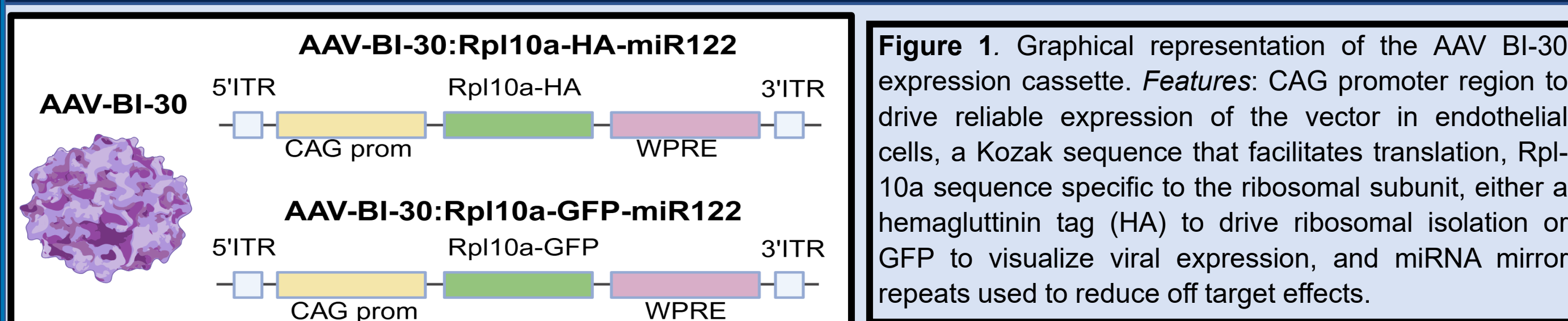


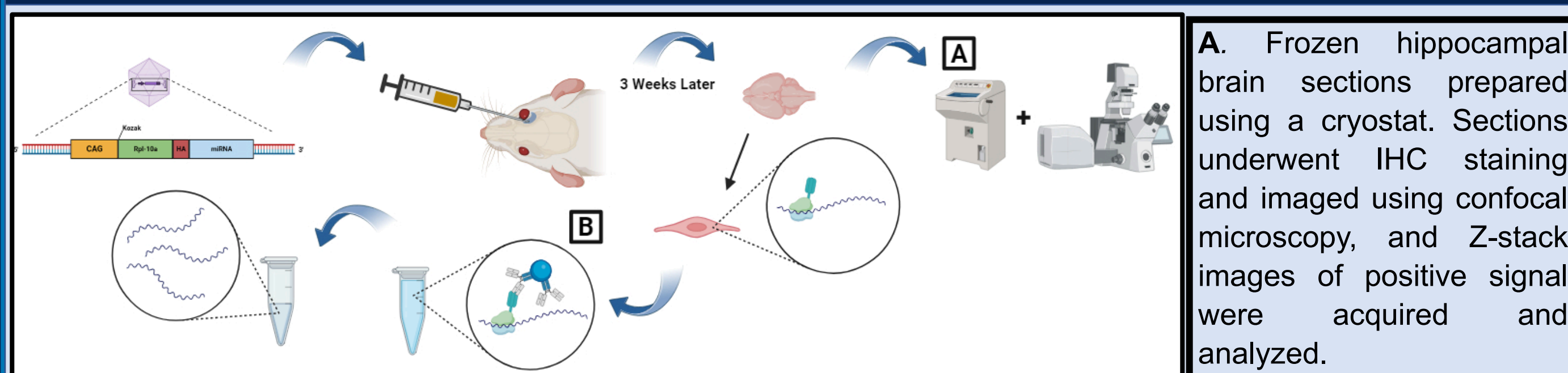
Introduction

- Cerebral microvascular disease (CMD)** is a leading cause of age-related cognitive decline and a major risk factor for stroke. Emerging evidence links CMD to the development and progression of Alzheimer's disease (AD).
- Brain endothelial cells** are the primary site of pathology in CMD
- Systematic identification and measurement of dysfunction molecular pathways in brain endothelia is challenging as these cells are difficult to isolate, resistant to traditional viral transduction approaches, and single-cell technologies require extensive tissue processing that can limit deep molecular profiling.
- To address this, we developed **viral translating ribosome affinity purification (vTRAP)** to **profile endothelial cells** in healthy and diseased states.
- This platform allows **systematic comparisons** of endothelial gene expression across species, highlighting **early molecular changes** in CMD and **AD-related dementias (ADRDs)**.
- We will focus on endothelial contributions in models of **tau**, **amyloid-beta**, and **hypertension**
- Our goal is to identify conserved molecular targets for therapeutic intervention in vascular contributions to cognitive impairment and dementia.

