



A highly potent engineered AAV capsid, STAC-150, enables high-throughput AAV production and arrayed epigenetic regulator screening directly in cultured neurons

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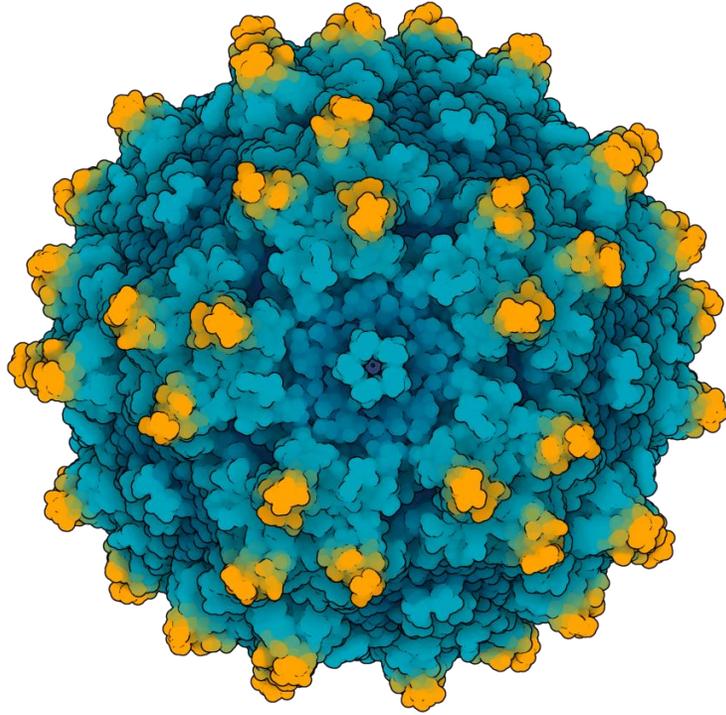
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¹Sangamo Therapeutics

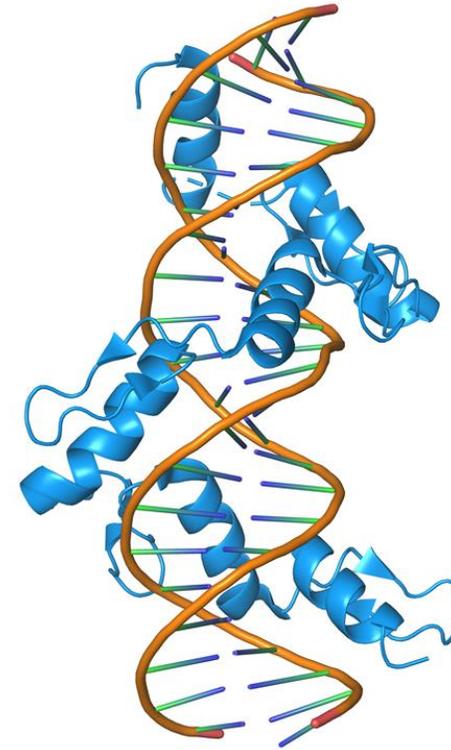
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I am a full-time employee of Sangamo Therapeutics

Next-generation medicines for neurology based on engineered AAV capsids and Zinc Finger epigenetic regulators



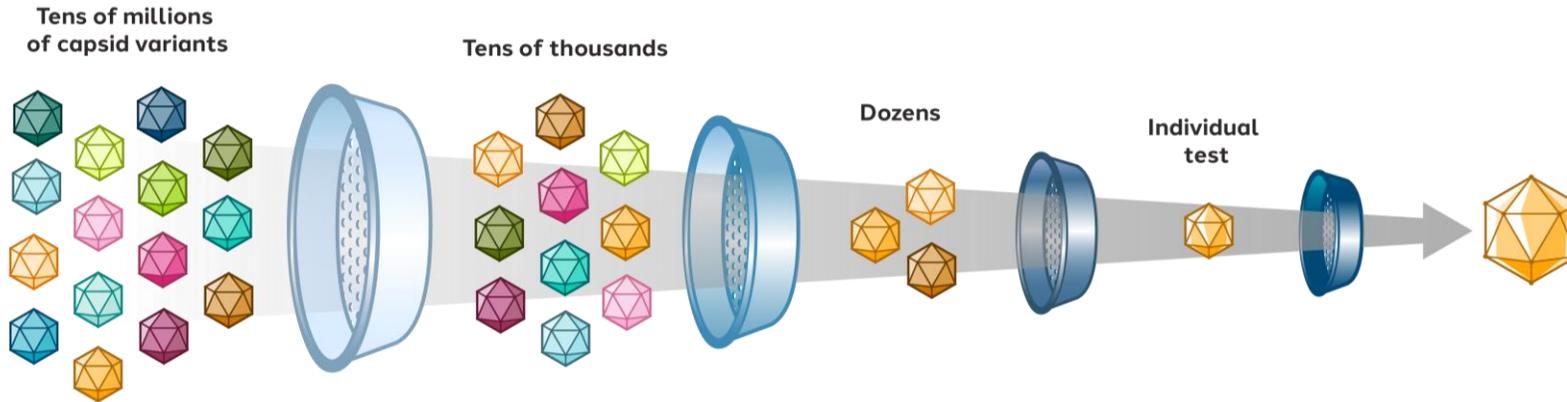
AAV Capsid



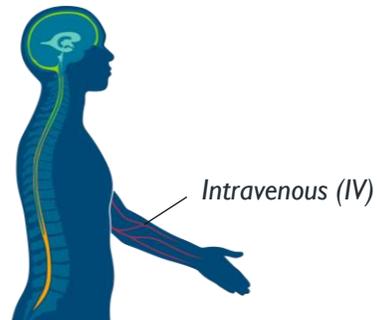
Zinc Finger
Epigenetic Regulators

Our SIFTER platform is generating engineered capsids for multiple CNS applications

