Dyno Therapeutics
The Capsids You Need

AI-Guided Design & In Vivo Validation of AAV Capsids for Better Delivery to Muscle, Eye & CNS

ASGCT 2023

Dyno:

(noun) in climbing, a powerful jump across a rock face to reach a hold

Our mission

Empowering **diverse teams** of

high-potential problem solvers to

transform patient lives with

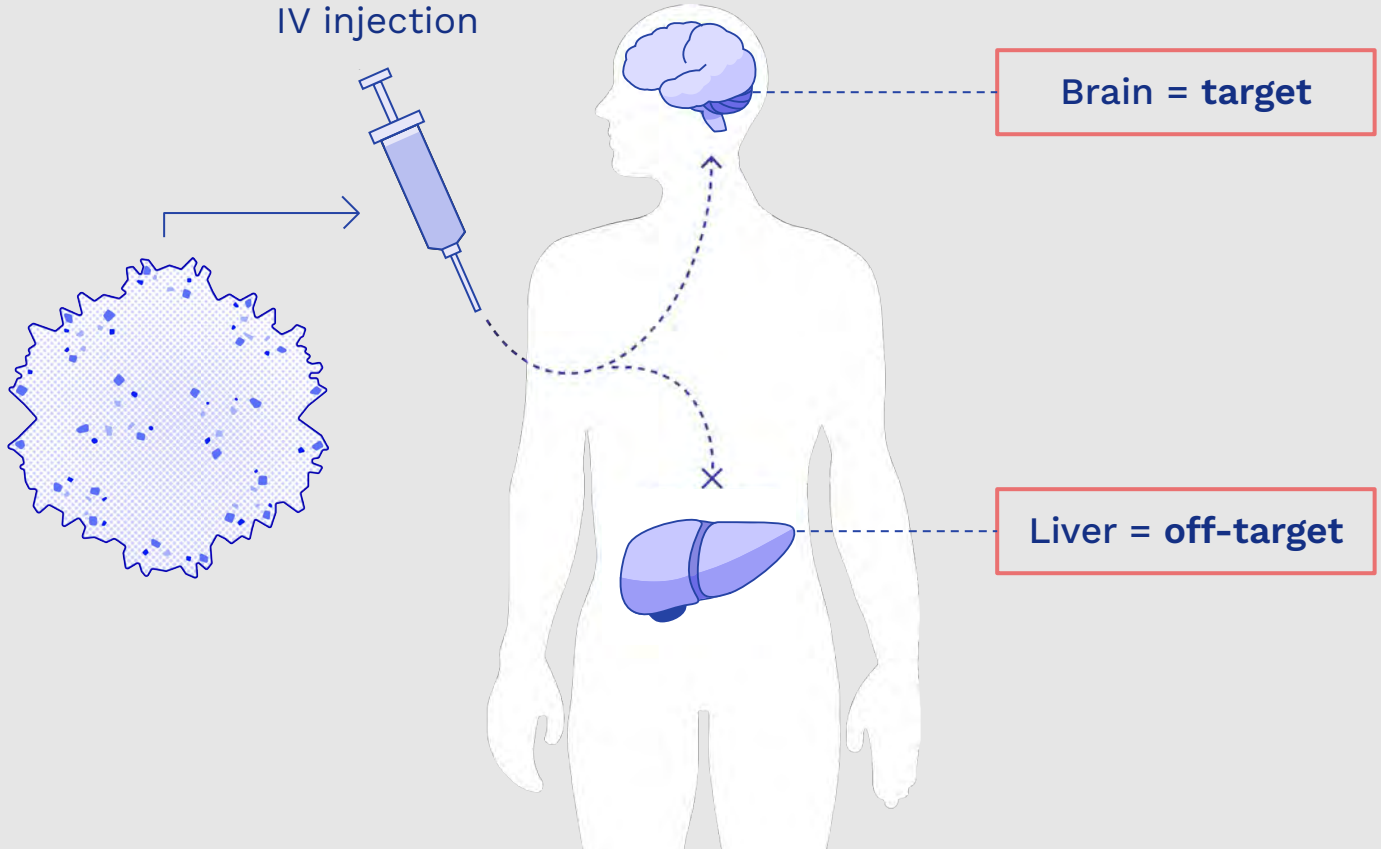
cutting-edge science

Why partner with Dyno?

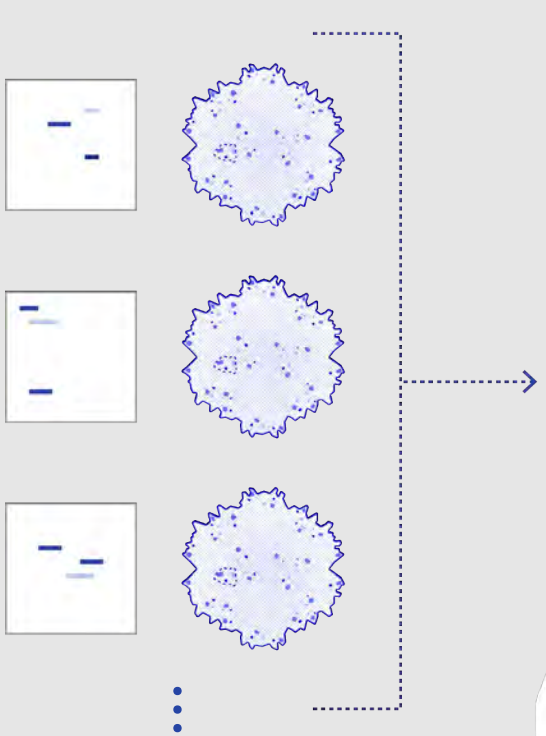
Partnership-centric business model = 100% alignment

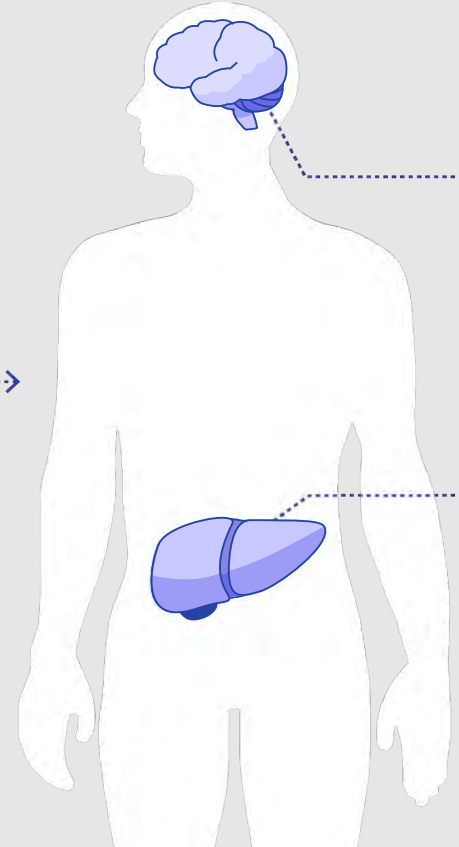
100% focused on capsid engineering

Our focus: solving the challenge of in vivo gene delivery



Our focus: solving the challenge of in vivo gene delivery



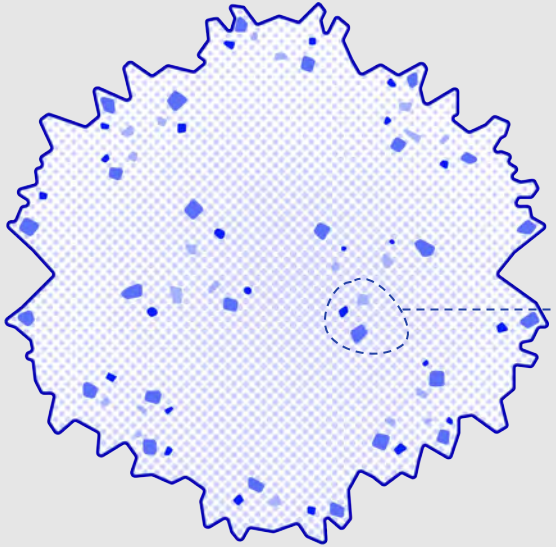


100X improvement in delivery CNS, crossing the blood-brain barrier

Same manufacturability as wild-type AAV9

10X lower off-target delivery to Liver to improve safety

AAV capsid: a ~735 letter search problem



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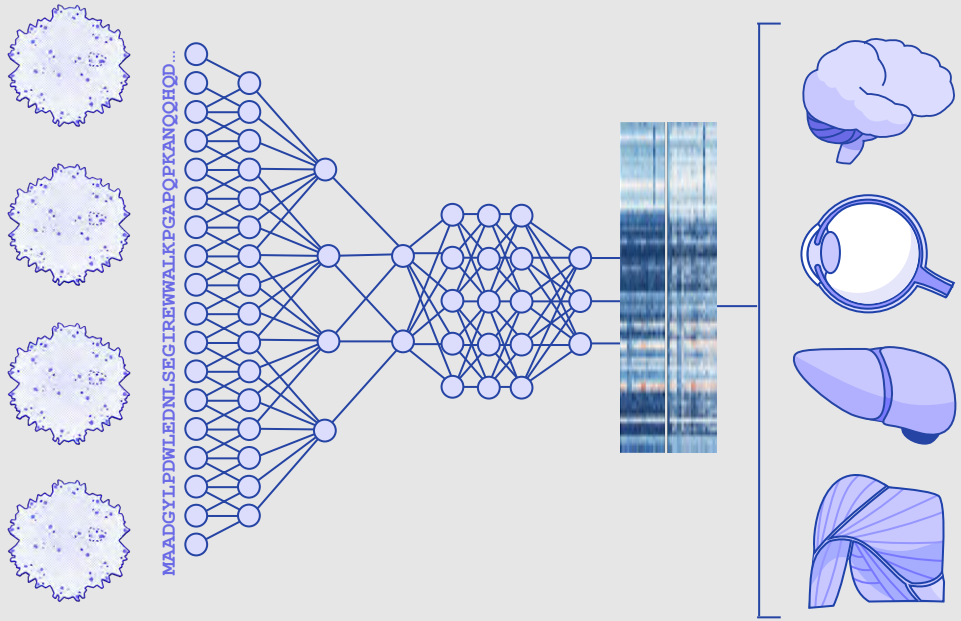
YKYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADA
EFQERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVE
HSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPS
GLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTR
TWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPR
DWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVF
TDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSF
YCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQY
LYYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSK
TSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGV
LIFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNR
QAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGF
GLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWEL
QKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL