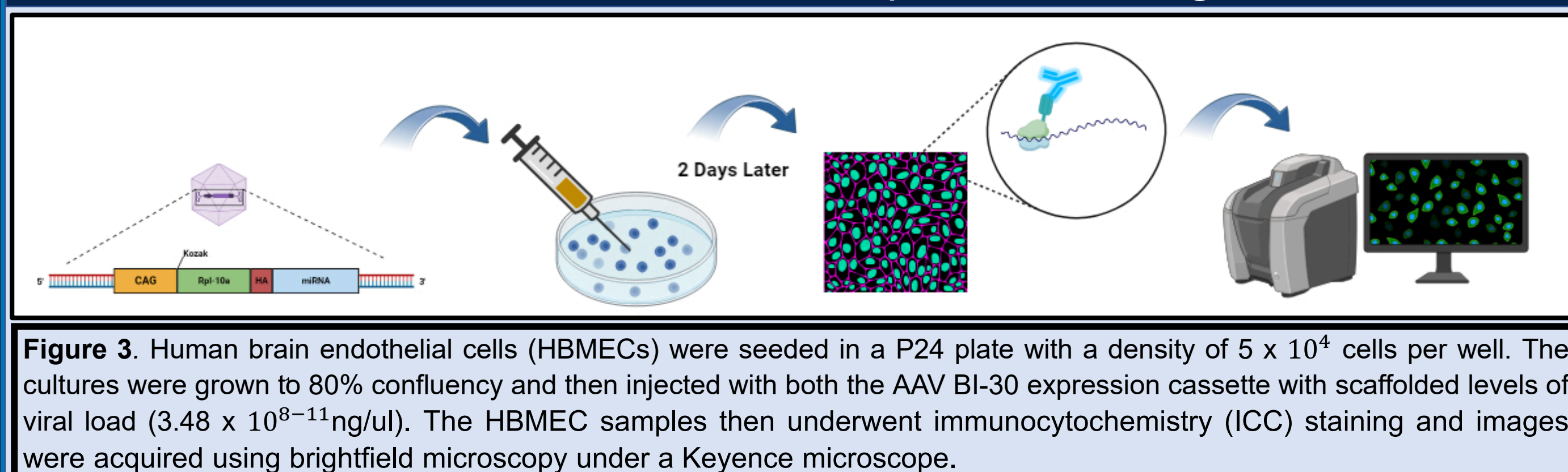
Methods



In-Vivo vTRAP Transcriptional Profiling



In-Vitro vTRAP Transcriptional Profiling



Results

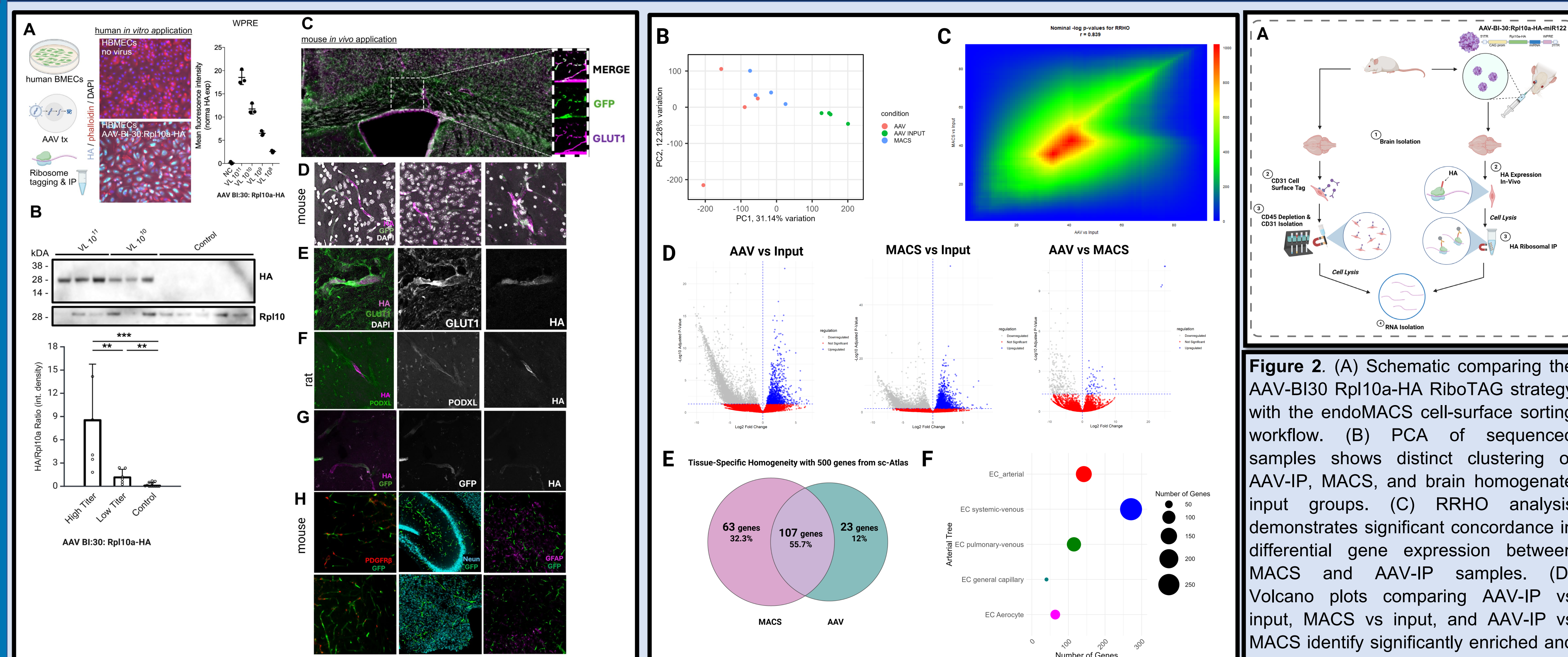


Figure 1. (A) AAV-BI30:Rpl10a-HA-miR122 transduces HBMECs 48 h after injection, producing dose-dependent HA expression. (B) Western blot of HBMECs 96 h after injection confirms increased HA expression at high and low AAV-BI30 titers compared with control. Significance was determined by two-tailed Mann-Whitney U test; **P = 0.02, ***P = 0.0001. (C) In mouse tissue, AAV-BI30:Rpl10a-GFP-miR122 and AAV-BI30:Rpl10a-HA-miR122 expression localizes to GLUT1-positive brain endothelial cells. (D, G) Mouse and rat cortex show GFP co-localization with HA-tagged ribosomal protein. (E, F) HA overlaps with vessel markers GLUT1/PODO in mouse and rat cortex, supporting endothelial specificity across species. (H) GFP does not co-localize with PDGFR β , NeuN, or GFAP, indicating minimal pericyte, neuronal, or astrocyte off-target labeling.

Figure 2. (A) Schematic comparing the AAV-BI30 Rpl10a-HA RiboTAG strategy with the endoMACS cell-surface sorting workflow. (B) PCA of sequenced samples shows distinct clustering of AAV-IP, MACS, and brain homogenate input groups. (C) RRHO analysis demonstrates significant concordance in differential gene expression between MACS and AAV-IP samples. (D) Volcano plots comparing AAV-IP vs input, MACS vs input, and AAV-IP vs MACS identify significantly enriched and depleted transcripts, with some heterogeneity between methods. (E) AAV-IP and MACS samples show overlapping enrichment of canonical endothelial genes relative to the top 500 tissue-specific genes from the single-cell endothelial atlas. (F) AAV-IP-enriched genes align with endothelial subtypes across the arterial tree from the scAtlas. (1)