SIFTER Capsid Engineering Platform

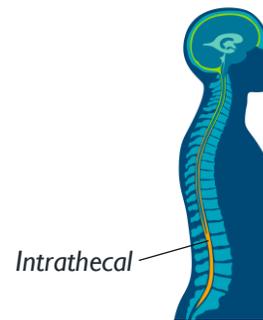


IV administration

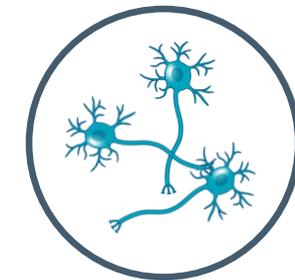


Abstract #117

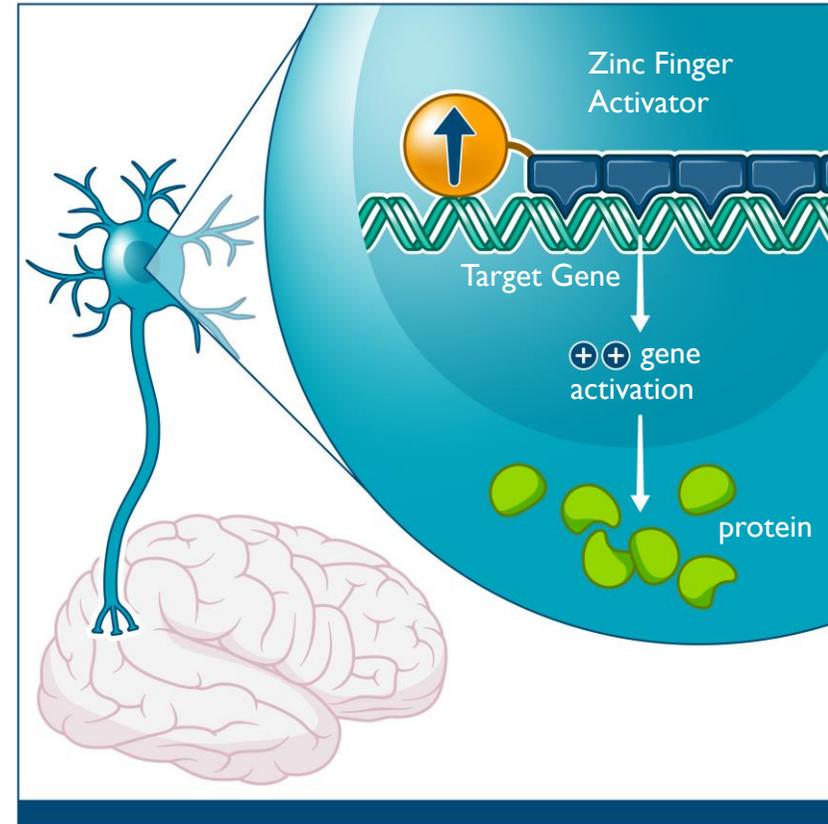
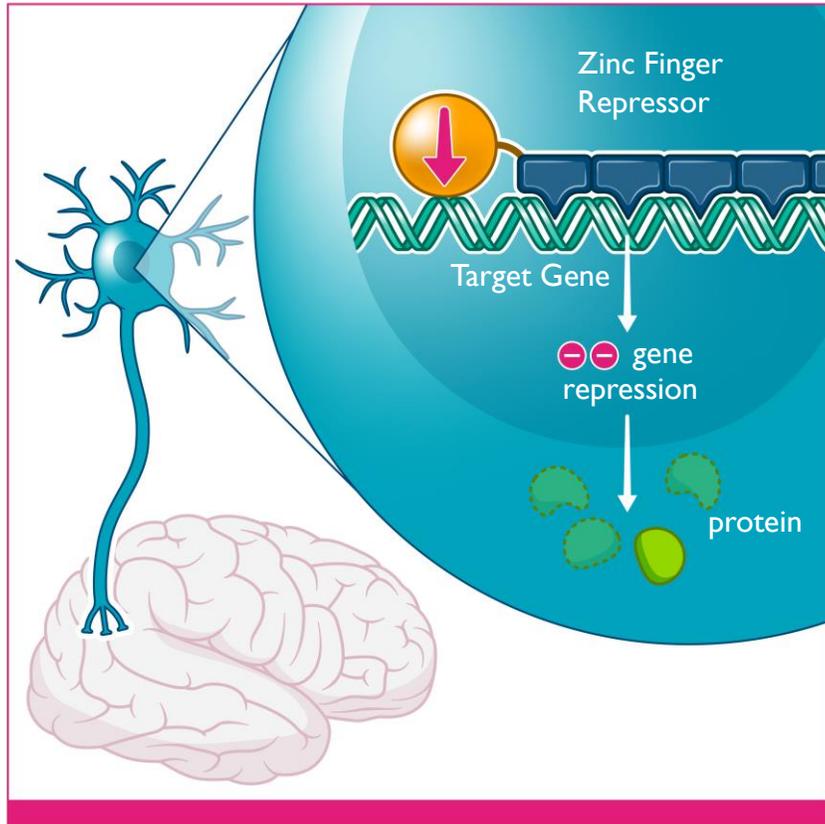
CSF administration



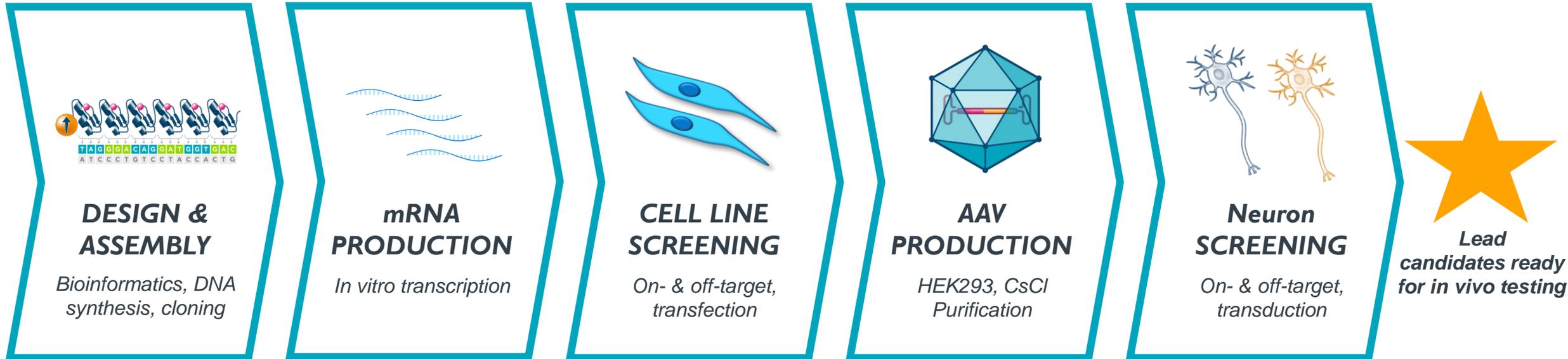
In vitro assays



Zinc finger epigenetic regulators have the potential to transform the treatment of neurodegenerative and neurodevelopmental disorders

