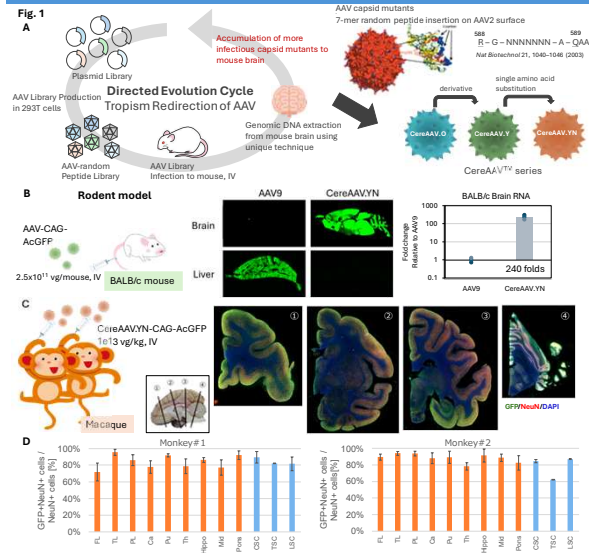


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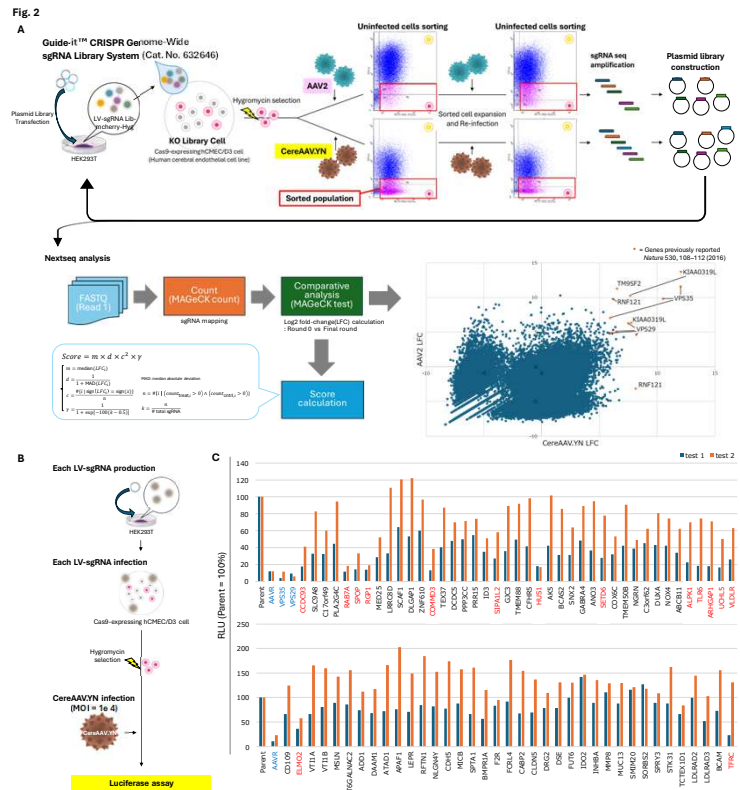
## Abstract

In this study, we aimed to elucidate the neuronal transduction mechanism of AAV2-derived CereAAV.YN vector. To this end, we established a Cas9-expressing human brain microvascular endothelial cell line (hCMEC/D3). These cells were transduced with a lentiviral sgRNA library targeting approximately 19,000 genes (~76,000 sgRNAs) to generate a pooled knockout (KO) cell population. The KO cells were then infected with either AAV2-CMV-AcGFP or CereAAV.YN-CMV-AcGFP, and the AcGFP-negative cells were isolated by flow cytometric cell sorting to obtain cells in which genes required for AAV transduction had been disrupted. Genomic DNA was extracted from the sorted cells, and the sgRNA regions were PCR-amplified and cloned into the lentiviral backbone to generate plasmid sublibraries for subsequent screening rounds. After up to three rounds of screening, the final sgRNA libraries were analyzed by next-generation sequencing. Based on the NGS analysis, we identified 78 candidate genes. In the first and second validation assays, knockout of *CCDC93*, *RAB7A*, *RGPI1*, or *COMMD3* resulted in altered transduction efficiency with CereAAV.YN compared with AAV2, whereas no significant differences were observed with AAV9. These findings indicate that, although CereAAV.YN is derived from AAV2, it possesses a cellular infection mechanism more closely resembling that of AAV9, which is known for its high blood-brain barrier permeability. Taken together, these results suggest that the AAV9-like cellular infection mechanism of CereAAV.YN may contribute to its enhanced brain-targeted gene delivery.