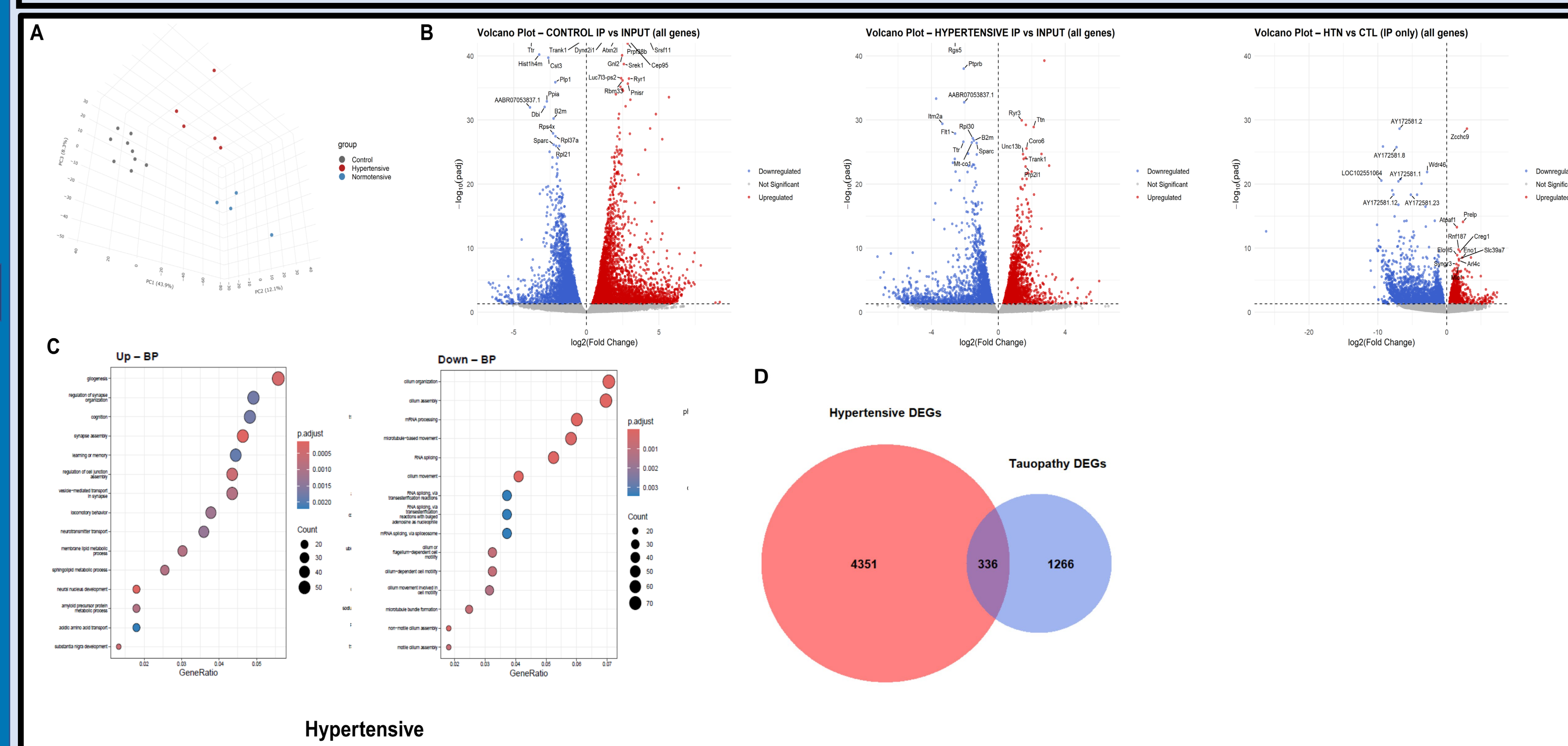


Figure 3. Hypertension-associated endothelial transcriptomic changes in the rat brain. (A) Three-dimensional PCA of RNA-seq samples shows separation between hypertensive IP RNA, normotensive IP RNA, and matched input samples. (B) Volcano plots show differential gene expression for normotensive (control) IP versus input, hypertensive IP versus input, and hypertensive versus normotensive IP samples, identifying enriched and depleted transcripts across conditions. (C) Gene Ontology enrichment analysis of DEGs from hypertensive versus normotensive IP samples highlights altered biological pathways, including vascular, metabolic, ciliary, and muscle/contractility-associated processes. (D) Venn diagram comparing hypertension-associated IP DEGs with PS19 tauopathy mouse endothelial DEGs isolated by MACS sorting shows 336 shared genes, with 4351 unique to the hypertensive cohort and 1266 unique to PS19 tauopathy.

Conclusions

- AAV-BI30 enables selective endothelial RiboTAG labeling in mouse and rat brain.
- AAV-BI30 produces dose-dependent endothelial transduction with minimal off-target labeling. AAV-IP captures an **endothelial-enriched transcriptome** comparable to MACS-based isolation.
- Hypertension induces a **distinct brain endothelial transcriptional signature**.
- Hypertensive endothelial DEGs are enriched for vascular, metabolic, ciliary, and contractility-related pathways.
- Hypertension and PS19 tauopathy share a subset of endothelial DEGs, suggesting **overlapping vascular stress programs**.
- Overall, AAV-BI30 RiboTAG provides a targeted platform for profiling disease-associated endothelial responses.

Next Steps

- Profile **tau** and **amyloid** mouse models using AAV-BI30 RiboTAG.
- Define endothelial transcriptomic signatures across distinct disease pathologies. Identify pathology-specific endothelial targets for therapeutic intervention.
- Prioritize **conserved endothelial stress pathways** shared across hypertension, tauopathy, and amyloid pathology.
- Validate candidate targets in disease-relevant vascular and neurodegenerative models.

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