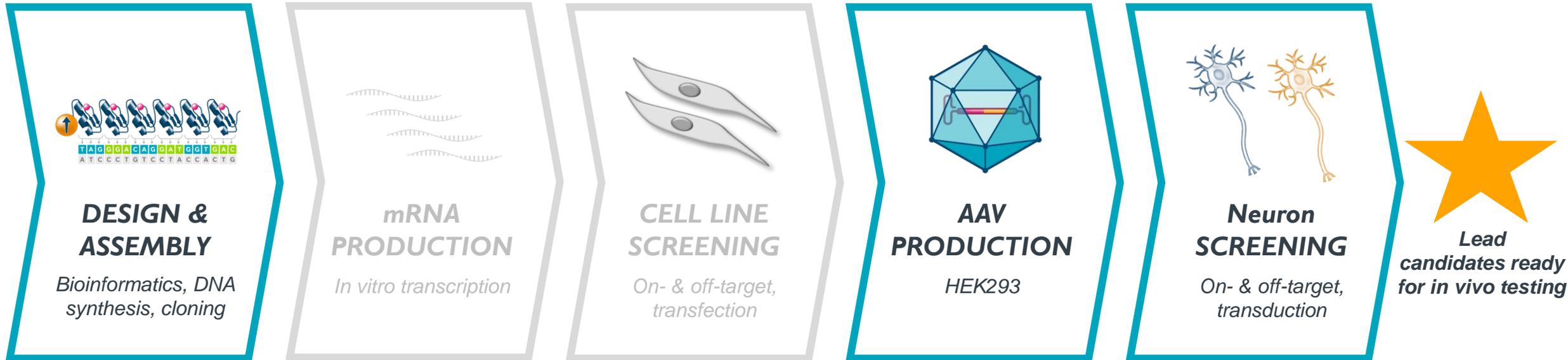


Current workflow for identifying potent and specific epigenetic regulators leverages cell lines prior to testing in neurons



- Initial screening in cell lines can be performed at scale
- Cell lines sometimes do not fully recapitulate native epigenetic signature present in more biologically relevant cells
- Primary cells better reflect biology, but can be difficult to transfect with mRNA

Screening epigenetic regulators directly in neurons could accelerate lead selection timelines and enable new targets



STAC-150 HT-AAV Production

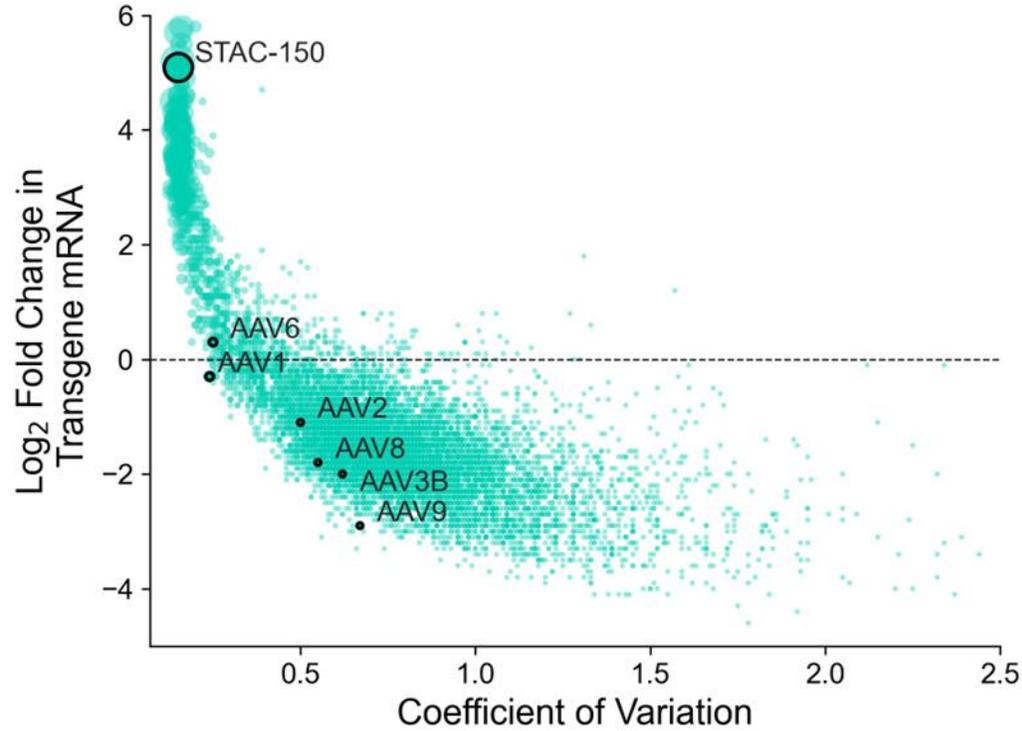
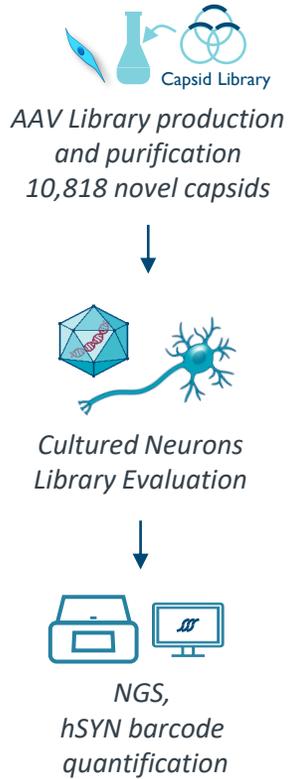
- Bypass the time and effort required to screen in cell lines (including assay development)
- Enable screening for targets that cannot be easily modeled in cell lines