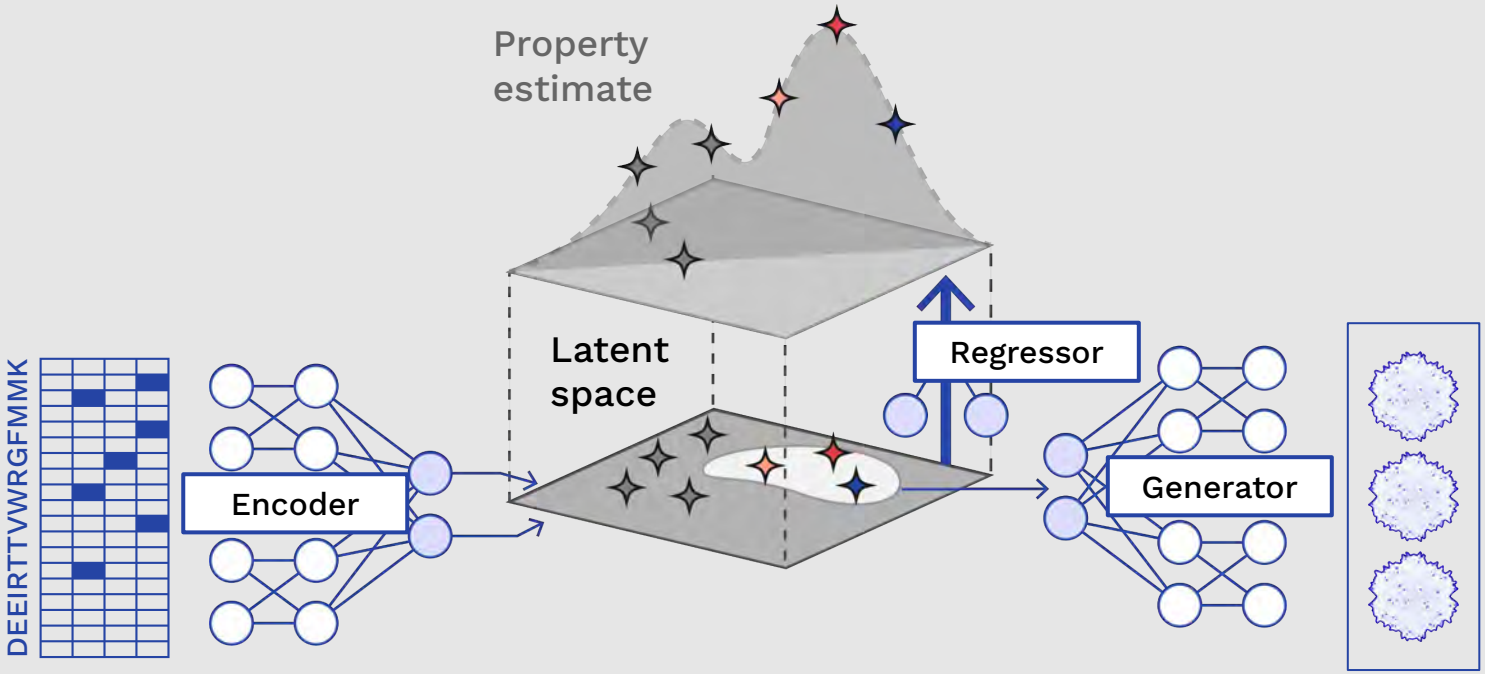
Dyno’s solution: AI-powered design



Dyno’s origins

Dyno Therapeutics Inc.
Multiplexing since May 2018

AI-powered design generates optimized capsid sequences

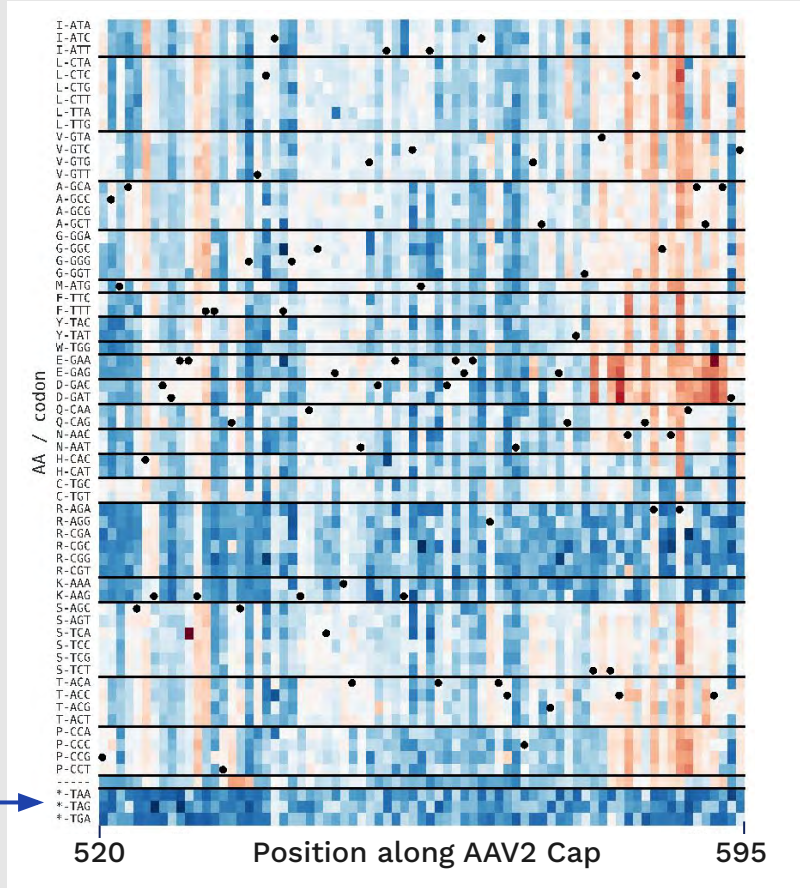


Sinai et al. arxiv 2017, Sinai et al. Biorxiv 2021, ASGCT23 Abstract #467

Multiplexing enables measurement of AAV capsid fitness landscapes

Production efficiency
of single codon substitutions
within 520-595 region of AAV2
(WT codon denoted by black dot)

