Development of Blood-Brain Barrier Penetrant AAVs through Receptor-Targeted Capsid Engineering

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Poster #985

Introduction

- The development of blood-brain barrier (BBB) penetrant AAV capsids has the potential to enable transformative genomic medicines for the treatment of neurological disorders.
- We have previously described SIFTER, a method for *in vivo* selection of AAV capsids that mediate improved central nervous system (CNS) delivery after administration into the vasculature or cerebrospinal fluid. *In vivo* SIFTER screens in non-human primates have yielded novel capsids that access the CNS after intravascular (STAC-BBB, Abstract #117) or cerebrospinal fluid (STAC-102 and STAC-103) administration.
- Here, we deployed a complementary library screening approach to engineer AAV capsids targeting receptors highly expressed in endothelial cells comprising the human blood-brain barrier.
- Multiplexed library screening was conducted for three distinct BBB receptors. For each receptor, library screens identified engineered capsids that exhibit enhanced transduction of cells that overexpress the receptor. A subset of these capsids also interact with macaque and mouse orthologs, suggesting the potential for cross-species CNS delivery.

Receptor-targeted capsid engineering approach

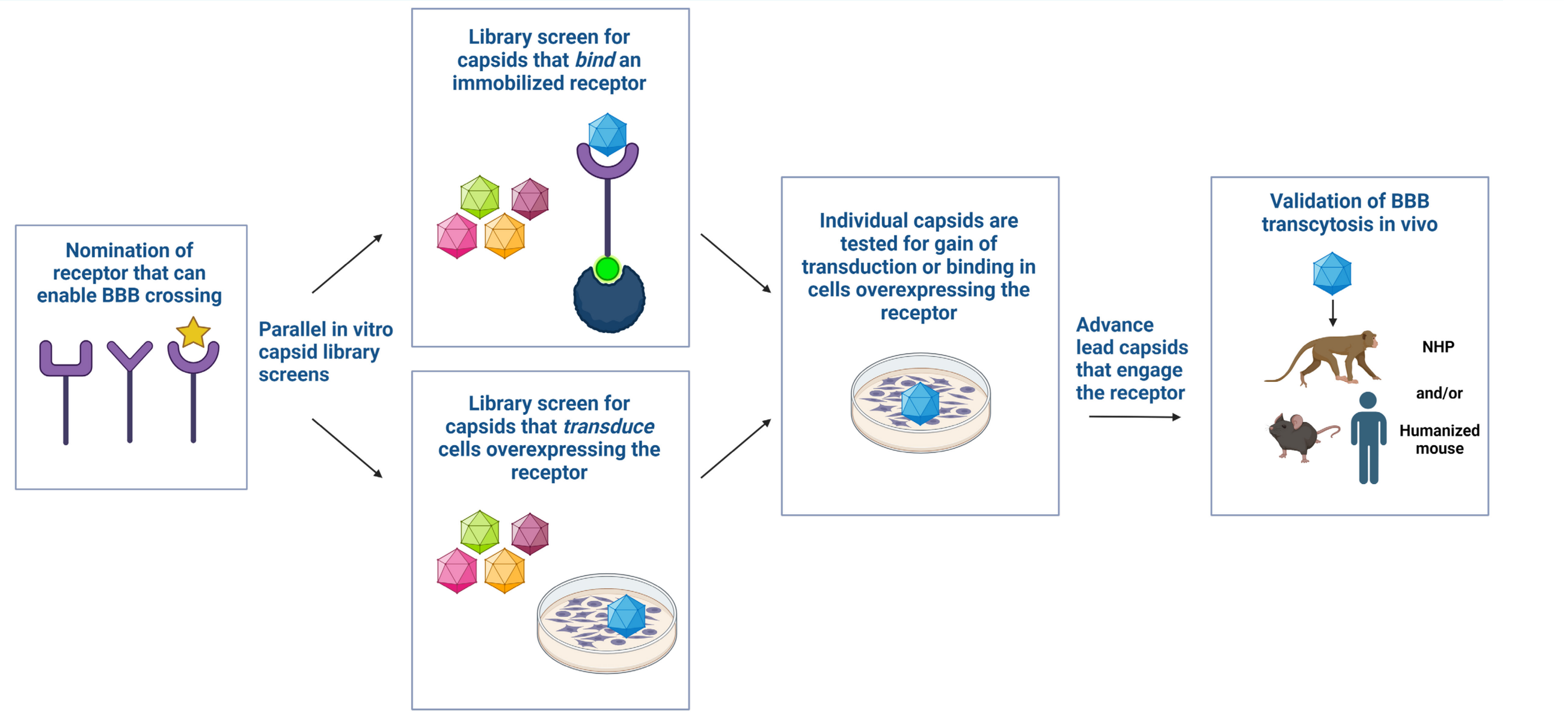

Figure 1. Receptor-targeted capsid engineering approach. Capsid libraries are screened in parallel for binding to the immobilized receptor and for transduction of cells that overexpress the receptor. Capsids that specifically target the receptor are next evaluated individually to confirm targeting specificity and assess cross-species performance. Lead capsids are advanced for *in vivo* assessment of BBB crossing and CNS delivery in animal models.