Key characteristics of a capsid engineered for in vitro screening

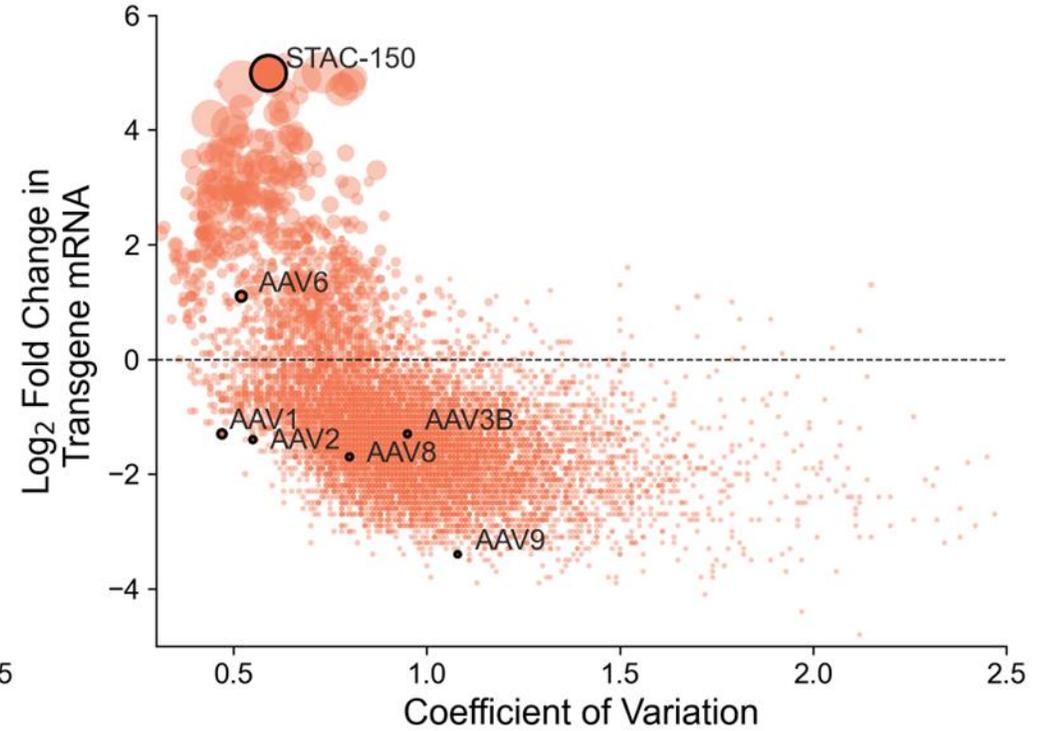
- Potent
- Secreted
- Non-toxic delivery
- Compatible with high throughput workflows



STAC-150 AAV outperforms in library evaluations in cultured neurons



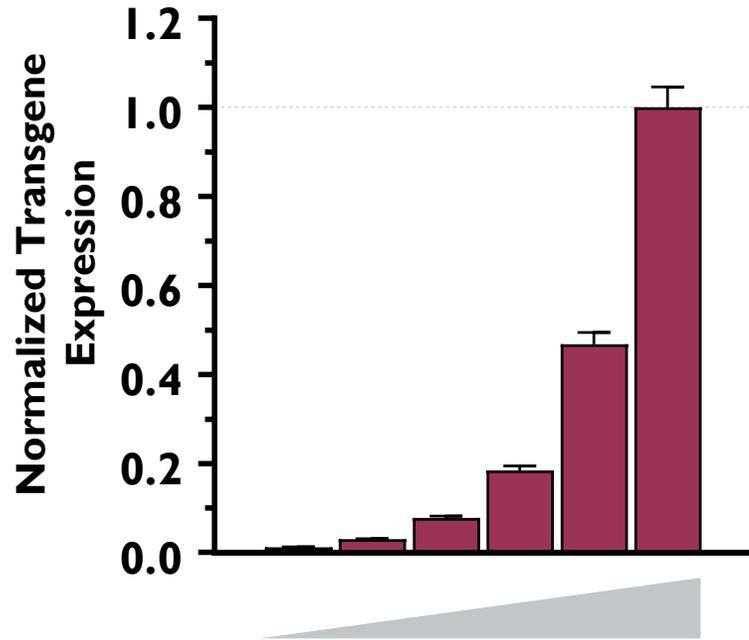
Mouse cortical neurons
7 days post-transduction
MOI 1E5



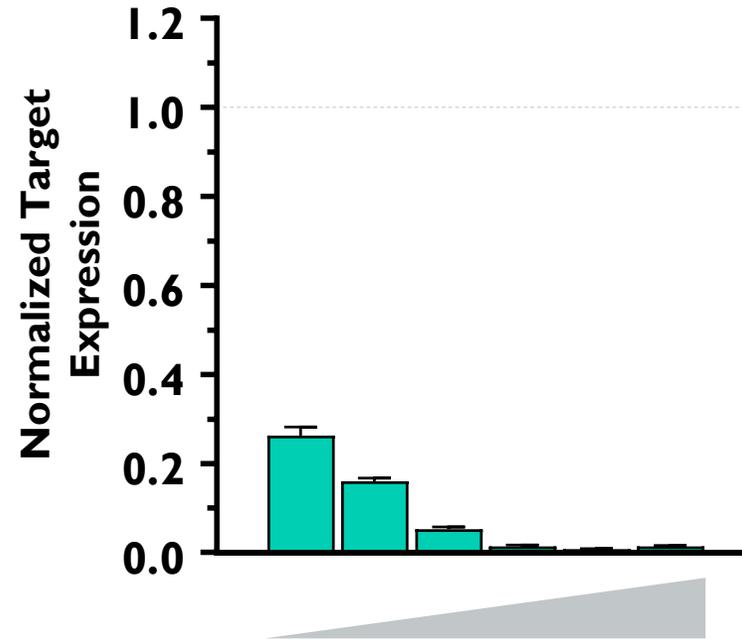
Human IPSC-derived neurons
30 days post-transduction
MOI 1E5

STAC-150 is exceptionally potent and efficiently released into the production medium

