Dyno bCap 1: Crossing the non-human primate blood brain barrier with machine-guided AAV capsid design

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DYNO THERAPEUTICS

Digital Poster

Summary

- **Dyno bCap 1** is a novel ML-designed CNS IV capsid variant of AAV9 that is available for immediate licensing.

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Dyno bCap 1 575 ESYGVVATNHQSAQAQAIVGALQSQGALP 603
      AAV9  575 ESYGQVATNHQSAQAQAQTGWVQNQGILP 603
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- Performance of the **Dyno bCap 1** capsid, at an IV dose of 1E13 vg/kg in Cynos and relative to AAV9
  - **100x** Improved brain transduction
  - **10x** Decreased liver transduction
  - **1x** Production efficiency
  - **4-14%** of cells expressed the delivered transgene pan-brain
  - **5-20%** of neurons across CNS sub-regions of basal ganglia, hippocampus, motor cortex, and spinal cord express the delivered transgene
- **Dyno bCap 1** showed enhanced transduction crossing the blood brain barrier in both **African Green Monkey** (*Chlorocebus sabaeus*) and **cynomolgus macaque**.
- Compared against an external engineered AAV capsid reported to have more improved brain transduction than other CNS capsids, **Dyno bCap 1** demonstrated consistent brain transduction across NHP replicates and across species, similar to or above the level of the external capsid, **with dramatically better production efficiency**.
- Together these properties show **Dyno bCap 1** is a transformative capsid for CNS IV delivery. Contact bd@dynotx.com for licensing and to learn more about emerging validated capsids from more recent NHP studies.

AI-Powered AAV Capsids for Improved NHP Brain Transduction

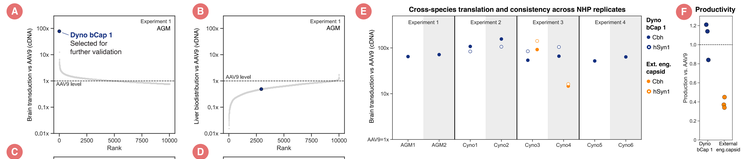


Figure 1. AI-powered capsid design generates AAV9 variants with greatly improved NHP brain transduction following IV administration.
(A, B, C, D) >1e5 AAV9 variants were designed and library barcodes measured in NHPs using NGS, showing top 1e4 variants. **(E)** Improved brain transduction by Dyno bCap 1 (blue) was observed consistently across AGM and Cyno replicates. Experiment 3 included an external engineered capsid variant (orange) that was chosen as a reference based on literature reports that it mediates widespread distribution in the brain with neuronal tropism significantly better than wild-type AAV9 following IV dosing, however, its performance between 2 NHPs was inconsistent (5x lower in Cyno #4). **(F)** Replicate single-capsid productions showed Dyno bCap 1 to be near 1x AAV9 production efficiency vs external capsid with ~40% AAV9 efficiency.

Multiplexed Validation of Dyno bCap 1 vs. AAV9

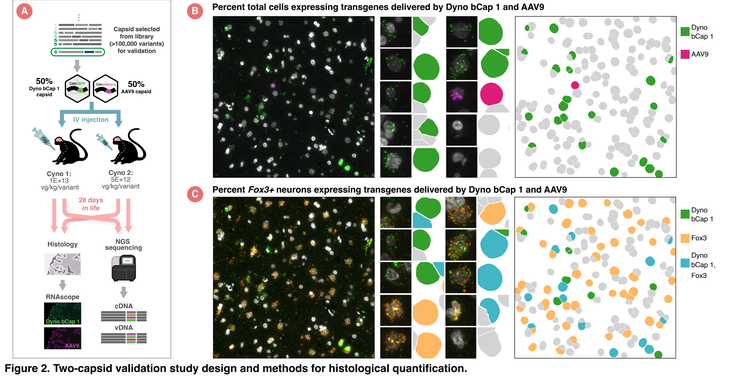


Figure 2. Two-capsid validation study design and methods for histological quantification.
(A) Dyno bCap 1 and AAV9 capsids were pooled in equal ratios into a single test article (confirmed by ddPCR and NGS) and used to deliver barcoded transgene reporters Cbh-NLS-eGFP and Cbh-NLS-mCherry, respectively. Cynos were dosed intravenously with 1E13 vg/kg per variant (2E13 vg/kg total) or 6E12 vg/kg per variant (1.2E13 vg/kg total). Following 28 days in-life, animals were sacrificed and tissues were processed for NGS and histology. NGS was used to measure transduction and biodistribution, and histological quantification was performed using multiplexed RNAscope and IHC, to enable direct comparisons between methods and animals. **(B, C)** Automated RNAscope image analysis methods for all cells **(B)** and neurons **(C)**. Representative RNAscope fluorescence in-situ hybridization images of motor cortex from a treated NHP are shown next to schematics of cells identified using an automated cell segmentation strategy. Multiple properties of the individually segmented cells determined by human counts were used to train classifiers to enable automated positive and negative cell calling for larger image datasets. Example cell images from each category (left) are enlarged and shown next to their schematic representations (right).