Methods

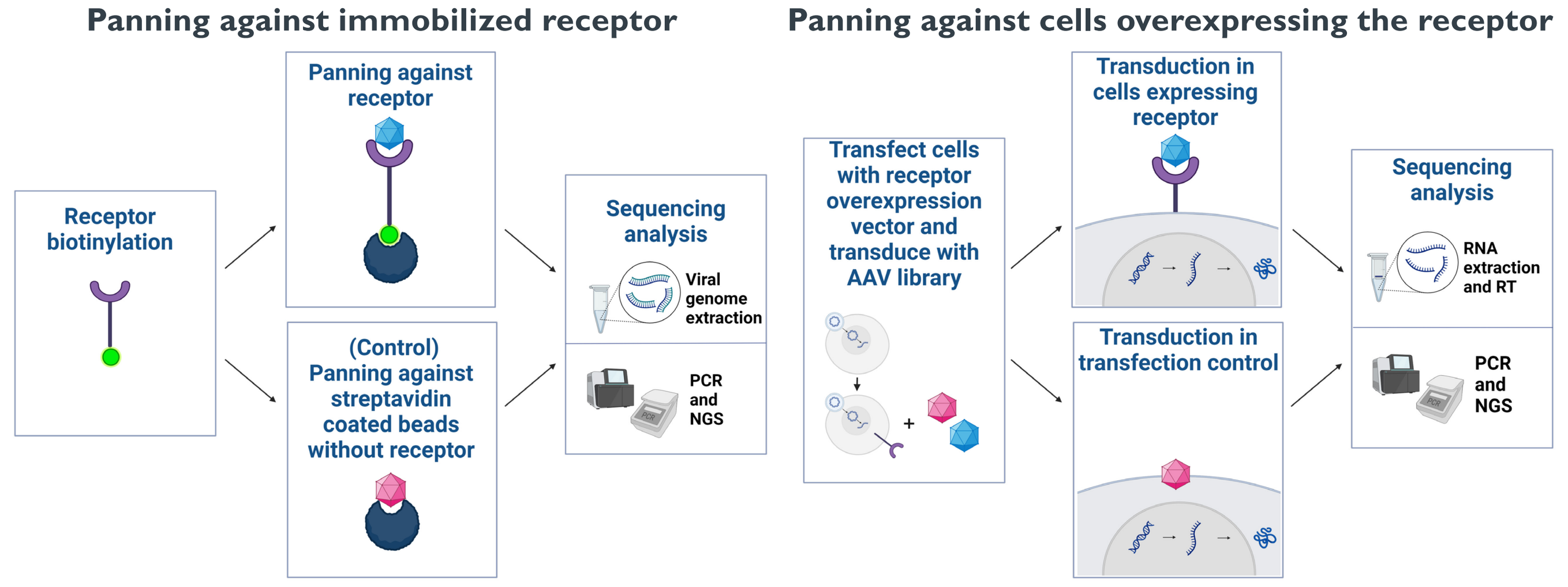

Figure 2. Workflows for library screening. AAV capsid libraries are screened for receptor targeting relative to background binding in control samples. Capsid performance is assessed by next-generation sequencing and bioinformatic analysis is used to identify capsid variants that specifically target the receptor.