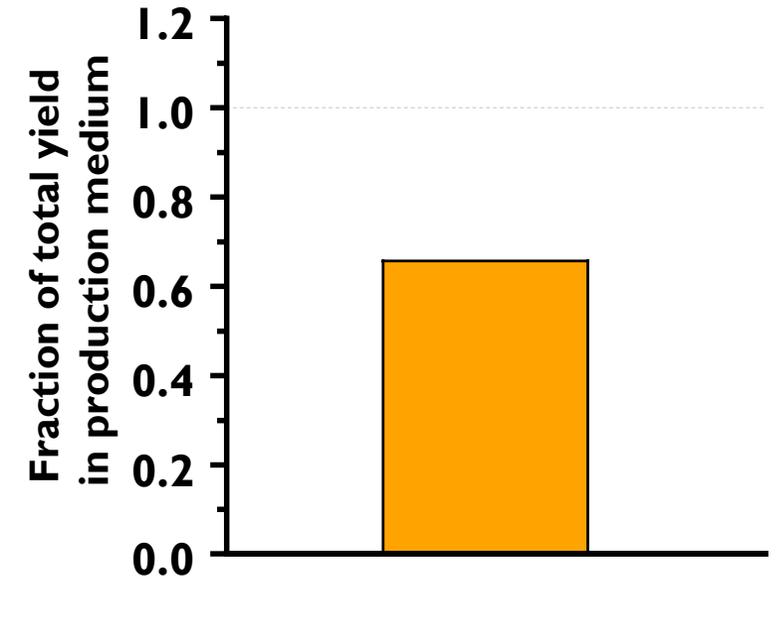
Potent transgene expression



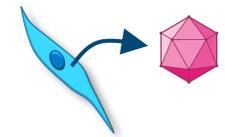
Effective delivery of epigenetic regulators



Released into production medium



Mouse cortical neurons
7 days post-transduction



A high-throughput AAV production workflow leverages STAC-150 AAVs to enable arrayed screening directly in neurons

Standard AAV workflow (Cesium Chloride gradient purification)



High-throughput AAV workflow



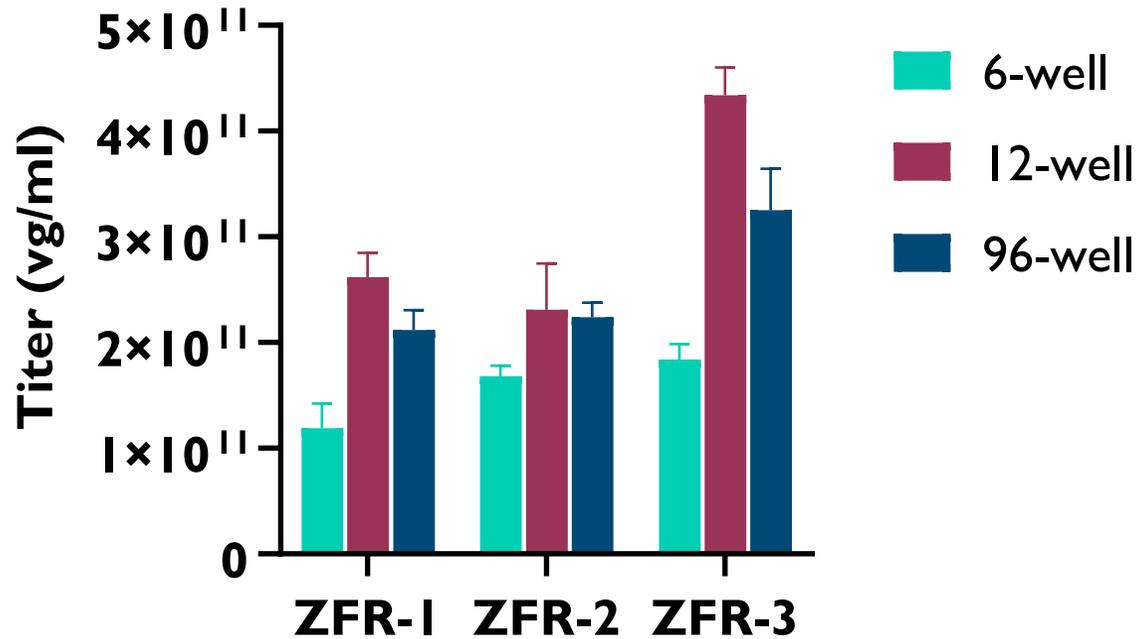
AAV production (96-well)
HEK293-suspension

AAV purification (96-well)
AAVX Magnetic Beads

	Standard	HT-AAV
Production volume	40mL +	500 – 1000µl
Yield (vg)	~10 ¹² +	~10 ¹¹
#AAVs/week	10 +	1,000 +
Time	1 – 2 weeks	2 – 3 days
Cost	\$\$\$\$	\$
Automation-compatible	Limited	Full
Empty capsid removal	Yes	No

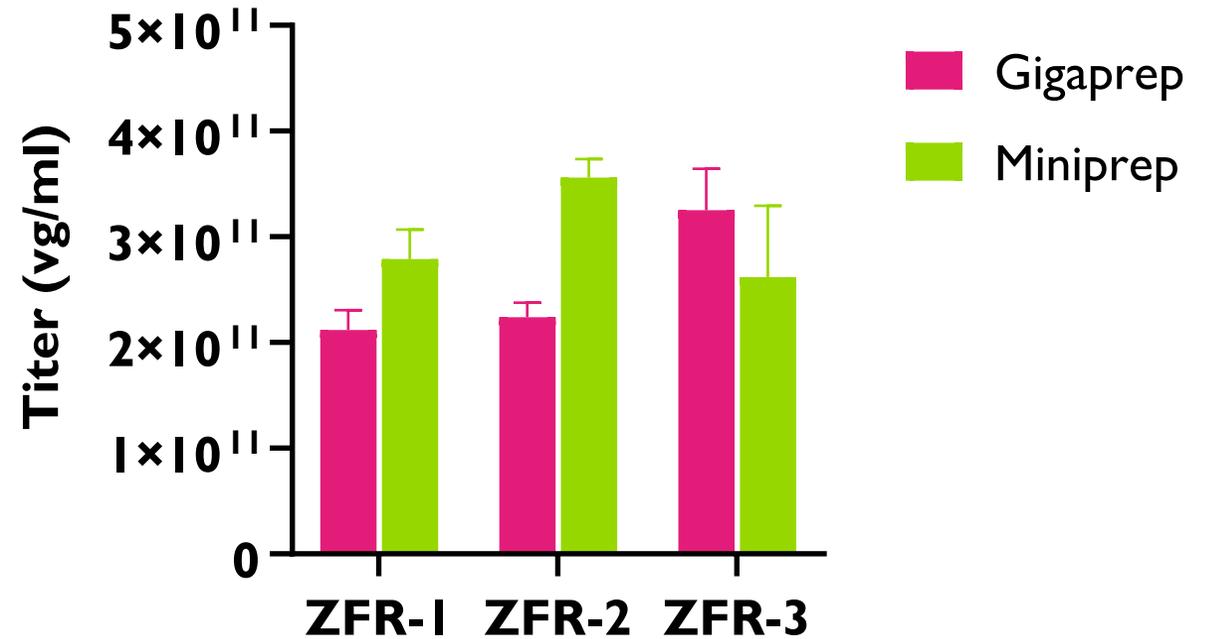
STAC-150 AAV enables cost-effective and high-throughput AAV production in 96-well format