worse than WT | WT | better than WT

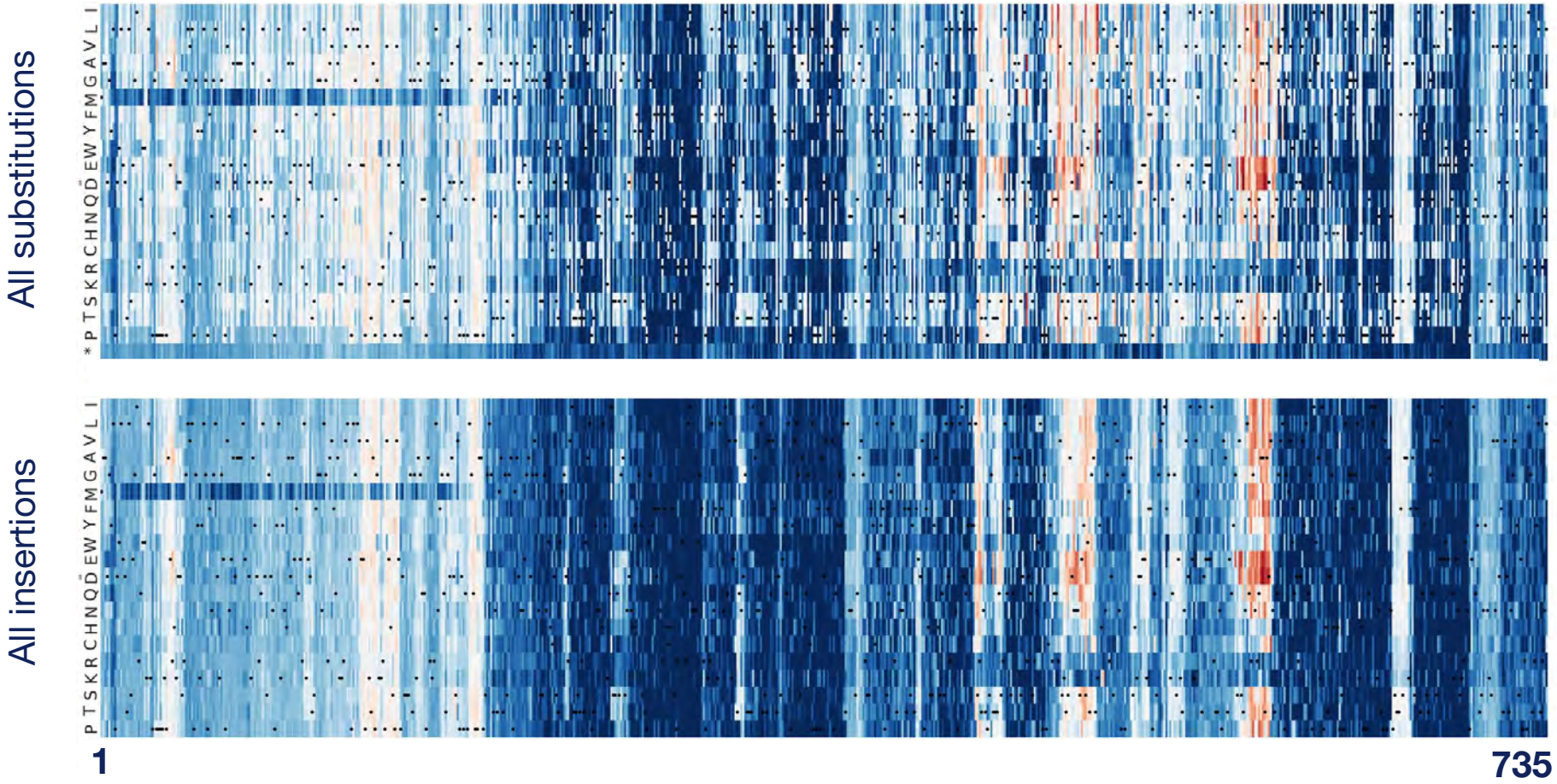


Stop codons

Ogden & Kelsic et al., Science 2019

Same data as prior slide, averaged by amino acid and position

Ogden & Kelsic et al., Science 2019

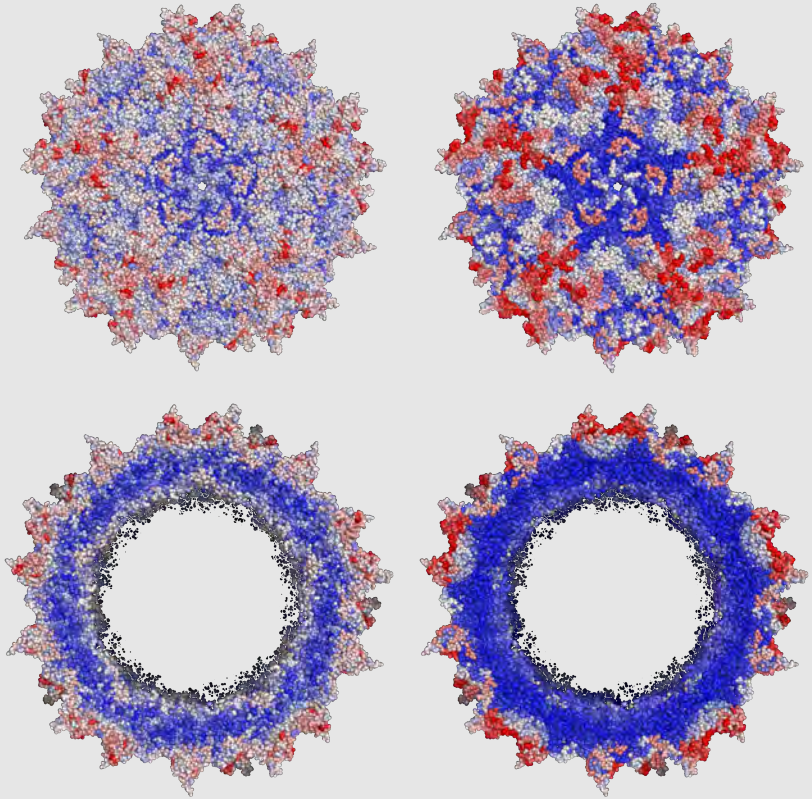


Ogden & Kelsic et al., Science 2019

Using multiplexing, in **one experiment:**

- Measured the fitness of **every possible single edit** to the AAV2 capsid sequence
- Learned **structural design principles** and more from data alone
- Discovered Membrane Associated Accessory Protein (**MAAP)**
- Provided **rich training data** for AI-powered sequence design

Substitutions **Insertions**

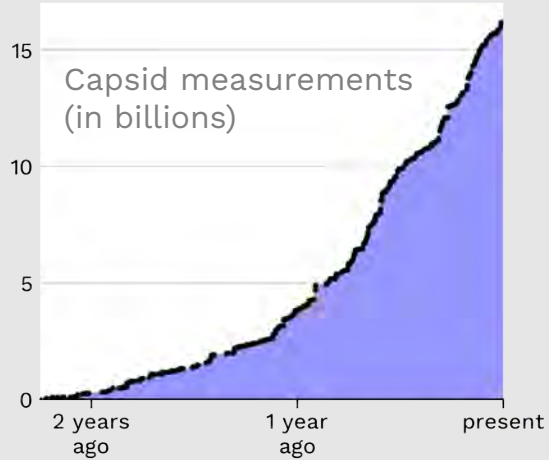


Ogden & Kelsic et al., Science 2019

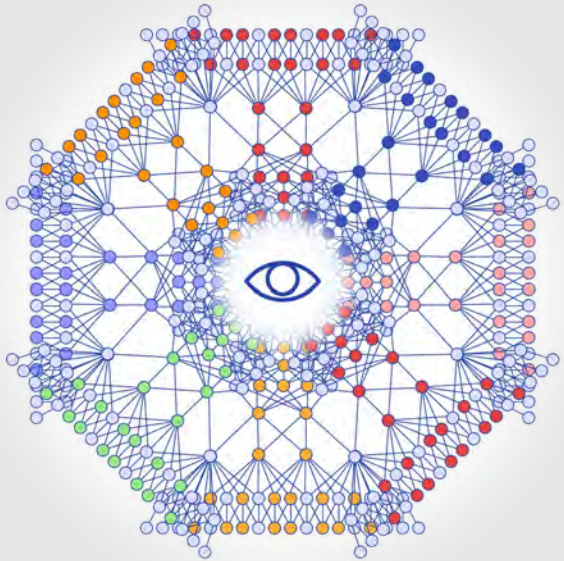
Dyno's partnerships to date...

Dyno's team has grown!