## Introduction



## Result 1: Host Gene Screening for CereAAV.YN Gene Transduction



## Result 2: Secondary Evaluation of AAV Transduction Using Bulk Knockout Cells

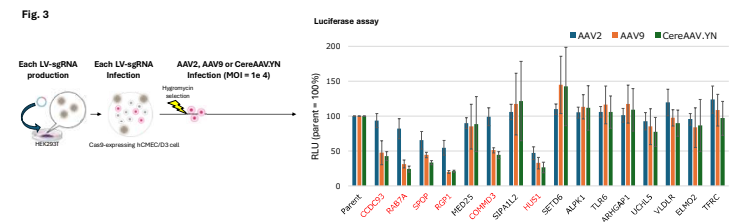


Figure 3. Assessment of CereAAV.YN-specific gene transduction compared with AAV2 and AAV9. Transduction efficiencies of AAV2, AAV9, and CereAAV.YN were evaluated in bulk knockout cells for 16 candidate genes identified through prior screening and validation. Knockout of gene HUS1 reduced transduction of all AAVs, whereas knockout of genes CCDC93, RAB7A, SPOP and RGP1 preferentially impaired AAV9 and CereAAV.YN transduction, with minimal effects on AAV2, suggesting an AAV9-like transduction mechanism for CereAAV.YN.

## Result 3: Validation of AAV Gene Transduction

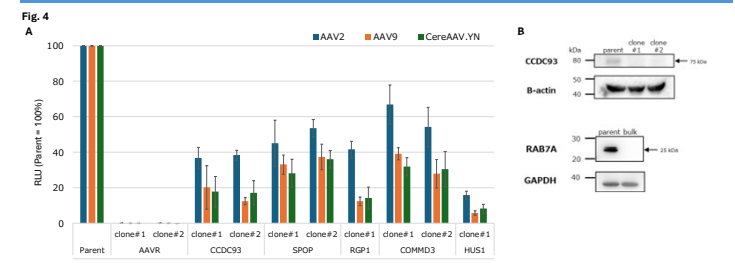


Figure 4. Validation of AAV2-, AAV9-, and CereAAV.YN-mediated gene transduction. (A) Transduction efficiencies of AAV2, AAV9, and CereAAV.YN were evaluated in single-cell clone of knockout cells generated for five candidate genes identified through prior bulk knockout validation studies. Consistent with the previous results, knockout single-clone cell lines for genes CCDC93, RGP1, and COMMD3 showed reduced gene transduction efficiencies of AAV9 and CereAAV.YN, whereas AAV2 transduction was less affected, revealing a clear difference between AAV2 and AAV9/CereAAV.YN. (B) Western blot analysis. Reduced expression of CDC93 and RAB7A proteins was confirmed in the corresponding CCDC93- (single) and RAB7A-knockout cells (bulk).

## Conclusion and Discussion

- A genome-wide CRISPR knockout screening successfully identified host genes involved in CereAAV.YN-mediated gene transduction.
- Known AAV2 host factors, including *AAVR*, *VPS35*, and *VPS29*, were rediscovered, validating the effectiveness and reliability of the screening approach.
- Comparative analysis using AAV2, AAV9, and CereAAV.YN revealed distinct host gene dependencies
- HUS1* gene knockout reduced transduction efficiencies of all AAVs tested.
- Knockout of genes *CCDC93*, *RGPI1*, *COMMD3* and *RAB7A* selectively impaired AAV9 and CereAAV.YN transduction, with minimal effects on AAV2.

*CCDC93*, *COMMD3*, and *RAB7A* are known to function in endosomal trafficking and maturation pathways. The selective impairment of AAV9 and CereAAV.YN transduction, but not AAV2, observed upon knockout of these genes suggests a similar dependency between AAV9 and CereAAV.YN for these endosomal processes. Given the established role of such pathways in efficient blood-brain barrier traversal, this AAV9-like host factor requirement may contribute to the enhanced brain tropism of CereAAV.YN.