Efficient AAV production in 96-well format



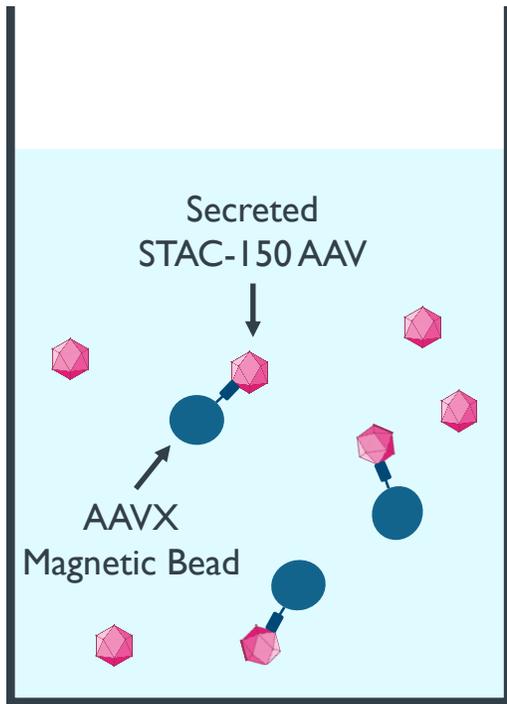
- 1 mL production medium yields enough vector genomes for on/off-target screening in cultured neurons

Compatible with multiple transgene plasmid preparation methods

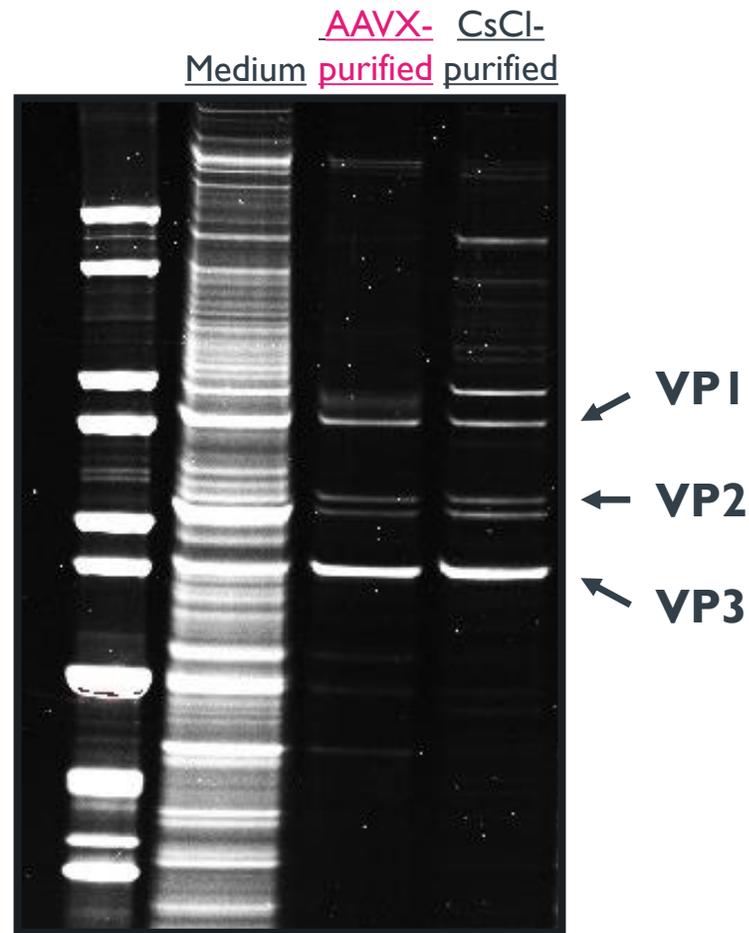


- Transgene plasmid prepared with a miniprep kit results in comparable titers

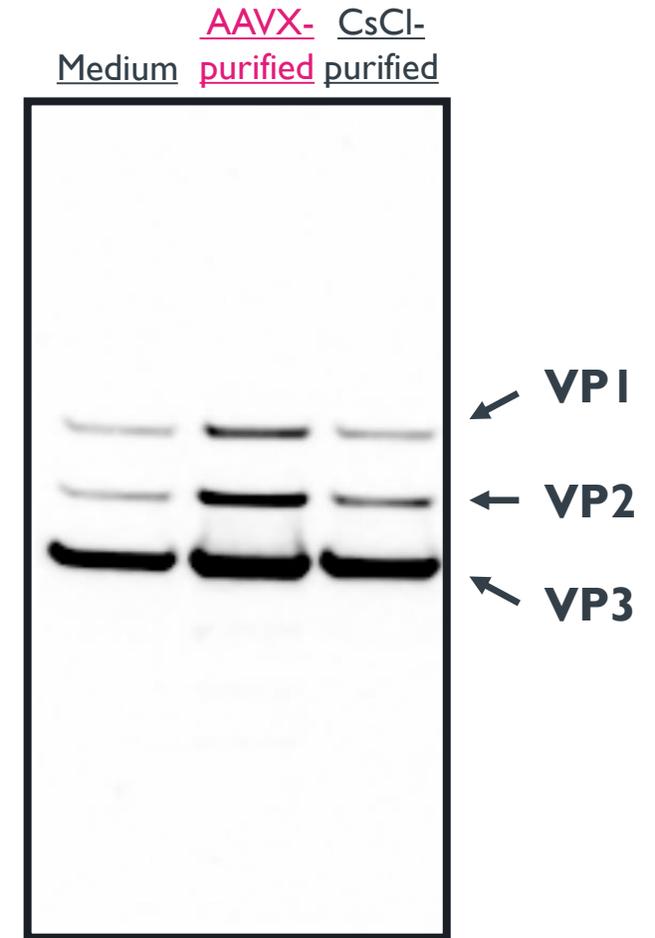
Secreted STAC-150 AAV particles enable fit-for-purpose purification from production medium