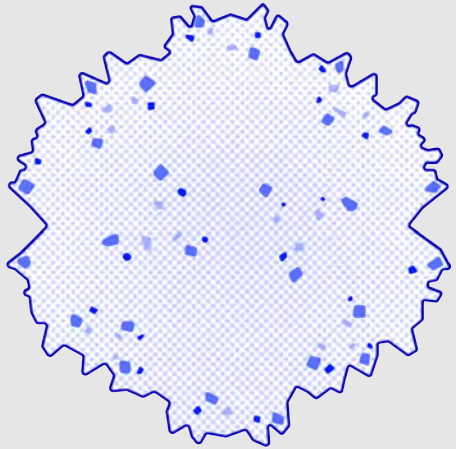
A lot has changed in 5 years



In vivo NHP Data

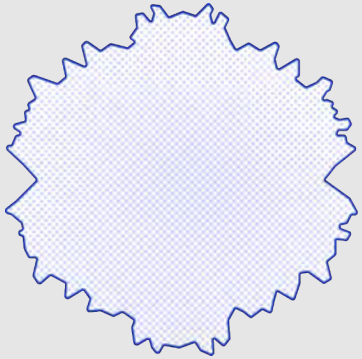


Artificial Intelligence

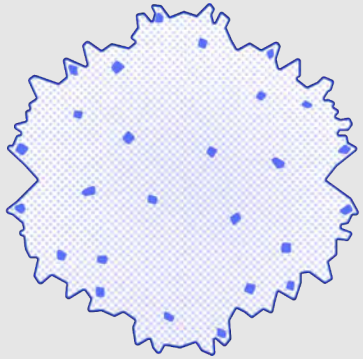


Better Capsids Are Here

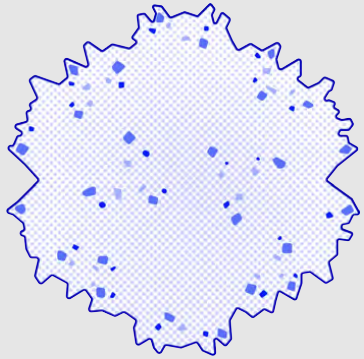
We compare capsids head-to-head



Natural capsids



External engineered capsids



Dyno capsids

Introducing Dyno’s best capsids in eye and CNS

Dyno-86m

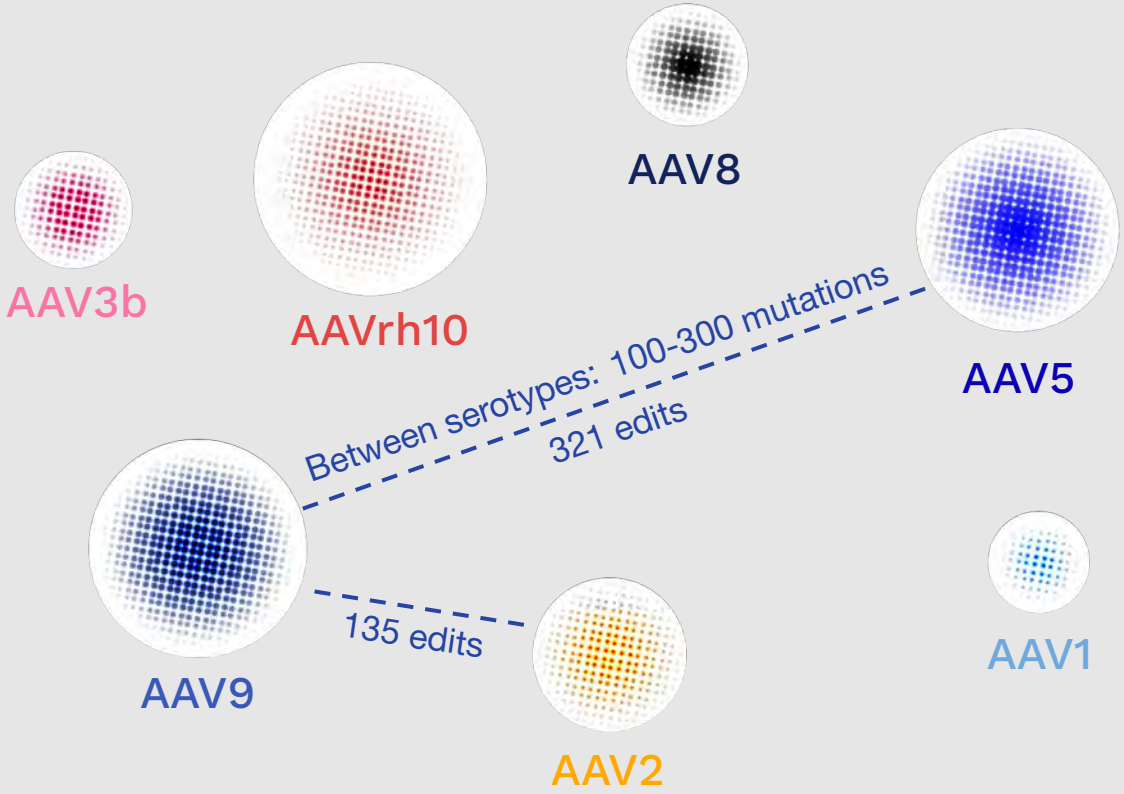
Dyno-gvk

Dyno bCap 1

Dyno-st2

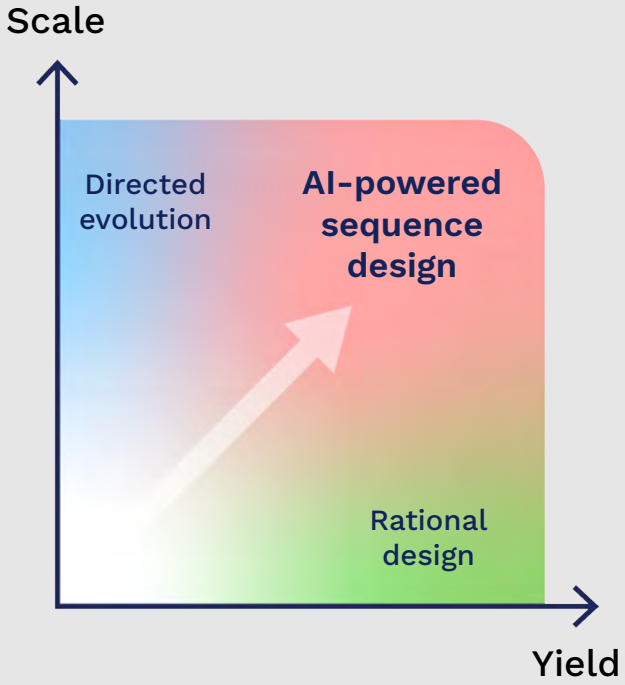

Powering Dyno’s
Platform

AAV capsid sequence space is vast and unexplored



Most AAV mutations produce non-functional Cap genes that do not assemble into stable capsids

Solving the sequence design challenge with AI-powered design



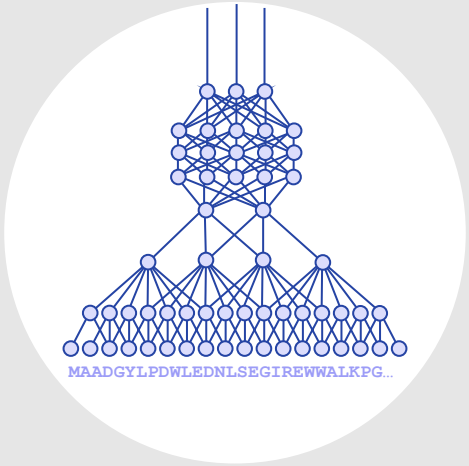
- **Directed evolution** can produce very large libraries, but most elements are non-functional due to incorporation of deleterious mutations
- **Rational design** can produce libraries with many functional elements, but their scope is hard to scale for efficient exploration of sequence space
- **Dyno solves this trade-off using AI-powered sequence design**

Overview of Dyno's Platform

Data excellence

Essential to power AI design

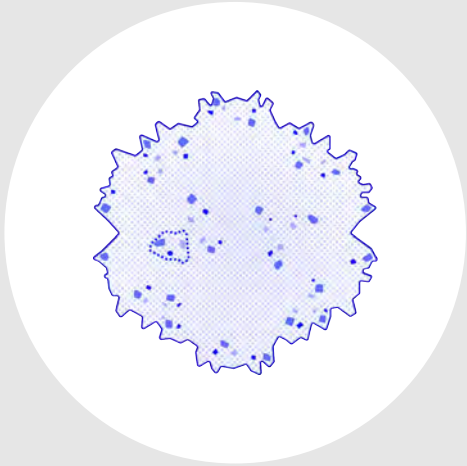
Coherence across four scales



AI excellence

Explores sequence space efficiently

Optimizes multiple properties

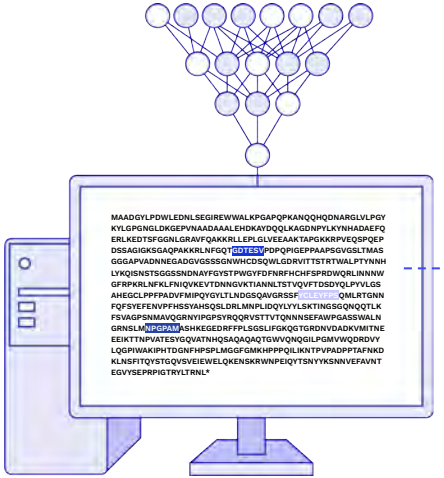


Better capsids

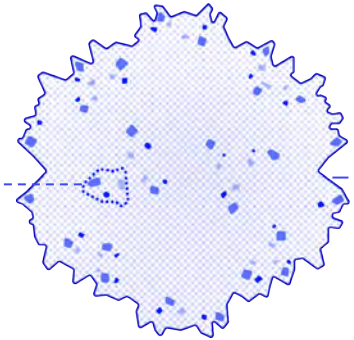
Confident comparisons of Dyno capsids to natural and external engineered capsids

