Engineered AAV2/7m8 serotype shows significantly higher transduction efficiency of ARPE-19 and HEK293 cell lines compared to AAV2/5, 2/8 and 2/9 serotypes

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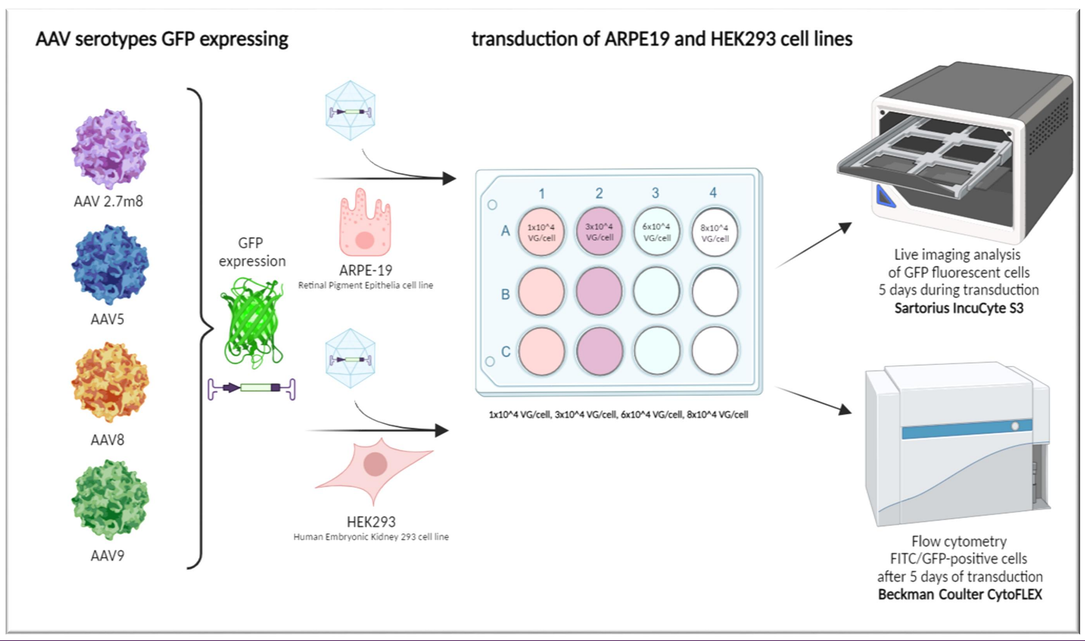
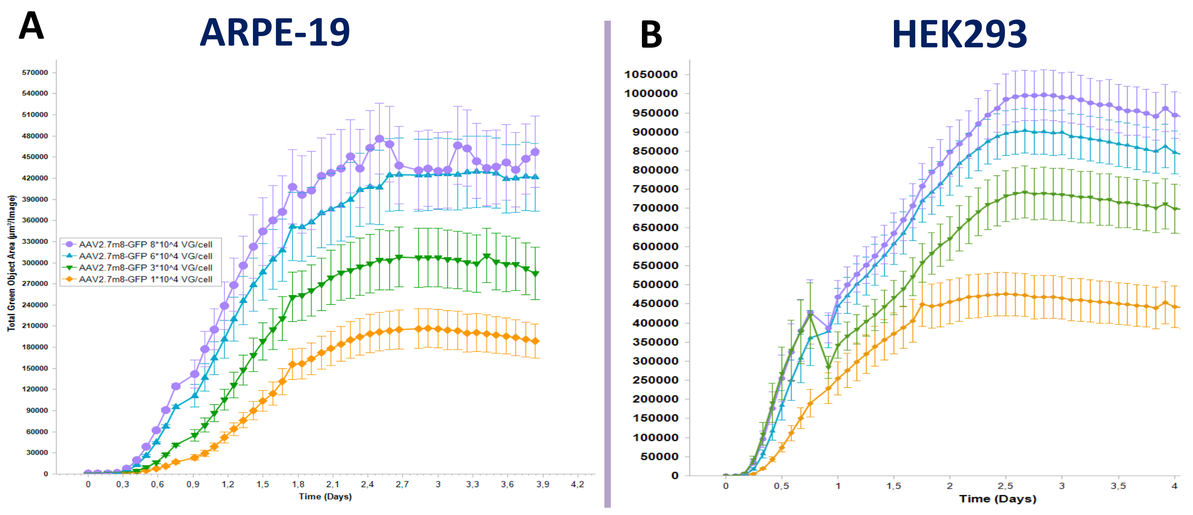
Introduction