Library screens for capsids targeting Receptor 1

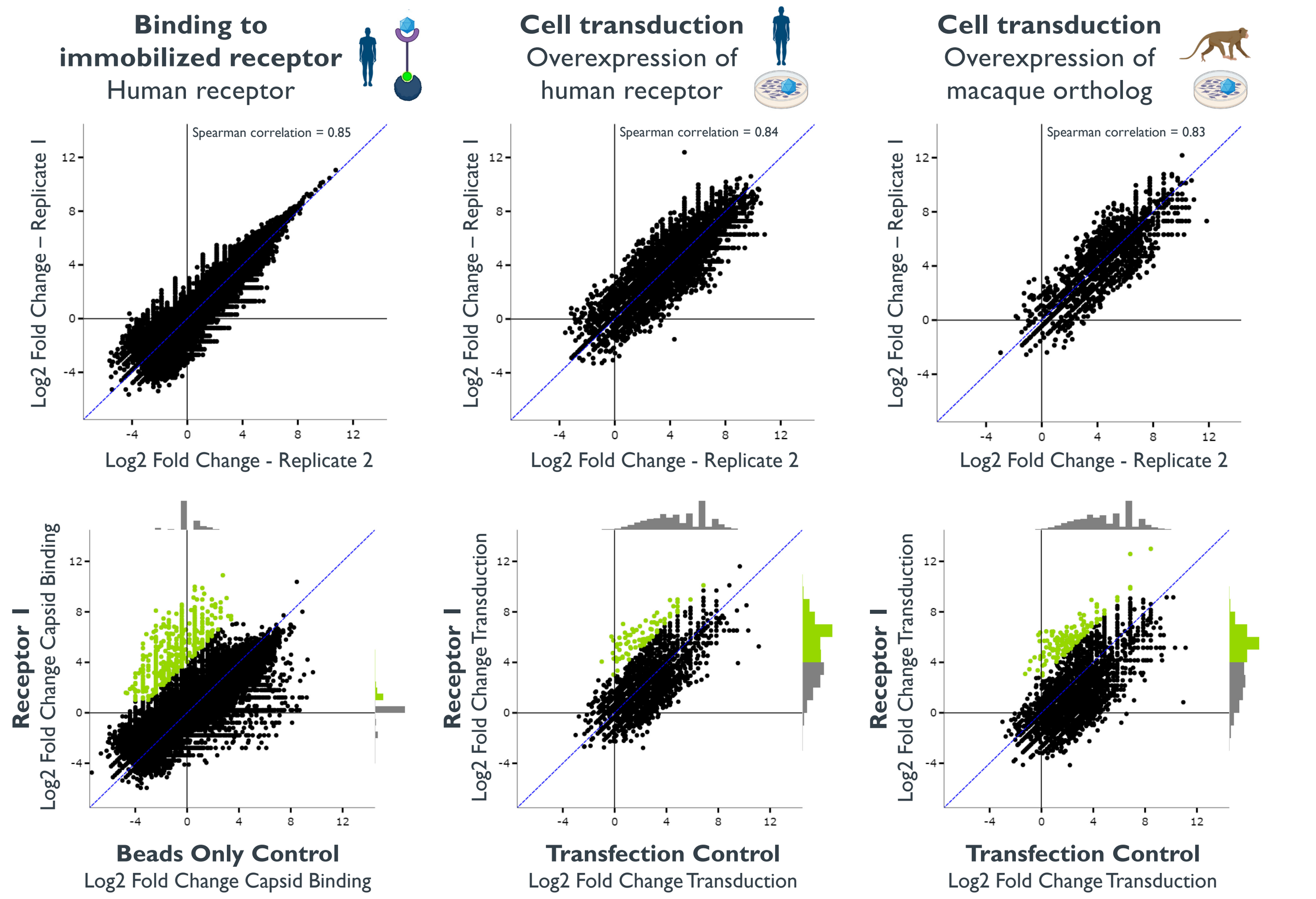

Figure 3. Library screens identify capsid variants that specifically target Receptor 1. (Top) Biological replicates are highly correlated in library screens targeting Receptor 1. (Bottom) Three parallel capsid library screens were conducted:
1) Immobilized human receptor versus a bead only control.
2) Transduction of cells overexpressing the human receptor versus a transfection control.
3) Transduction of cells overexpressing the macaque receptor versus a transfection control.
Capsids exhibiting specific enrichment for Receptor 1 are colored in green.