Dyno’s success starts with our team

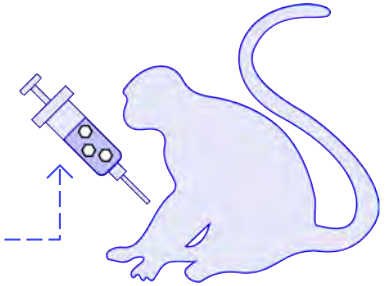
Design



Build



Test

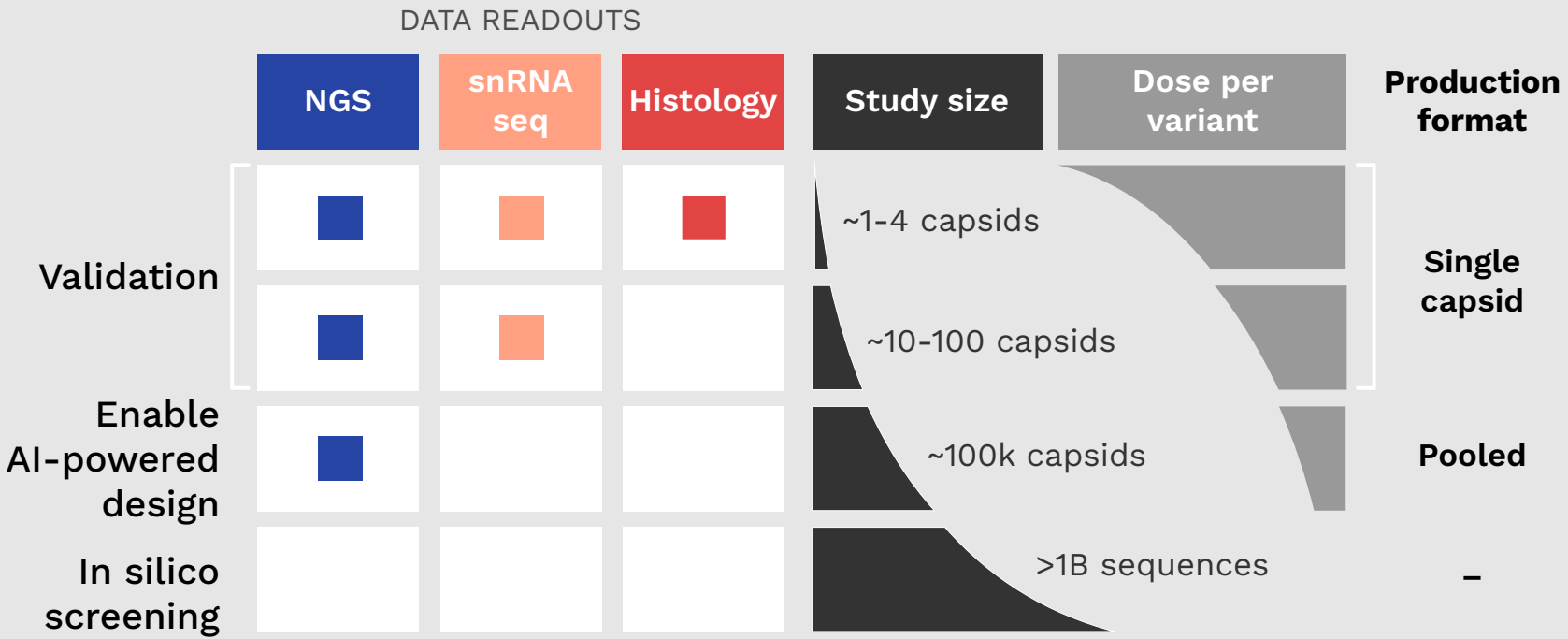


NGS

snRNA seq

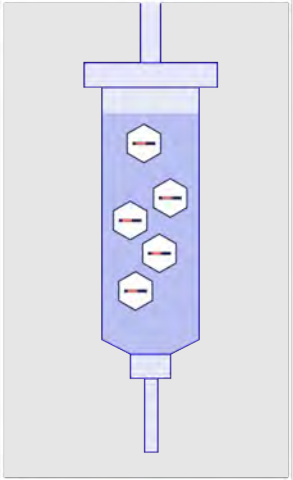
Histology

Our platform operates at four scales



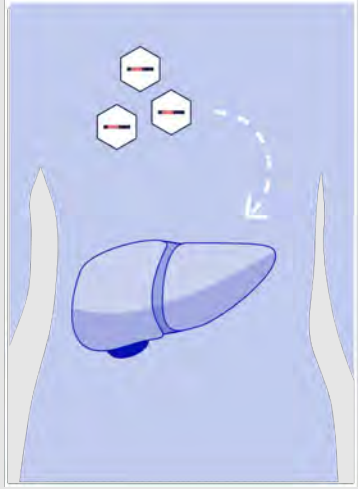
We measure key capsid properties

Production



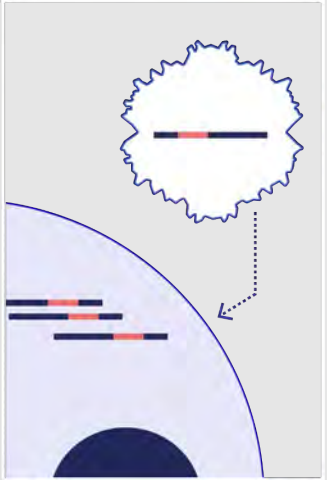
Abundance of capsid in viral test article

Biodistribution



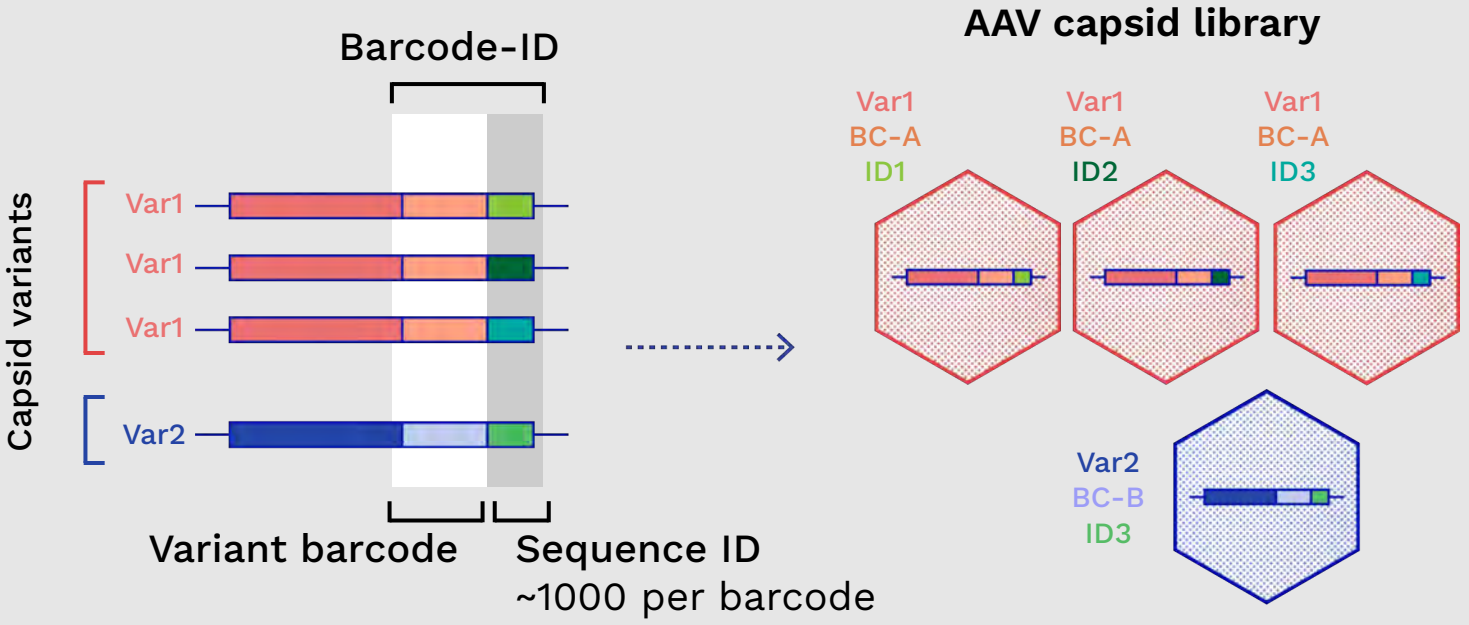
Location of capsids *in vivo* by measuring viral genome