- AAV viruses are a powerful tool of gene replacement therapy for the treatment of inherited retinal diseases (IRDs)
- The choice of AAV serotype and the outcome of gene replacement therapy for IRDs depend on the level of transduction efficiency of the target retinal cells
- Engineered serotype AAV2/7m8 has increased tropism for retinal cells compared to other AAV serotypes
- Previously, AAV 2/7m8, 2/5, 2/8 and 2/9 serotypes were studied in HEK293 cells and murine models but not ARPE-19 cells (human retinal pigment epithelium), which have low transduction efficiency compared to HEK293 cells
- **The aim** of this study was to compare the tropism, efficacy and rate of viral invasion of rAAV 2/7m8, 2/5, 2/8 and 2/9, delivering GFP, on ARPE-19 and HEK293 cells

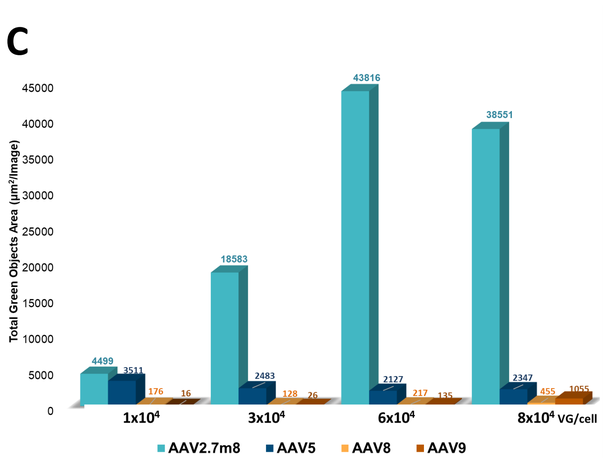
Methods

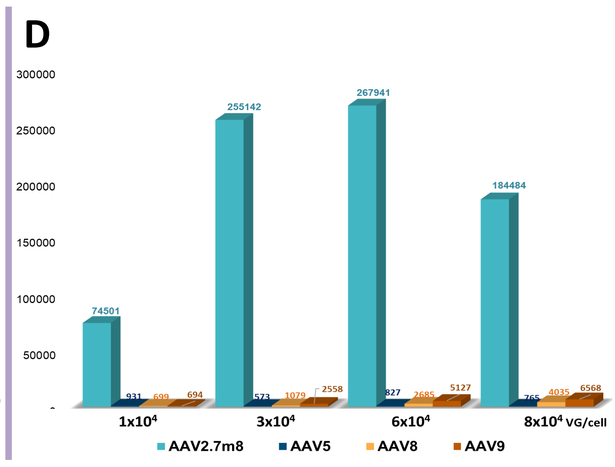


AAV2/7m8 demonstrates superior transduction efficiency in dose-dependent manner

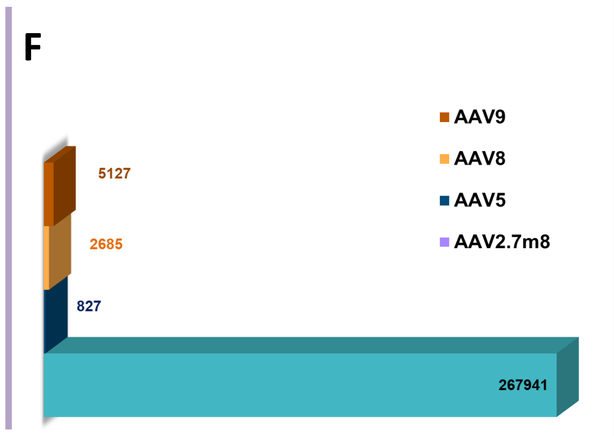


GFP expression (Total Green Objects Area, µm²/image) in ARPE-19 (A) and HEK293 (B) cell lines transduced with AAV2/7m8 at 1×10⁴, 3×10⁴, 6×10⁴ and 8×10⁴ VG/cell