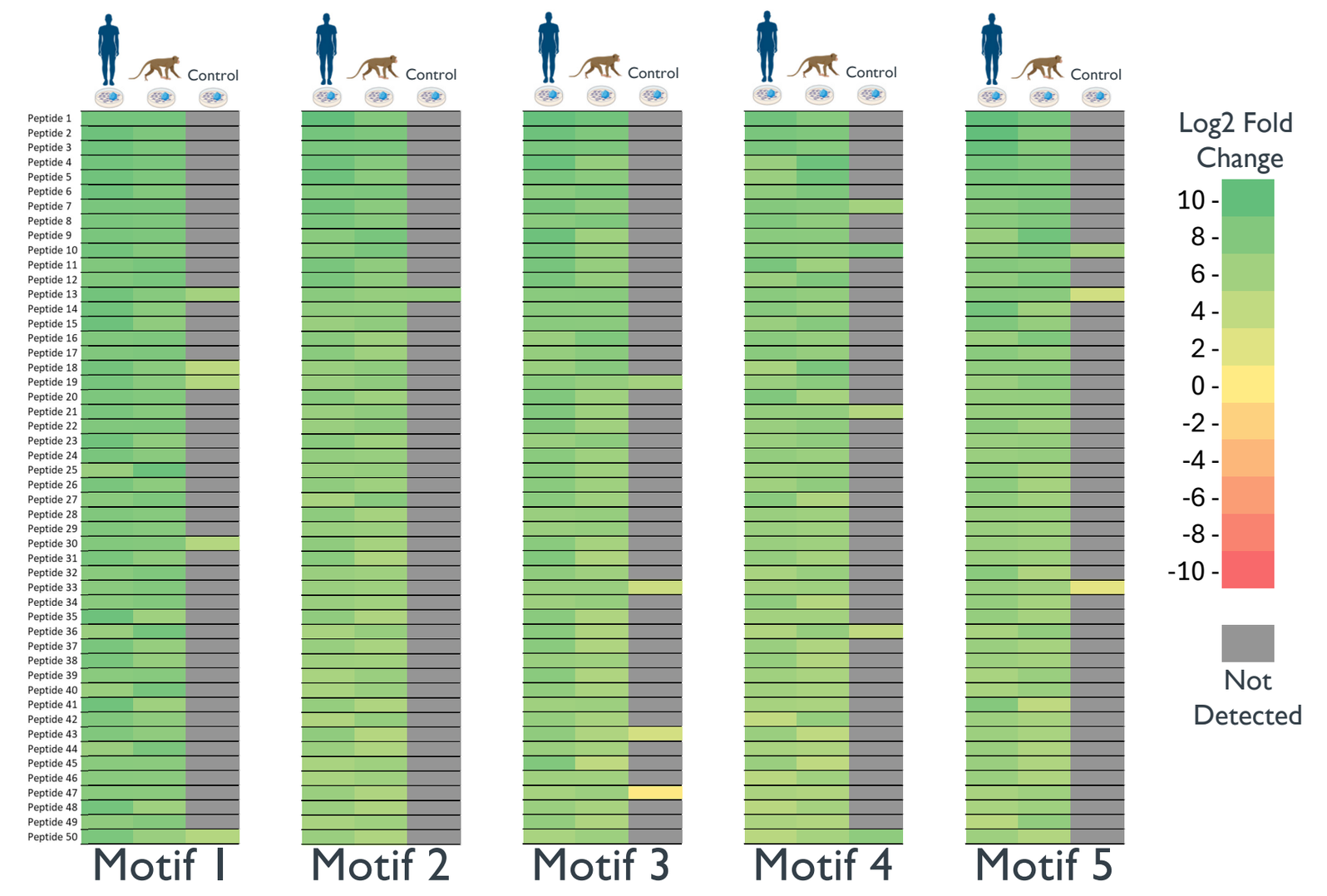

Figure 4. Peptide motifs are enriched in engineered capsids targeting Receptor 1. The top 50 capsids with cross-species targeting of Receptor 1 are shown for each motif.