Transduction

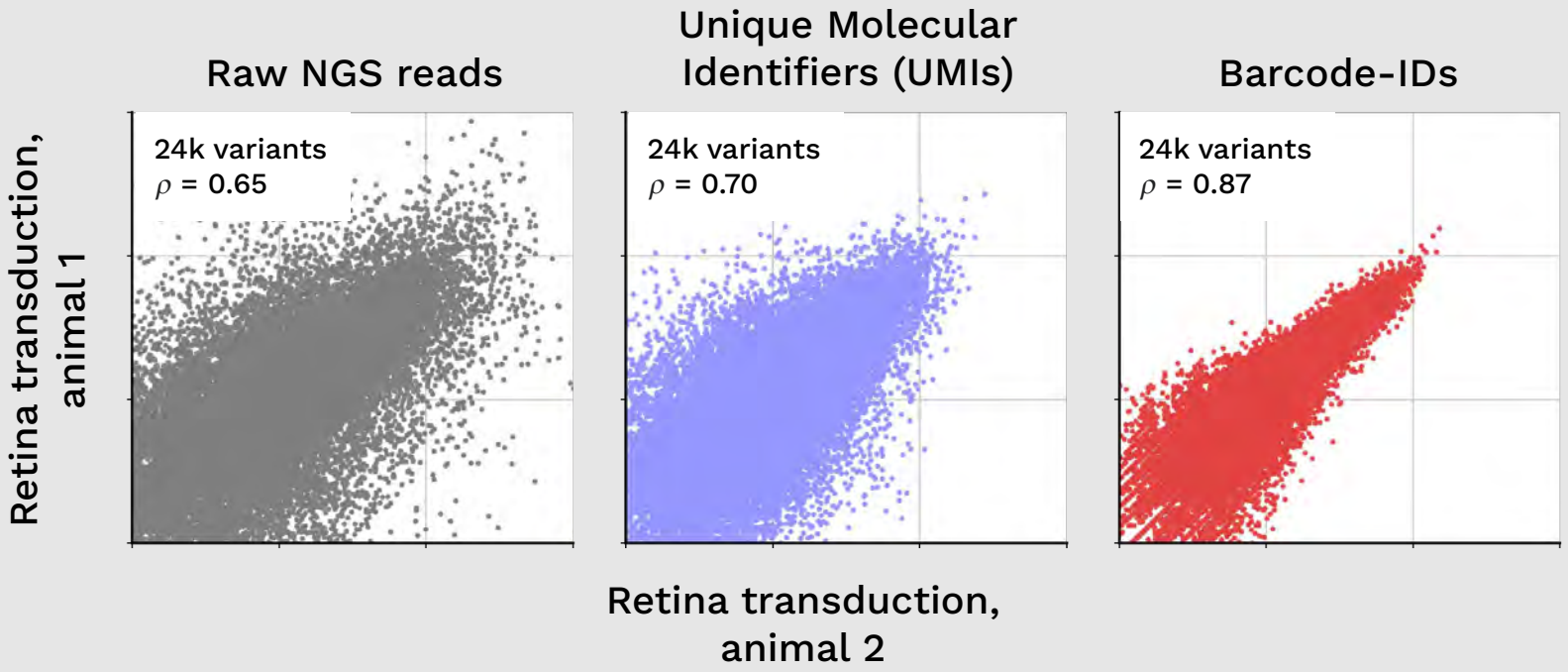


Infection of cells *in vivo* by measuring capsid-derived mRNA expression

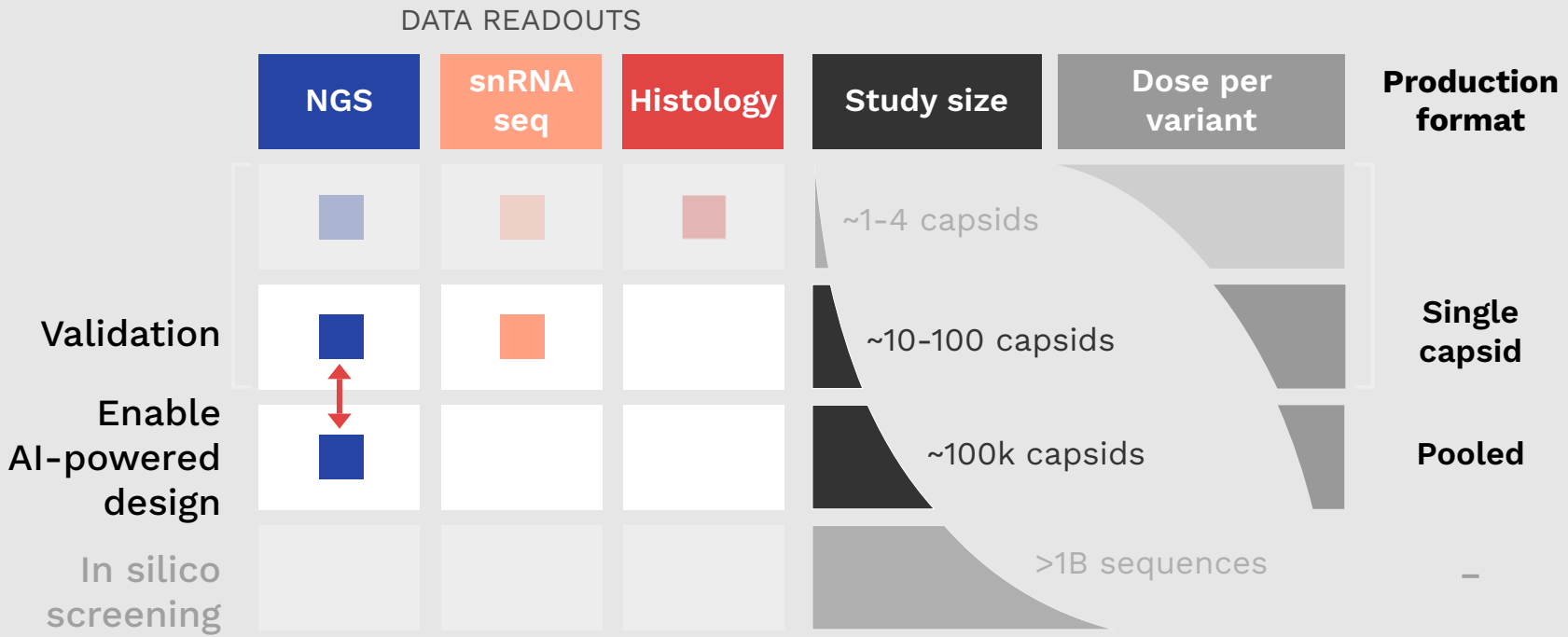
Molecular barcoding via barcode-IDs



Quantification of transduction with barcode-ids increases data reproducibility

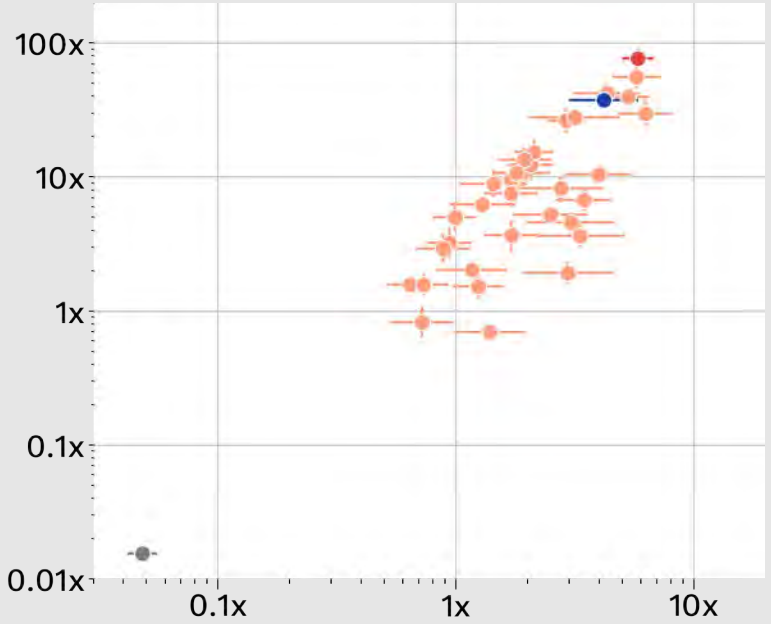


Our platform operates at four scales



Data agree across different scales

Retina transduction measured by bulk NGS, fold-change vs AAV2



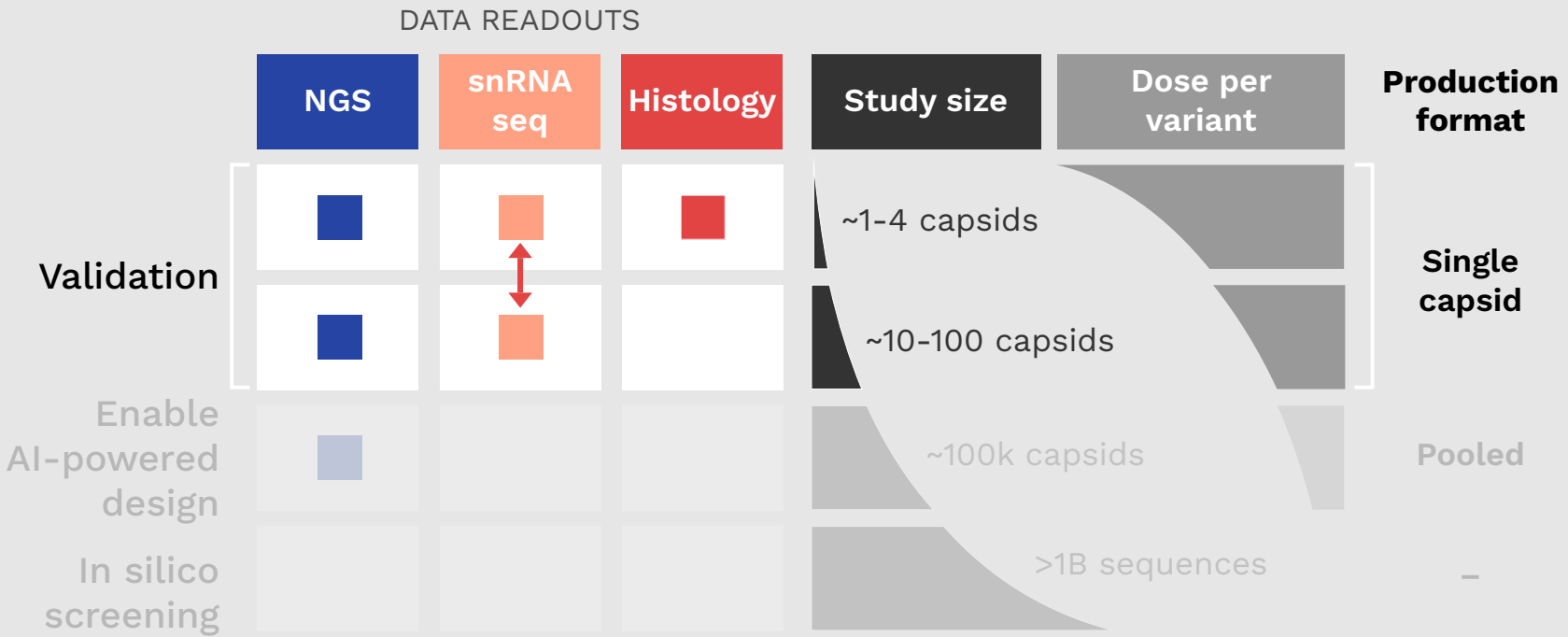
- **Dyno-86m**
- Dyno capsids
- External engineered capsid
- Negative control

Intravitreal delivery

Measured with **~10-100** capsids multiplexed

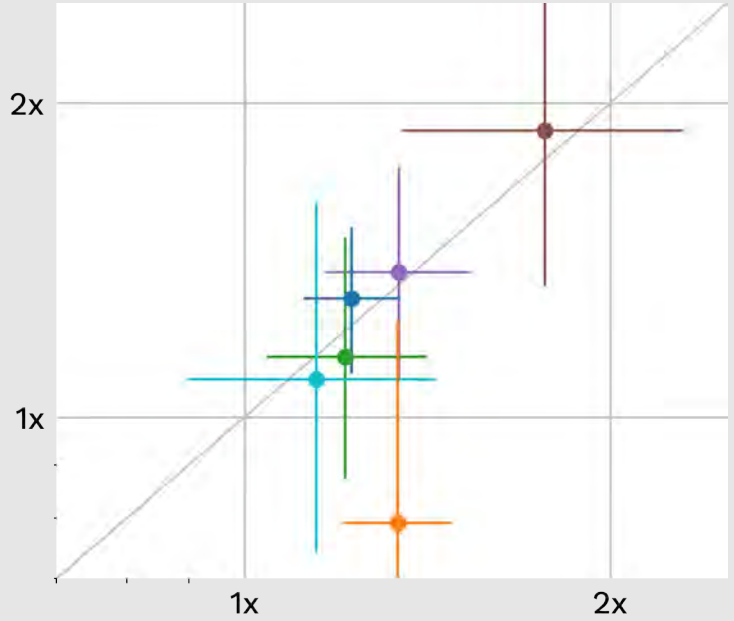
Measured with **>100,000** capsids multiplexed

Our platform operates at four scales



Single-nuclei RNA-seq data agree at different scales

Fold-change per cell type,
~10-100 capsids multiplexed



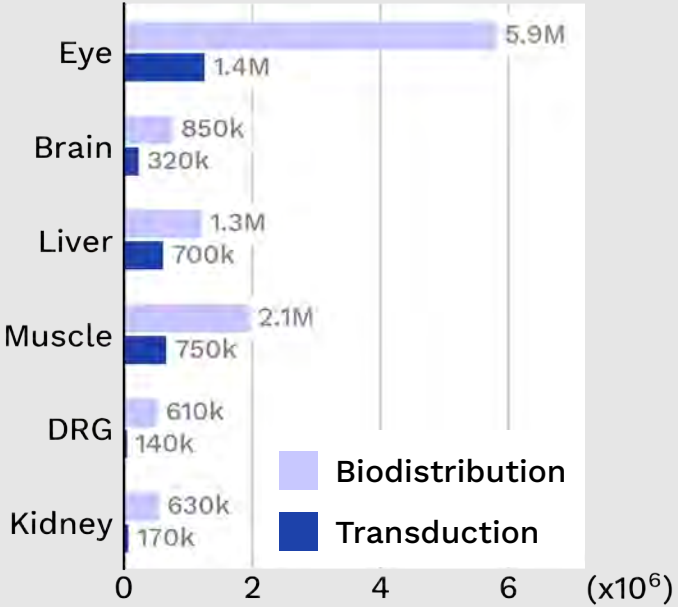
Fold-change per cell type,
2 capsids multiplexed