Total Protein



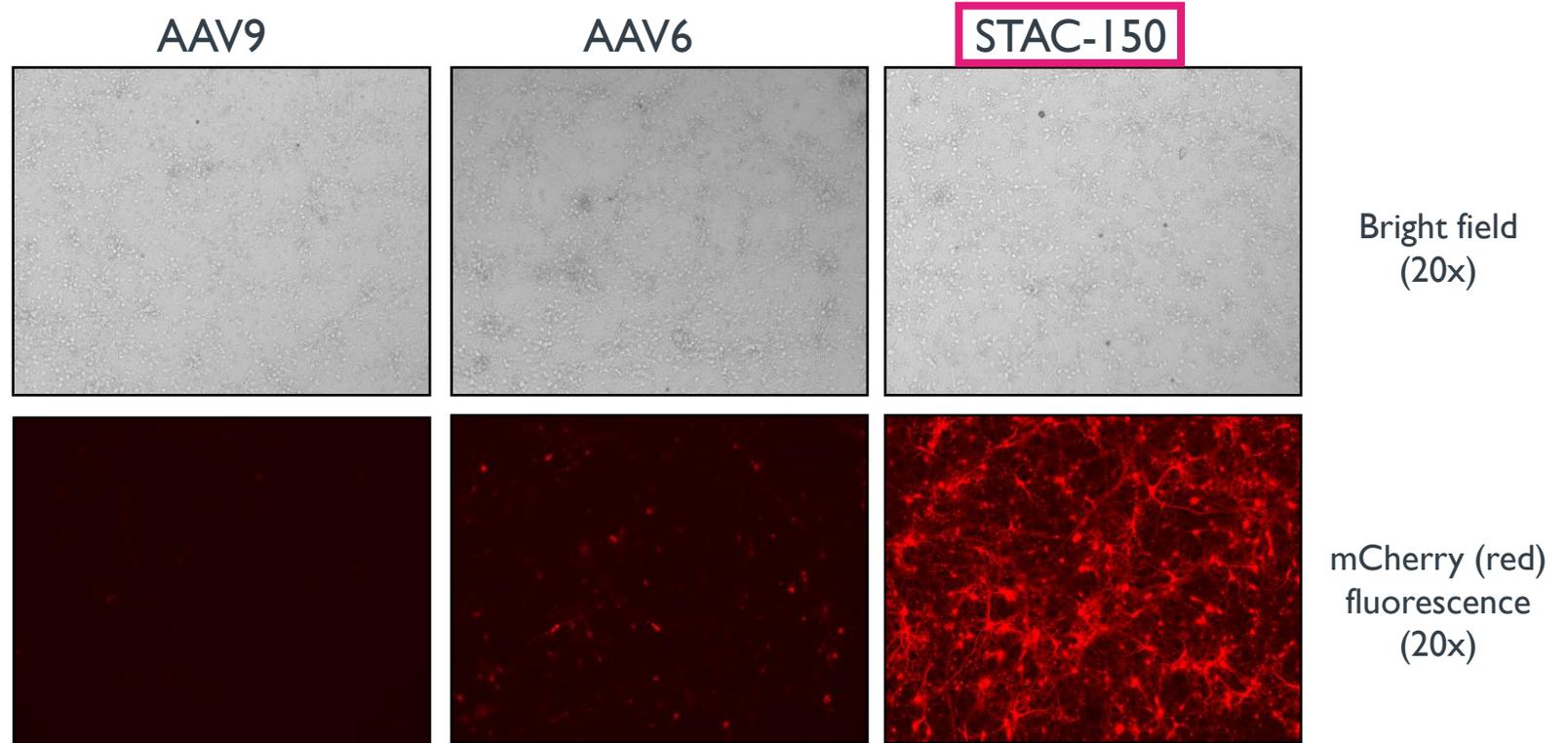
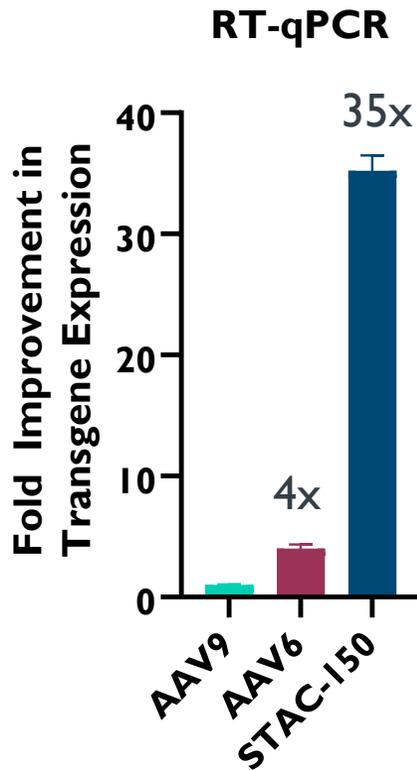
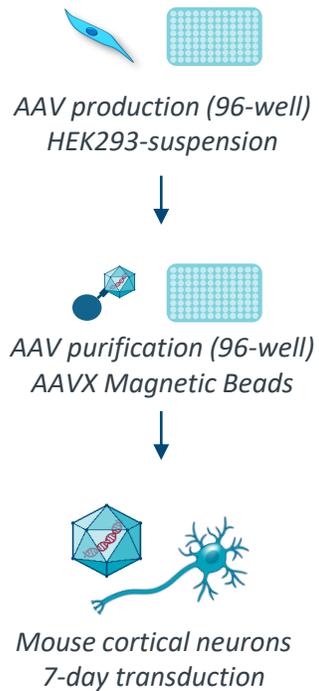
Western Blot (anti-VP1/2/3)