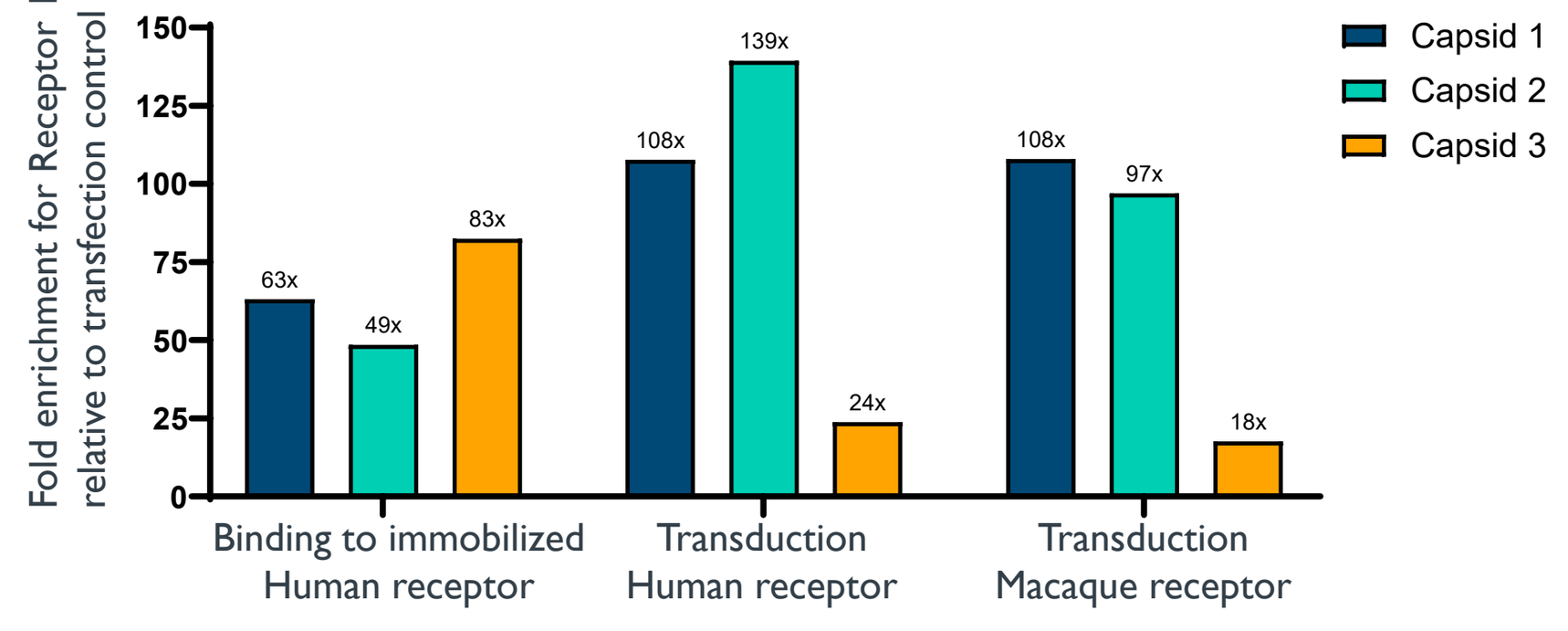

Figure 5. Examples of capsids that exhibited enrichment in all three Receptor 1 screens and were advanced for subsequent individual evaluation experiments.