Fold-change of Dyno capsid relative to external engineered capsid

Intravitreal delivery

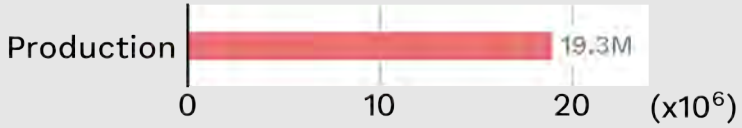
- Retinal ganglion cells
- Amacrine cells
- Rods
- Bipolar cells
- Cones
- Muller glia

Characterization of millions of AI-designed capsids



Unique AAV capsids measurements (in millions)

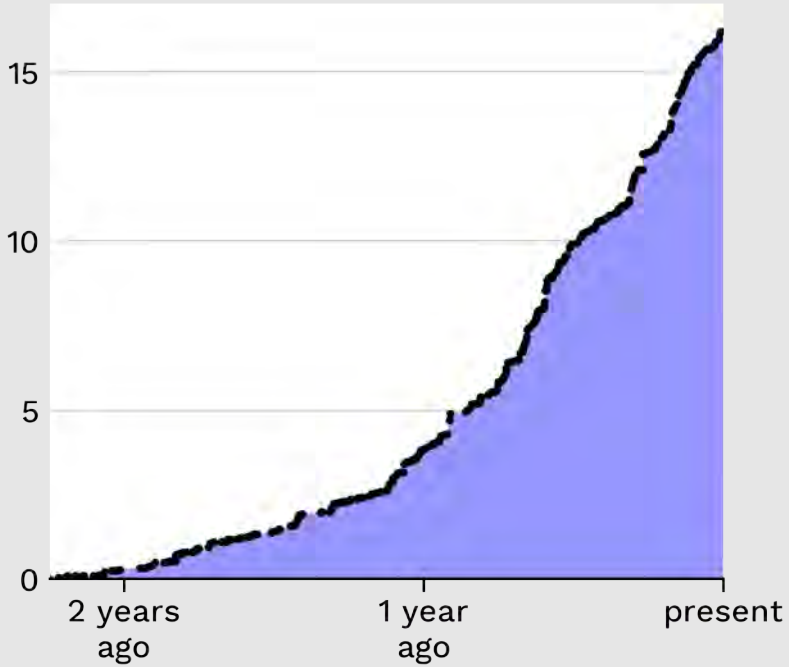
Capsids with high quality measurements that pass all quality checks but many more are detected



Unique AAV capsids measurements (in millions)

We make billions of measurements every month

Total capsid measurements (in billions)



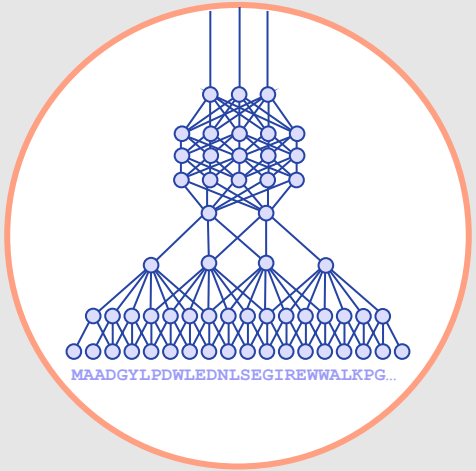
We make billions of [illegible] every month

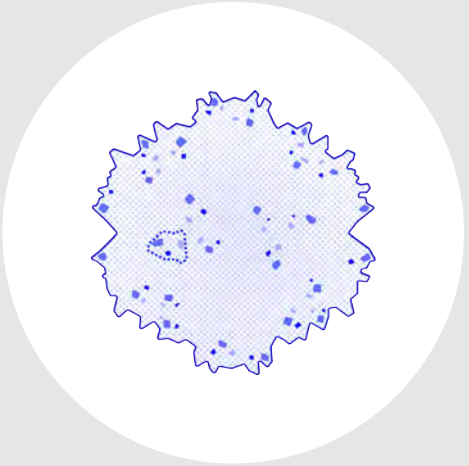
Total capsid measurements (in billions)

Abstract 887:

“Automated Micro-TFF System Streamlines Purification and Operator Time in a Lean rAAV Manufacturing Operation”