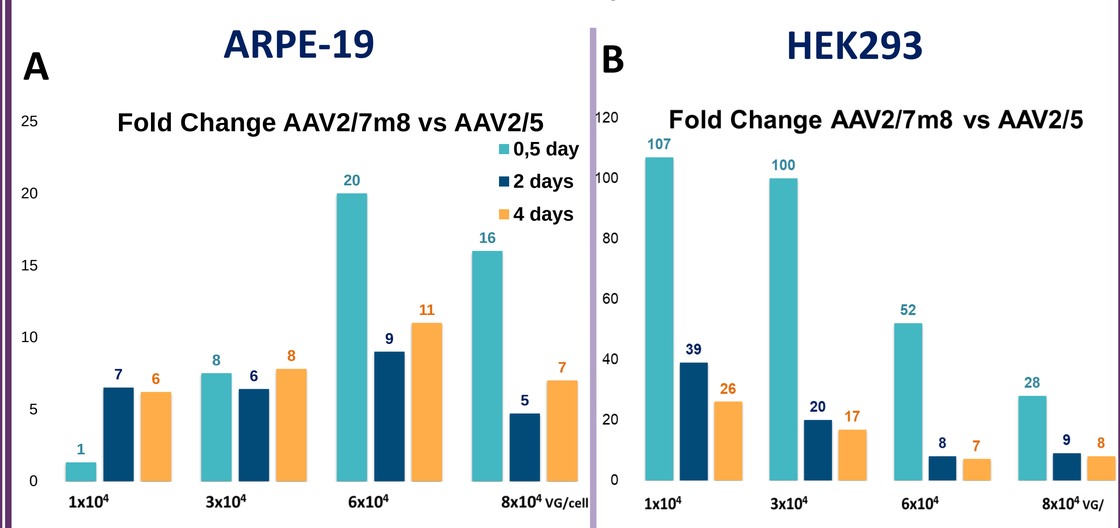


AAV2/7m8 demonstrates highest transduction efficiency at four viral concentrations compared to other AAV serotypes at 12 hours post transduction