Individual evaluation of capsids targeting Receptor 1

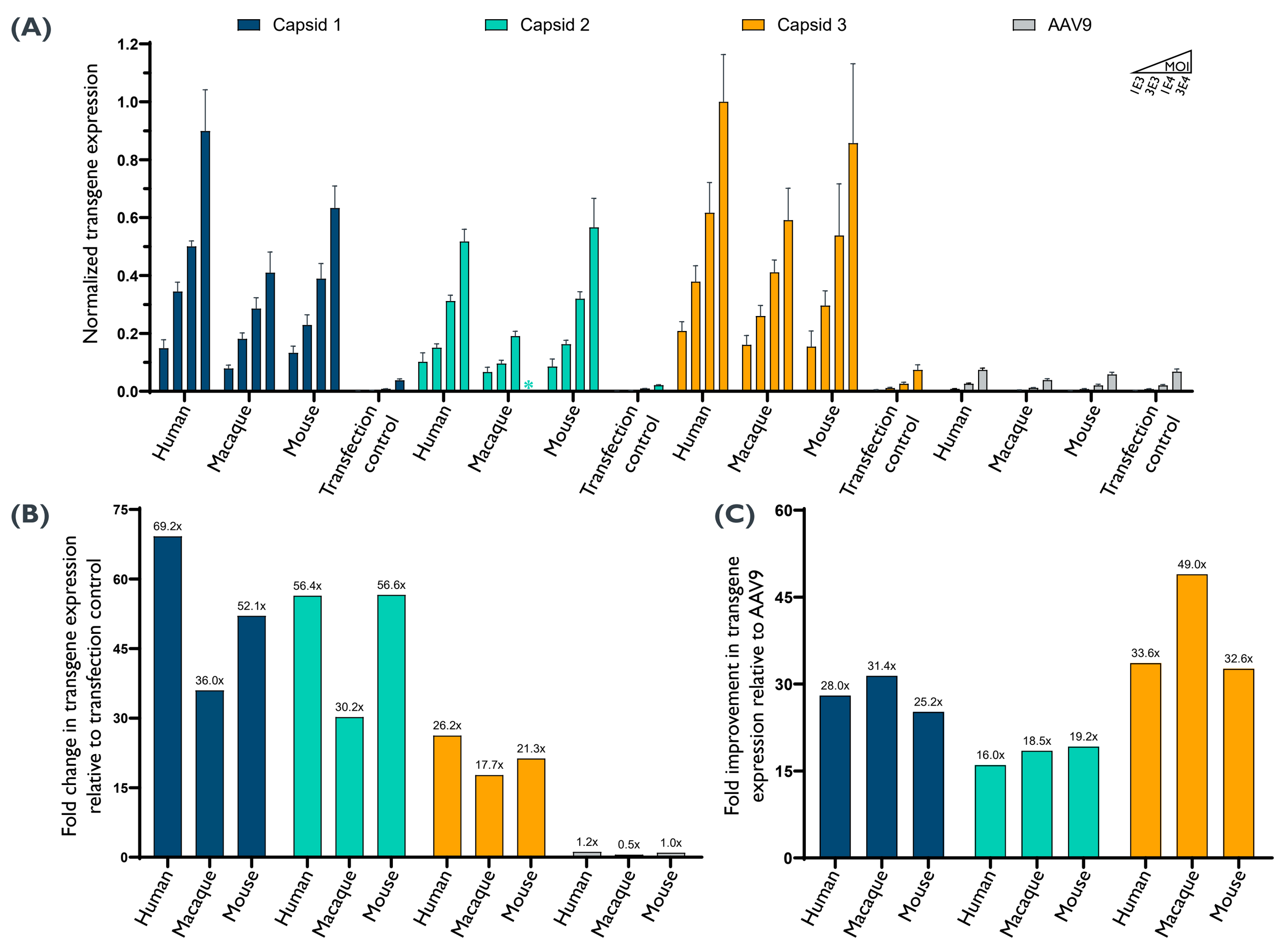

Figure 6. Engineered capsids exhibit enhanced transduction of cells overexpressing the human, macaque, or mouse ortholog of Receptor 1. Cells were transiently transfected with an overexpression plasmid encoding Receptor 1. Transfected cells were transduced with each AAV and transgene mRNA expression was assessed 72 hours later by RT-qPCR. *Sample lost during processing.

(A) Capsids 1-3 were evaluated individually and compared against the parental serotype AAV9. Receptor-targeted capsids exhibit a marked enhancement in transgene mRNA expression in cells overexpressing Receptor 1, but not in the transfection control condition. Moreover, this gain of function was conserved across human, macaque, and mouse orthologs of Receptor 1.

(B) Fold increase in transgene expression relative to control. A nonlinear regression model was used to interpolate relative transgene expression values for each capsid-receptor condition. These values were then scaled to the transfection control value for each capsid.

(C) Engineered capsids mediate higher transgene expression than AAV9 in cells overexpressing human, macaque, or mouse Receptor 1.

