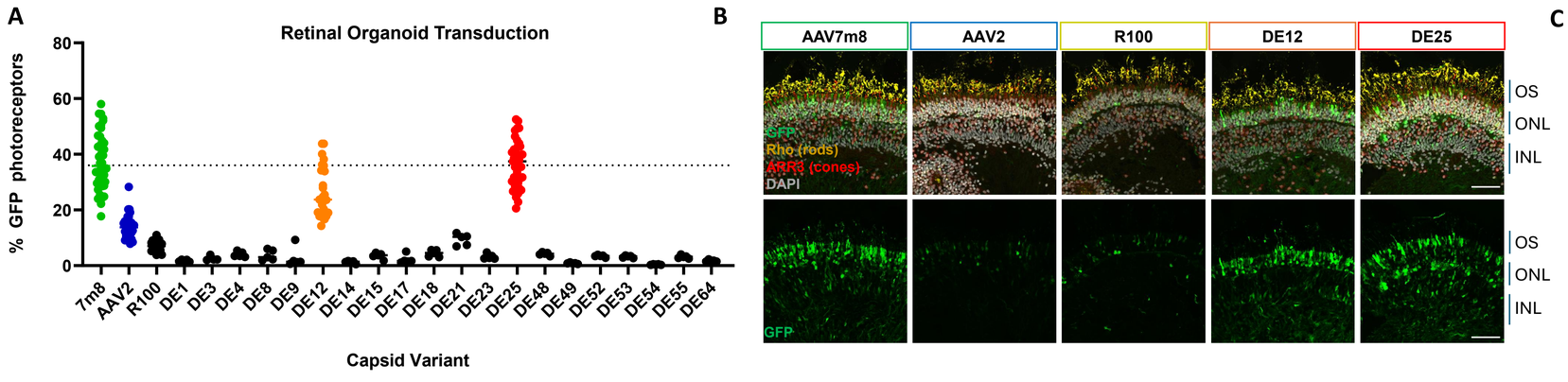
## Directed evolution capsid screen in non-human primates identified variants with significantly more potent transduction compared to the best performing IVT capsids

- In vivo directed evolution screen in non-human primates to identify AAV capsids targeting the retina beyond the fovea
- Constructed a 7mer peptide display library (~$10^6$ variants) by insertion into AAV2 capsid at position 588
- Administered capsid library intravitreally; fovea excluded to enrich for variants with broader retinal tropism
- Recovered and sequenced viral genomes to identify top-performing capsids with high transduction efficiency
- Validated lead variants in human retinal organoids and IPSC-RPE cells using imaging, flow cytometry, histology, and single-cell RNA-seq
- Identified capsids with higher transduction efficiency in the retina than AAV2 and 7m8
- Confirmed elevated retinal expression in mice following intravitreal injection

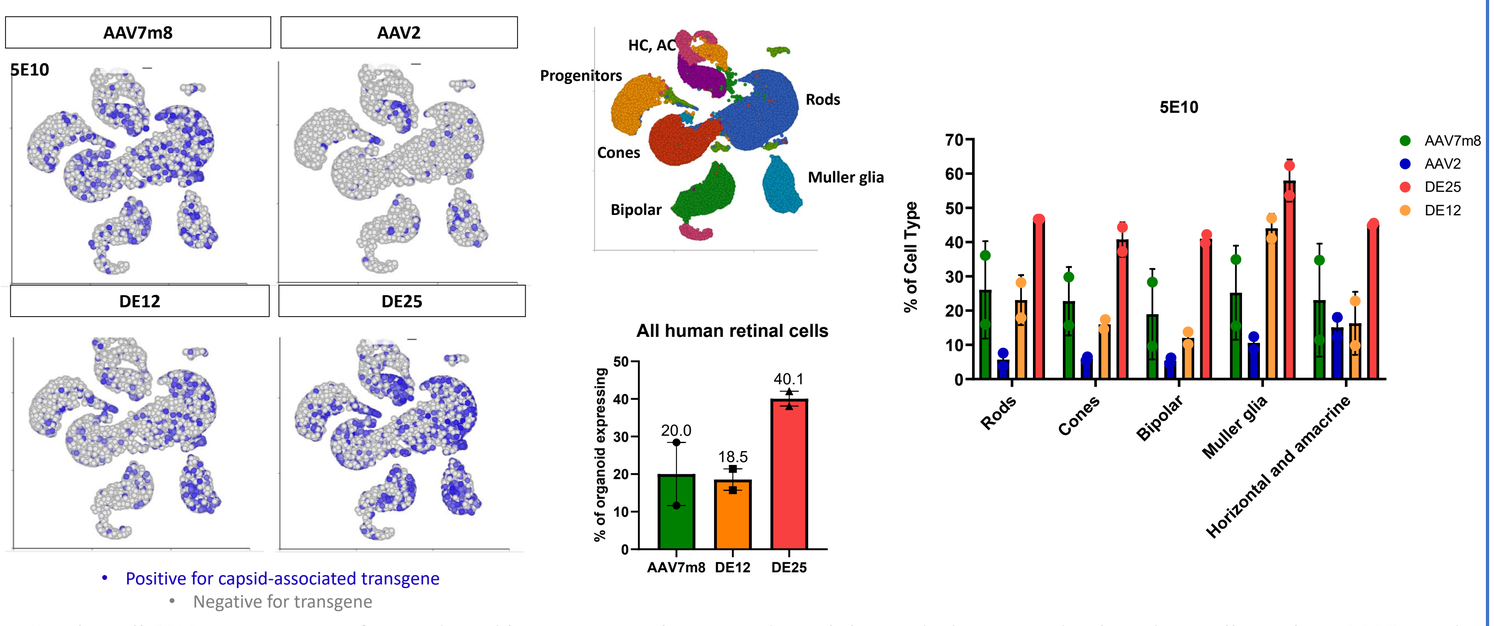



## Top performing capsids identified in the NHP screen validate superior transduction of lead capsids in human retinal organoids