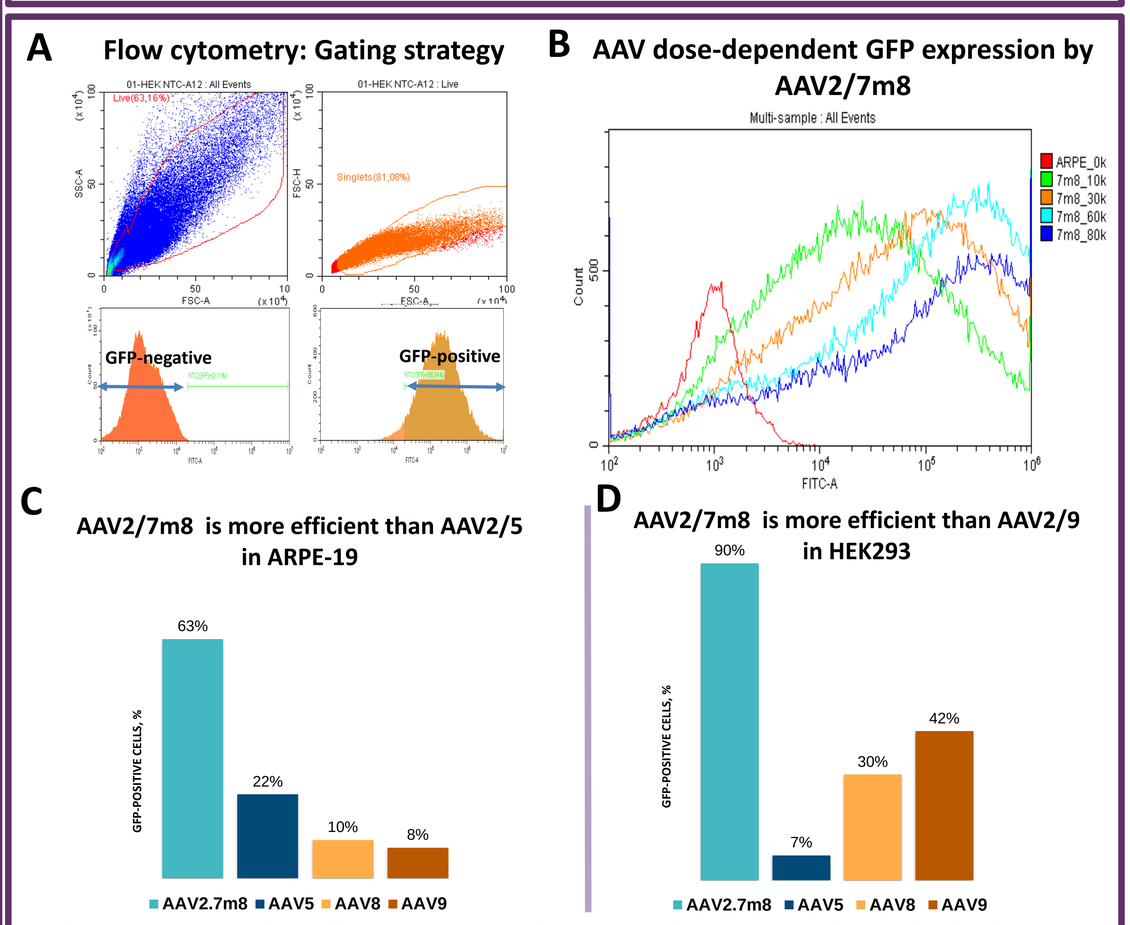


Transduction efficiency of AAV2/7m8 at 6×10⁴ VG/cell at 12 hours post transduction was 20, 201 and 323 times higher in ARPE-19 (E) and 324, 99 and 52 times higher in HEK293 (F) cells compared to that of AAV2/5, AAV2/8 and AAV2/9, respectively

Higher transduction efficiency of AAV 2/7m8 as compared to AAV2/5



Comparison of transduction efficiencies of AAV2/7m8 and AAV2/5 at four viral concentrations (1×10⁴, 3×10⁴, 6×10⁴ and 8×10⁴ VG/cell) at 0.5, 2 and 4 days



Flow cytometry analysis of GFP-positive populations at 4 days post transduction by the four AAV serotypes in ARPE-19 (C) and HEK293 (D) cells. (B) Histogram overlay for AAV2/7m8-transduced GFP-positive populations

Conclusion

- **AAV2/7m8 demonstrated superior transduction efficiency at four concentrations (1 , 3 , 6 and 8 ×10⁴ VG/cell) in dose-dependent manner followed by AAV2/5 in ARPE-19 and AAV2/9 in HEK293 cells at 12 hours post transduction**
- **The efficiency of AAV2/7m8 transduction at the dose of 6×10⁴ VG/cell was 20, 201 and 323 times higher in ARPE-19 cells and 324, 99 and 52 times higher in HEK293 cells than that of AAV2/5, AAV2/8 and AAV2/9, respectively**
- **As additionally shown by flow cytometry, this trend remained for 4 days at all viral concentrations**
- **In ARPE-19 cells, AAV2/7m8 (63% GFP+ cells) was nearly 3 times as efficient as AAV2/5 (22% GFP+ cells) and 10 times as efficient as AAV2/9 or AAV2/8 (10% and 8%, respectively) at the dose of 6×10⁴ VG/cell**
- **In HEK293 cells, 33% of cells transduced by AAV2/7m8 were GFP-positive, followed by AAV2/9 (10%), AAV2/8 (9%) and AAV2/5 (3%)**
- **Therefore, AAV2/7m8 proved itself as the most powerful gene delivery tool which should be utilized for gene replacement therapy of such IRDs as RDH12-associated retinopathy**

References

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