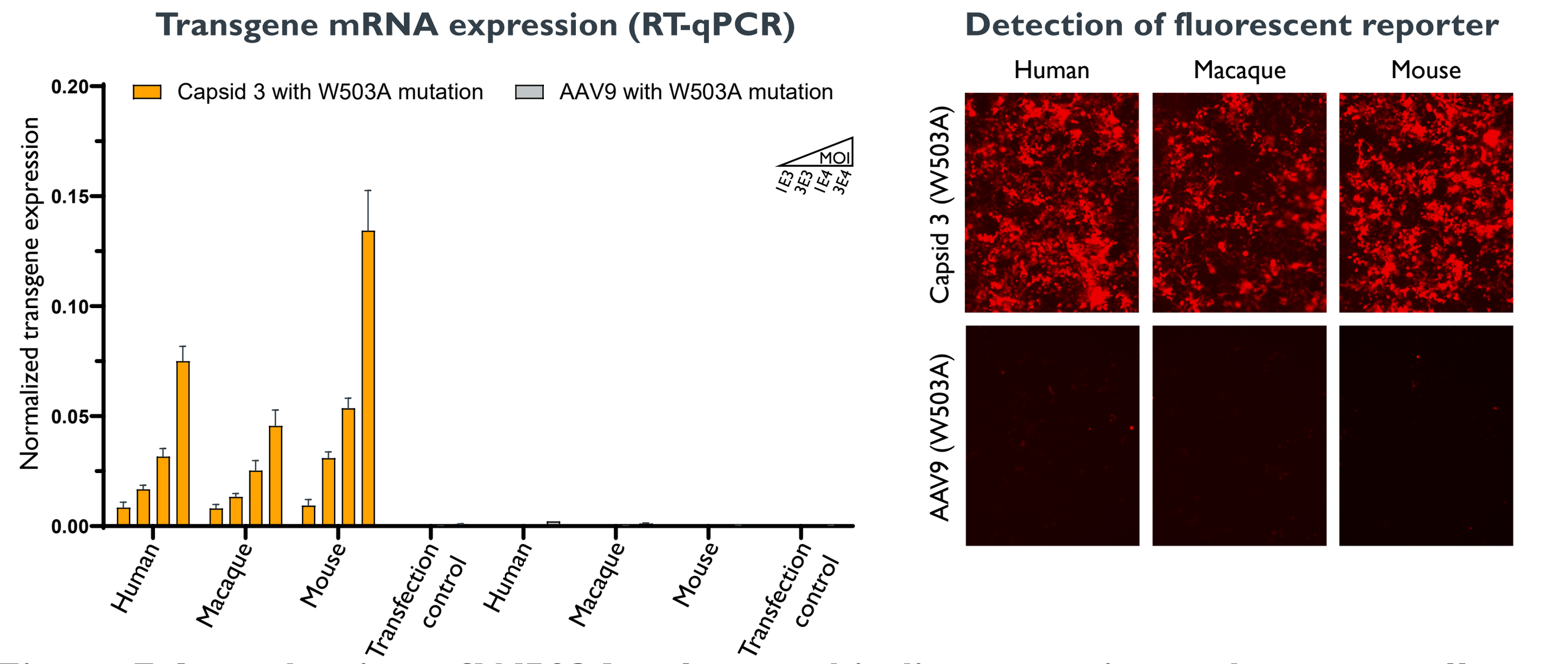

Figure 7. Introduction of W503A galactose binding mutation reduces overall potency, but a gain of function remains in cells overexpressing Receptor 1. These results suggest that Capsid 3 can utilize both galactose and Receptor 1 for cell entry.