- Efficient purification in 96-well plates using AAVX magnetic beads

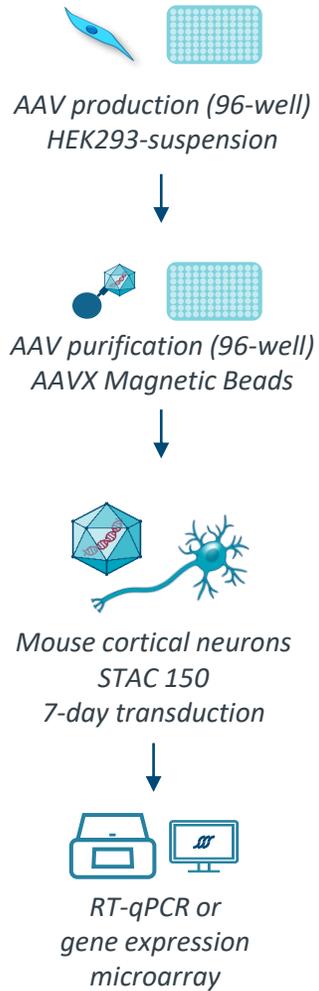
- Purified virus shows the expected viral protein ratios

Small-scale STAC-150 AAVs are highly potent in cultured mouse cortical neurons

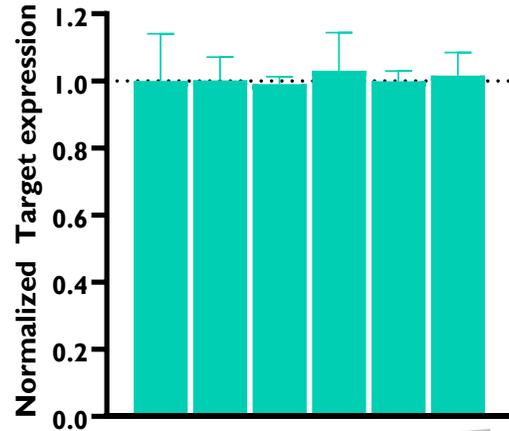


- STAC-150 is significantly more potent than AAV9 and AAV6 in cultured primary mouse cortical neurons

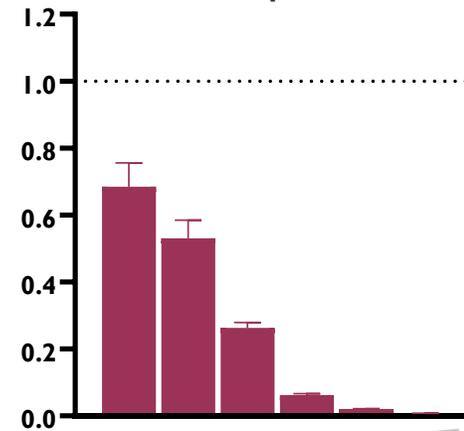
STAC-150 enables epigenetic regulator screening directly in cultured neurons



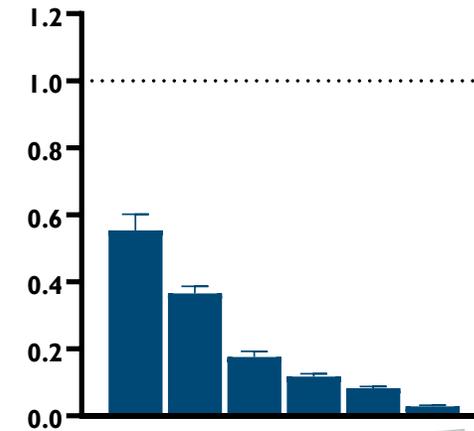
Negative Control



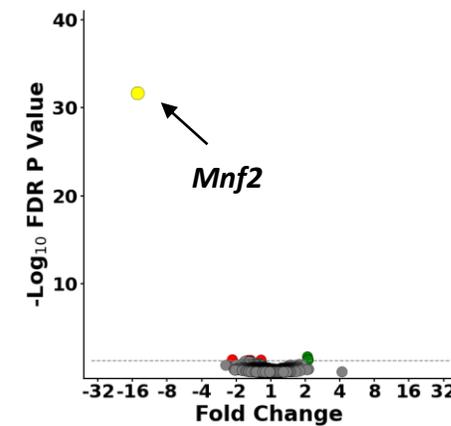
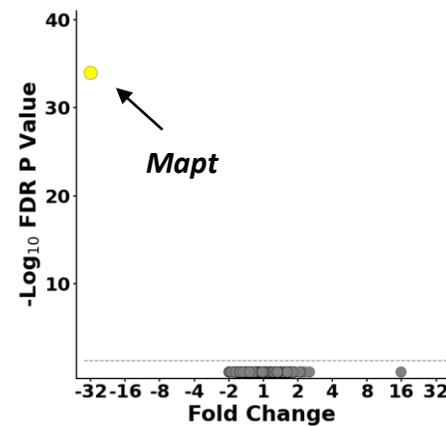
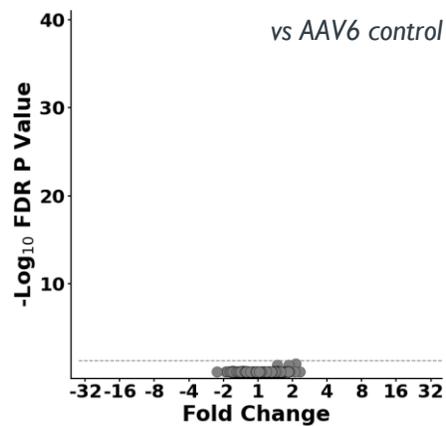
ZFR Target:
Mapt



ZFR Target:
Mfn2

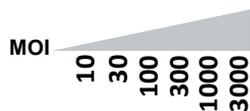


On-target
Repression



Off-target
Analysis

- Genes down-regulated
- Genes up-regulated
- Target



Conclusions

- We engineered STAC-I50, a novel neurotropic AAV capsid, with two important properties:
 - High potency *in vitro*
 - Released into HEK293 production medium
- We leveraged STAC-I50 to develop a high-throughput AAV production workflow
 - 96-well AAV production in HEK293 suspension cells
 - 96-well AAV purification using AAVX magnetic beads
- We showed that STAC-I50 effectively delivers epigenetic regulator payloads to mouse cortical neurons for potent and highly specific repression of target genes