IV-Delivered Dyno bCap 1 Efficiently Transduces the NHP CNS in a Multiplexed Validation Study

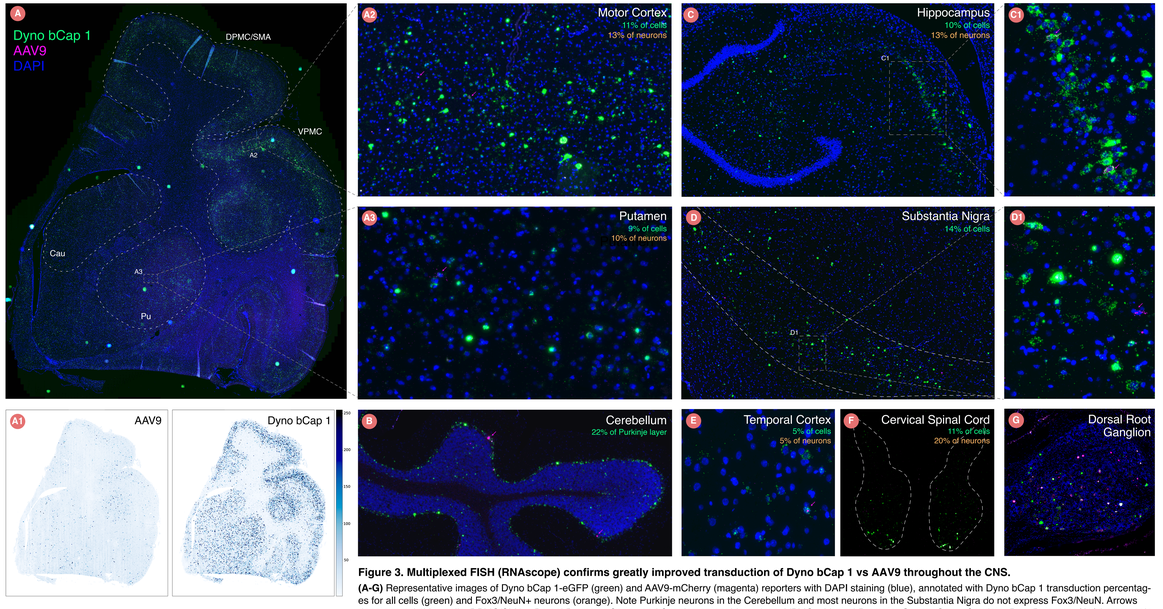


Figure 3. Multiplexed FISH (RNAscope) confirms greatly improved transduction of Dyno bCap 1 vs AAV9 throughout the CNS.
(A-G) Representative images of Dyno bCap 1-eGFP (green) and AAV9-mCherry (magenta) reporters with DAPI staining (blue), annotated with Dyno bCap 1 transduction percentages for all cells (green) and Fox3/NeuN+ neurons (orange). Note Purkinje neurons in the Cerebellum and most neurons in the Substantia Nigra do not express Fox3/NeuN. Arrows denote rare AAV9+ cells. DPMC/SMA = Dorsal Premotor Cortex and Supplementary Motor Area, VPMC = Ventral Premotor Cortex, Cau = Caudate, Pu = Putamen. **(A1)** Large-scale visualization of QuPath-based automated cell detection and transduction quantification for AAV9 vs Dyno bCap 1, plotting markers for each cell colored by maximum pixel intensity for each transgene.