**A.** Flow cytometry data showing GFP expression in CD73+ve photoreceptors 3 weeks post transduction. Organoids were transduced individually with AAV capsid variants (5E10 VGs per organoid) **B.** Immunohistochemistry in transduced retinal organoid. Cryosections from transduced retinal organoids. Scale bar = 10um. **C.** Live GFP imaging week 3 post transduction. Whole retinal organoids imaged in brightfield overlayed with GFP. (5E10 VGs per organoid)

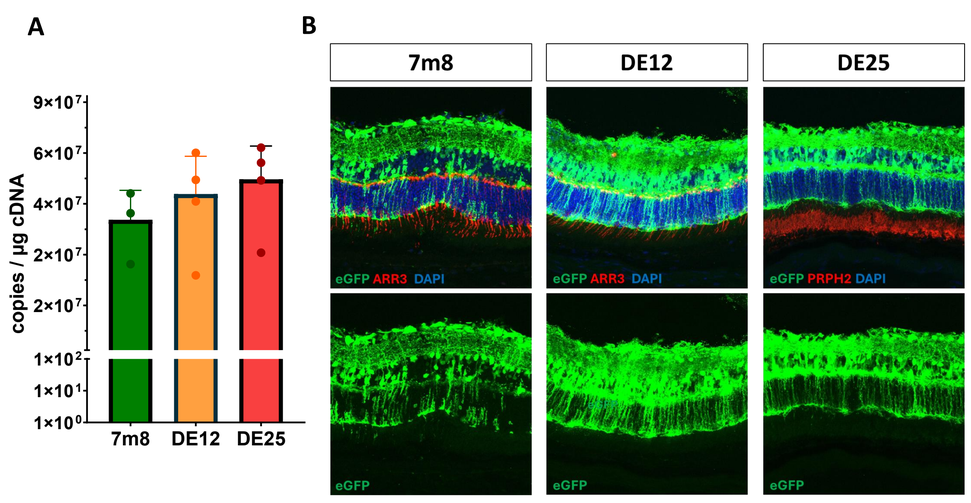
## Single-Cell Transcriptomic Profiling Reveals >2-Fold Increase in Retinal Transduction Efficiency Compared to 7m8




- Positive for capsid-associated transgene
- Negative for transgene

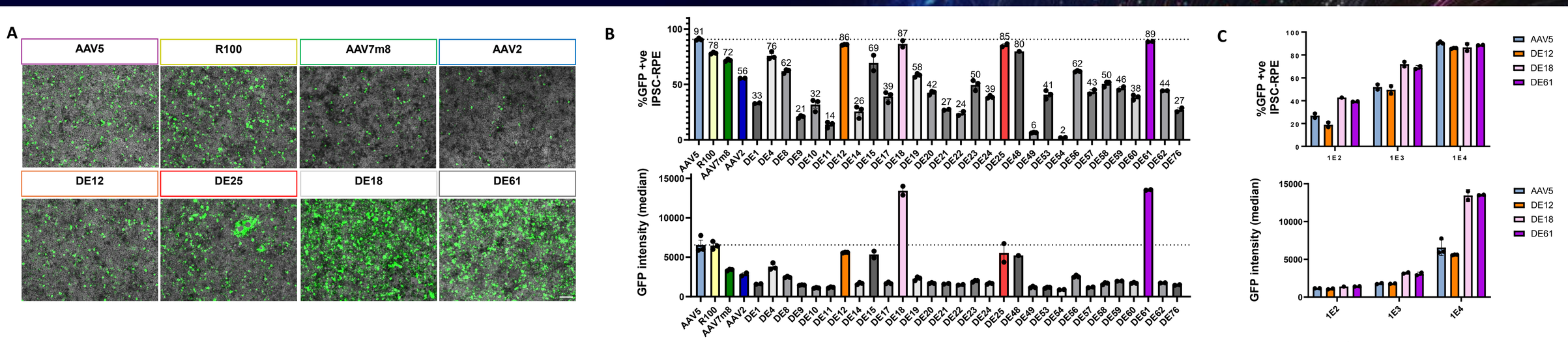
Single-cell RNA sequencing of transduced human retinal organoids with barcoded vectors displayed visually in the UMAPs and expression levels were quantified in the whole organoid as well as in specific retinal cell subtypes.

## Novel capsids show higher levels of retinal expression in mice following IVT injection




A. qPCR analysis of GFP expression in transduced mouse retinas. Absolute quantification of GFP mRNA expression in mouse retinas 3 weeks post administration with intravitreal delivery. 2E10 VGs per eye, n=3-4 per vector.
B. Cryosections from IVT injected mouse retina.

## RPE Screening Identifies Capsids with Enhanced Transduction Efficiency in IPSC-Derived RPE Cells




**A.** Live GFP imaging week 3 post transduction. **B.** Flow cytometry data. IPSC-RPE cells transduced at week 8 at an MOI of 1E4 (5.5E9 per well). Dotted line = AAV5 median intensity/% GFP. **C.** Flow cytometry data transduced with 3 different MOIs.

- **Differentiated directed evolution approach that allows for identification of capsids with broader transduction profile by exclusion of the fovea.**
- **Validation across multiple models, including in human retinal organoids, ensures performance across human, NHP and mouse retinas as well as high resolution data on specific cell populations being transduced.**
- **All capsids were additionally validated for packageability and high yield manufacturing using MeiraGTx's proprietary manufacturing process.**
- **Novel capsids can increase potency and lower required dose for gene therapies treating retinal disorders due to their enhanced specificity and broad cell-type coverage.**

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