Library screens for capsids targeting Receptor 2 or 3

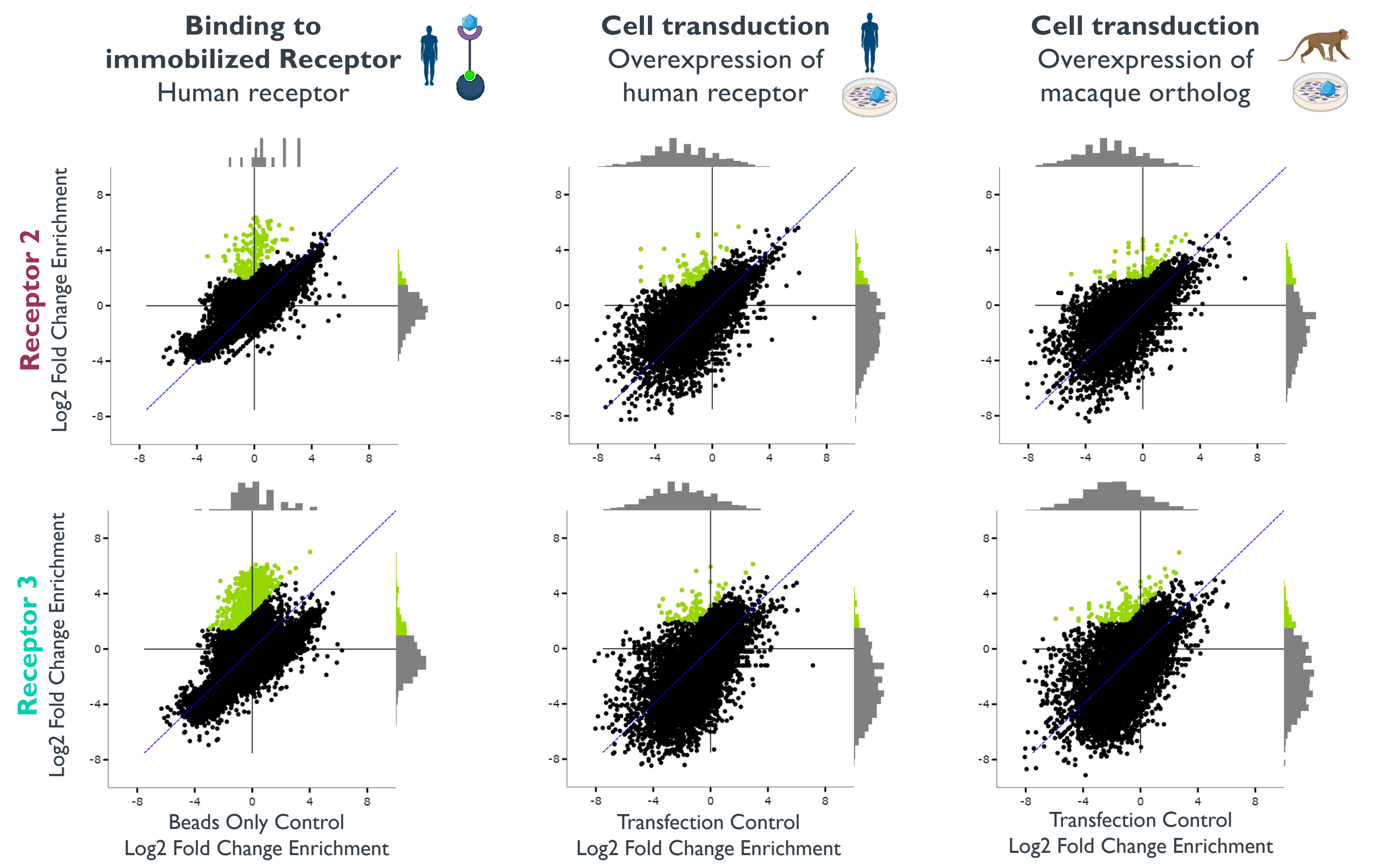

Figure 8. Library screens identify capsid variants that specifically target Receptor 2 or Receptor 3. Three parallel capsid library screens were conducted for each receptor:
1) Immobilized human receptor versus a bead only control.
2) Transduction of cells overexpressing the human receptor versus a transfection control.
3) Transduction of cells overexpressing the macaque receptor versus a transfection control.
Capsids exhibiting specific enrichment for Receptor 2 or 3 are colored in green.

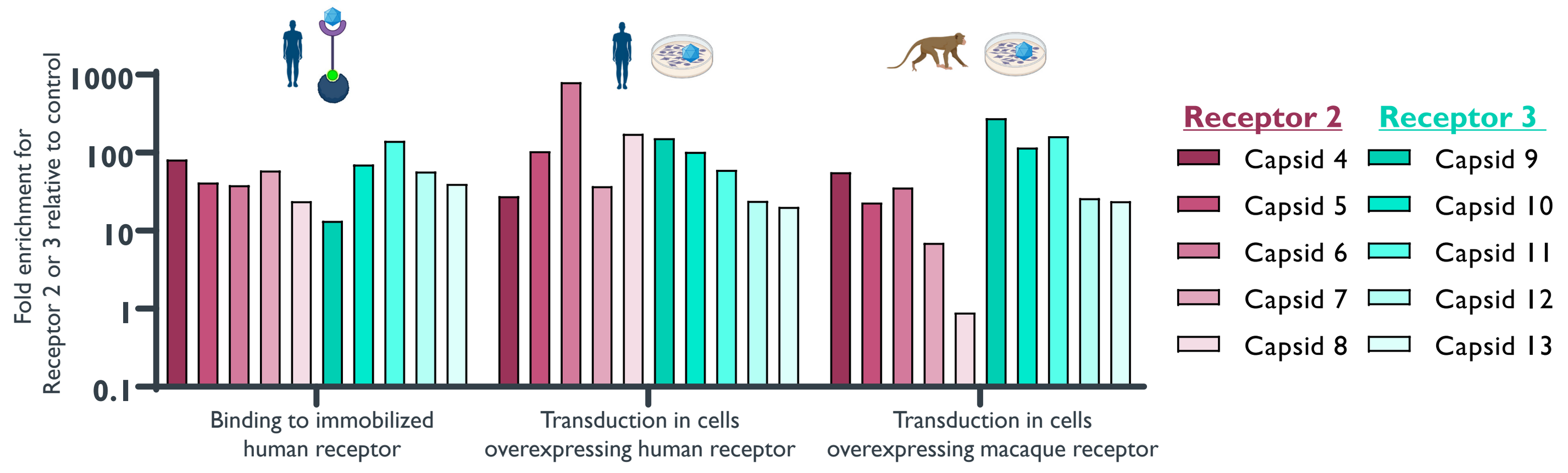

Figure 9. Examples of capsids that exhibited enrichment in Receptor 2 or Receptor 3 screens and are being advanced for individual evaluation. A subset of identified capsids exhibit cross-species interaction with both the human and macaque orthologs.

Conclusions and next steps

- We have engineered receptor-targeted AAV capsids for three receptors that have the potential to mediate transport across the blood-brain barrier.
- Capsids targeting Receptor 1 exhibit enhanced transduction in cells overexpressing the human, macaque, and mouse orthologs. A subset of capsids targeting Receptors 2 and 3 display cross-species interaction with both the human and macaque orthologs.
- Receptor-targeted capsids will be evaluated *in vivo* to assess BBB crossing efficiency and tropism for CNS cell types.
- These receptor-targeted capsid families are distinct from STAC-BBB and likewise have the potential to mediate widespread CNS delivery for the treatment of neurological disorders.

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