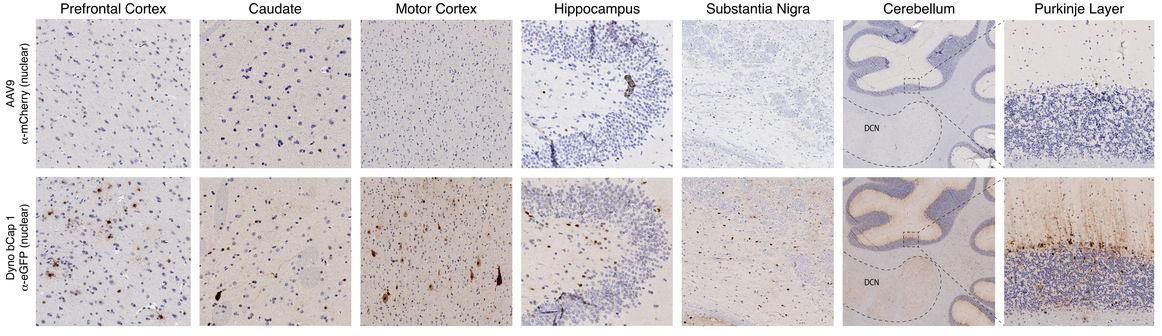


Figure 4. IHC confirms greatly improved pan-brain transduction of Dyno bCap 1 vs AAV9.
Immunohistochemistry targeting the nuclear-localized transgenes delivered by Dyno bCap 1 (NLS-eGFP) or AAV9 (NLS-mCherry) was separately performed on matched adjacent 5 µm sections cut from FFPE fixed brain slabs. Transgene proteins expressed in the indicated regions are detected using DAB chromogenic staining (brown); cell nuclei are detected using hematoxylin (blue). DCN = Deep Cerebellar Nuclei in the cerebellum.

Validation Study Quantification

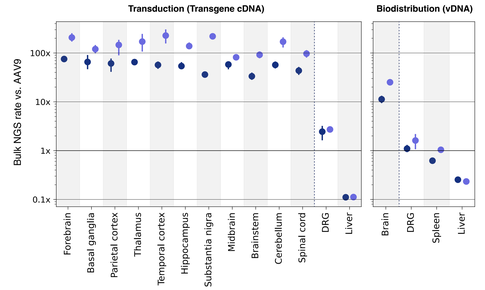
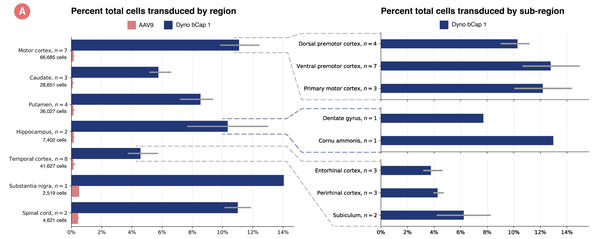
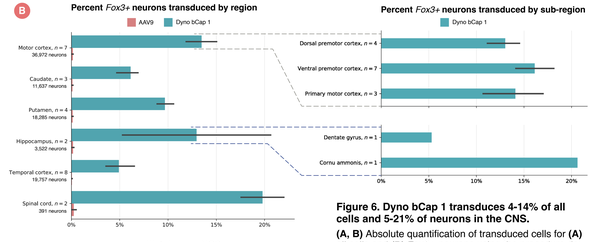


Figure 5. Bulk NGS measurements confirm 100x improvement in pan-brain/spinal cord transduction and 10x liver detargeting for Dyno bCap 1 vs AAV9.
Brain regions of interest were microdissected from 4mm coronal slabs preserved in RNA*later* . Transduction and biodistribution rates were measured using NGS of variant associated barcodes from cDNA (expressed transgenes) and vDNA (viral genomes) extracted from bulk tissue samples respectively. Total counts from tissues were normalized to NGS-determined ratios of variant barcodes (Dyno bCap 1 and AAV9) in the test article input. Rates of Dyno bCap 1 transduction and biodistribution are reported relative to AAV9. In addition to improved brain and spinal cord transduction observed in all regions sampled, 10x liver detargeting is observed along with comparable DRG targeting.





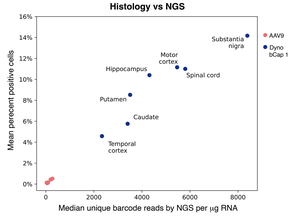


Figure 6. Dyno bCap 1 transduces 4-14% of all cells and 5-21% of neurons in the CNS.
(A, B) Absolute quantification of transduced cells for **(A)** all cells and **(B)** Fox3+ neurons using the procedure described in Figure 2. Larger regions were further stratified to show transduction patterns for sub-regions (right). We report the number of images and total number of cells from each region. Error bars denote 95% confidence interval between quantified images. In negative control images from untreated NHPs (not shown), the same automated quantification identified the following numbers of Dyno bCap 1+ cells/total cells counted: Frontal cortex 3/7123, Hippocampus 0/7443, Temporal cortex 1/6443.

Figure 7. Dyno's transduction quantification methods are consistent between histology and NGS.
Correlation between median barcode-IDs for each variant per microgram of RNA, as determined by NGS (x-axis) and mean percent transduced cells by each variant, as determined by histology (y-axis). Pearson correlation = 0.93 for Dyno bCap 1.

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