Overview of Dyno's Platform





Data excellence

Essential to power AI design

Coherence across four scales

AI excellence

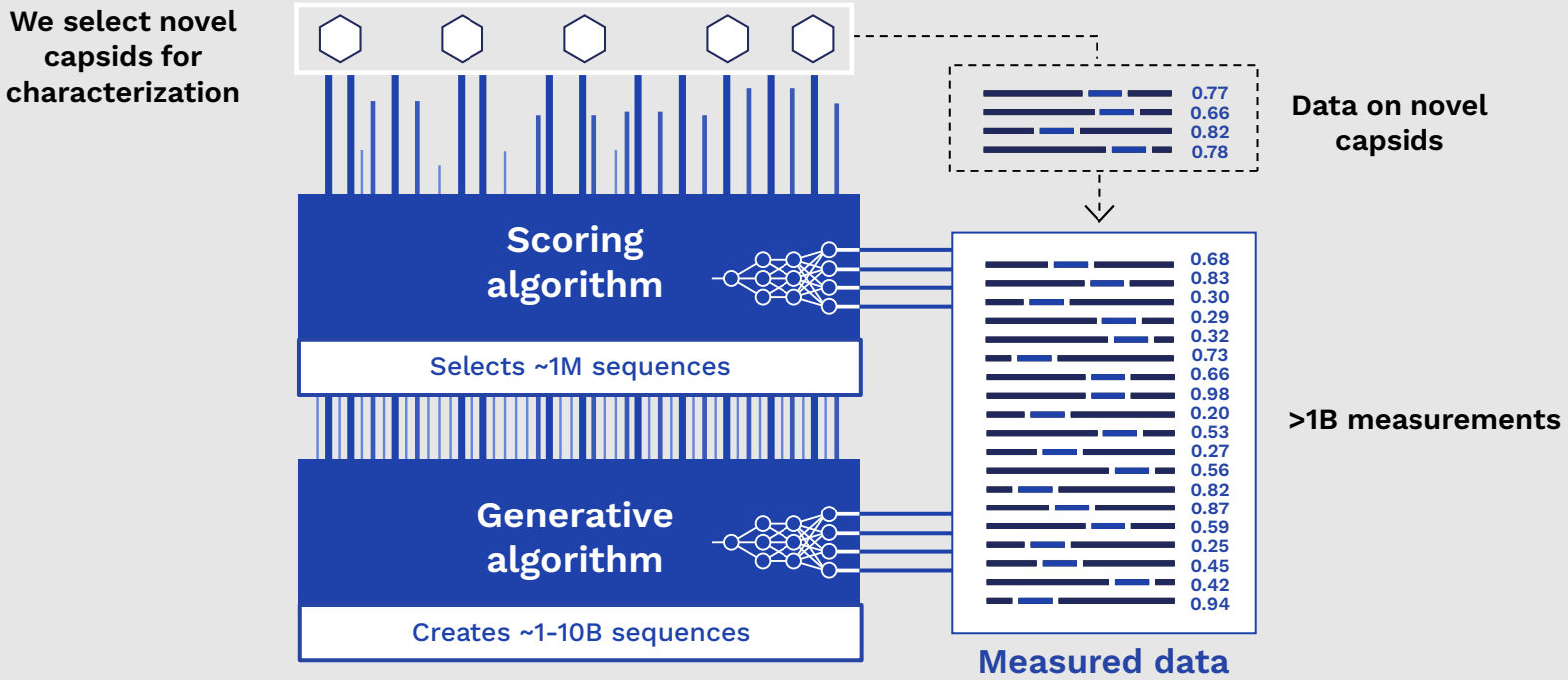
Explores sequence space efficiently

Optimizes multiple properties

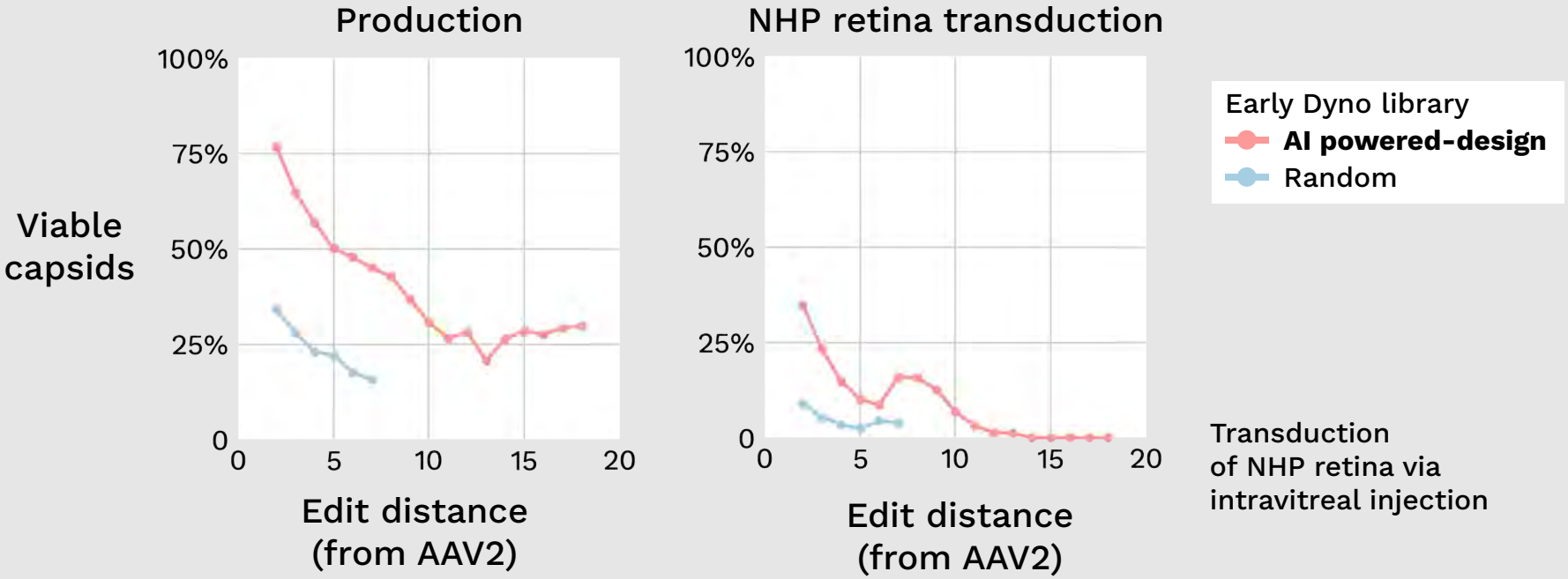
Better capsids

Confident comparisons of Dyno capsids to natural and external engineered capsids

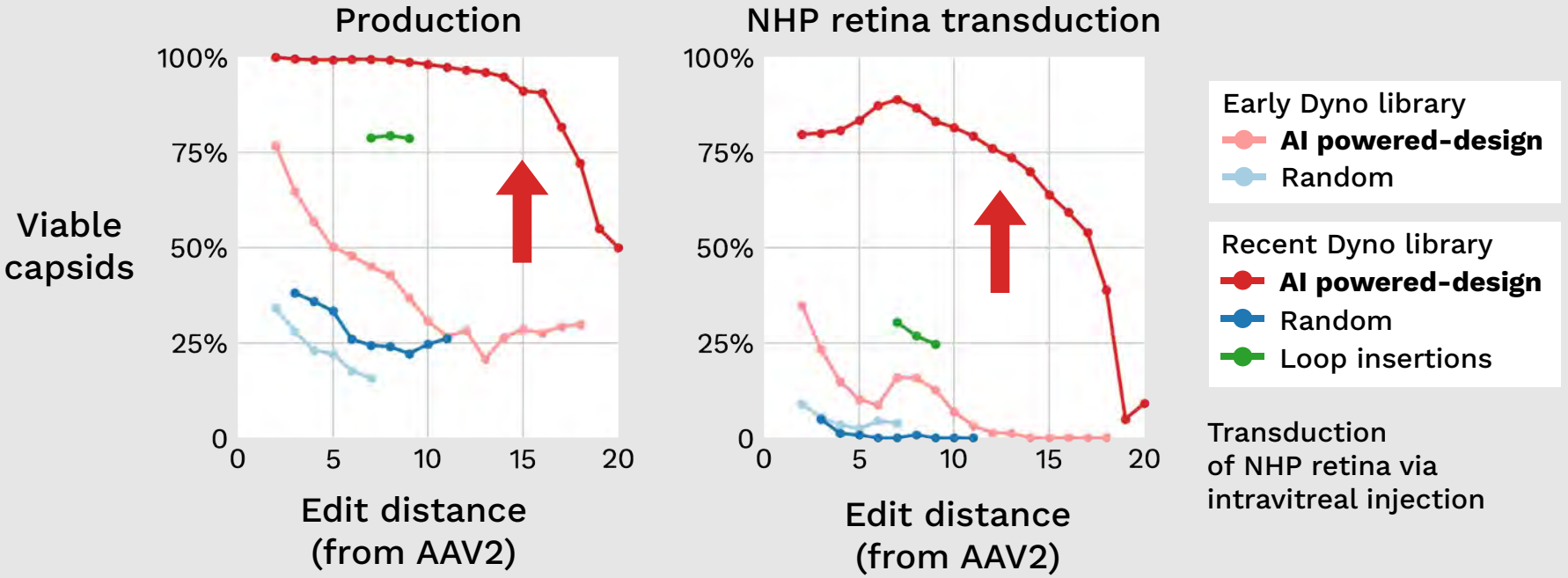
Continuous improvement of models by collection of new data



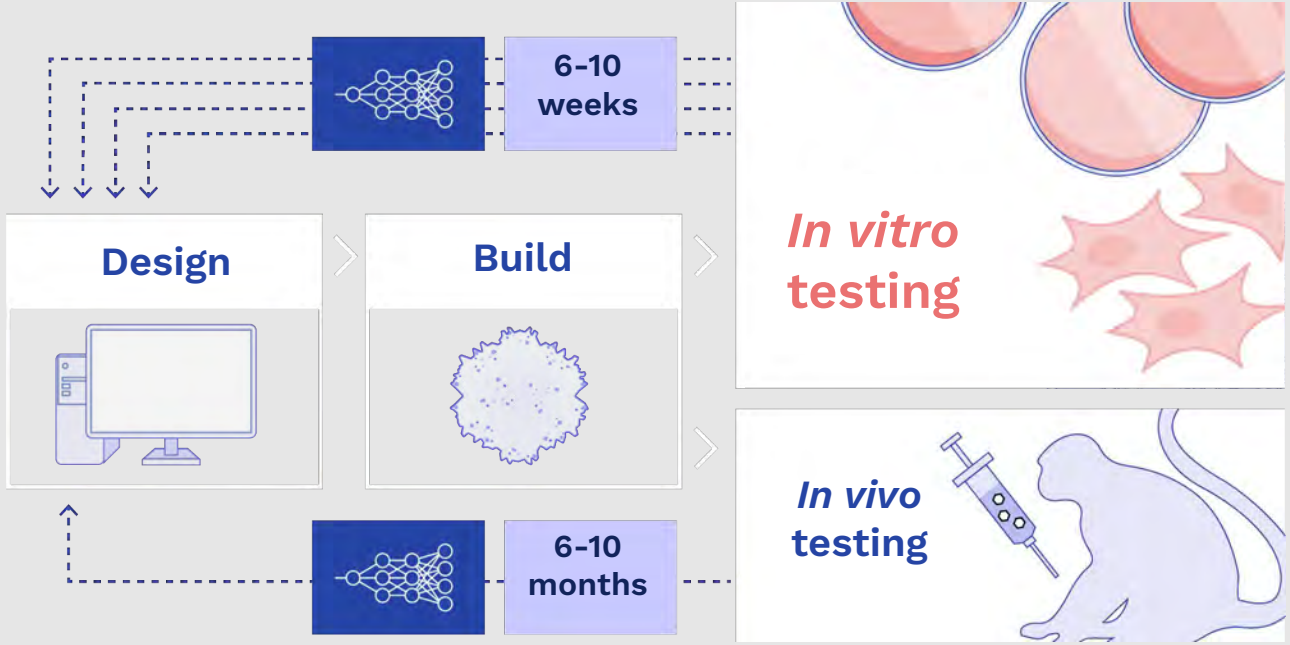
Efficient exploration of sequence space to design new variants for ocular gene delivery



Efficient exploration of sequence space to design new variants for ocular gene delivery



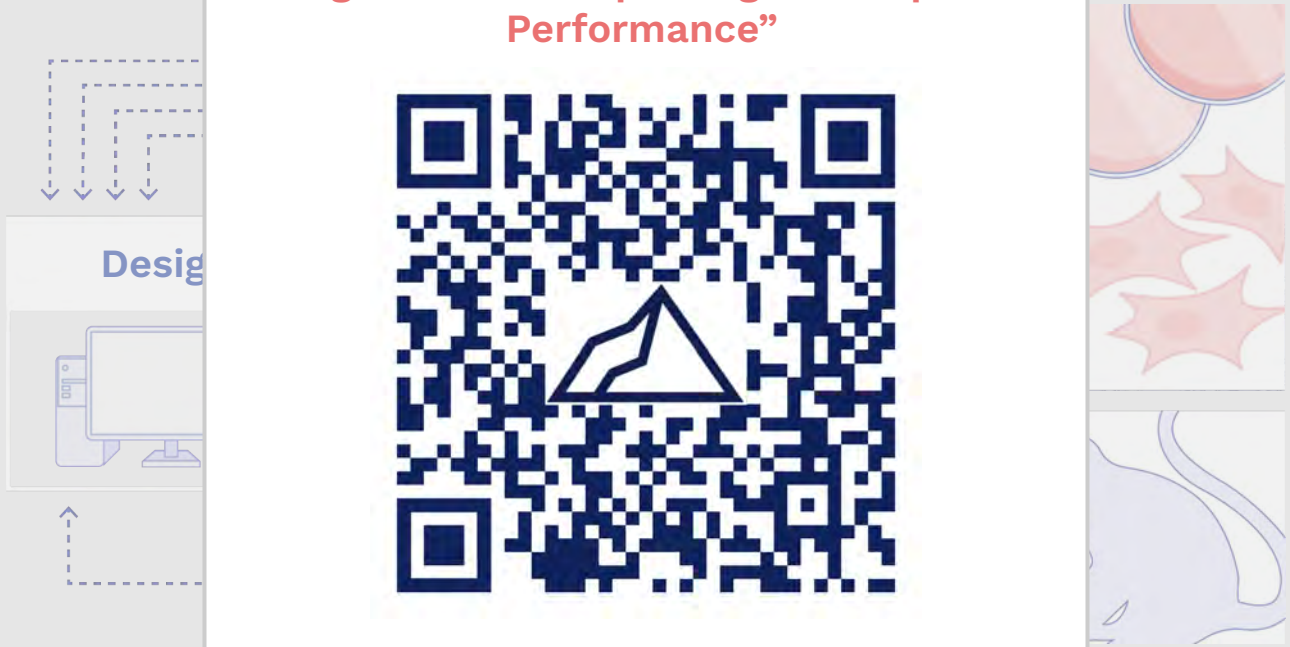
In-vitro feedback loop enables method improvement



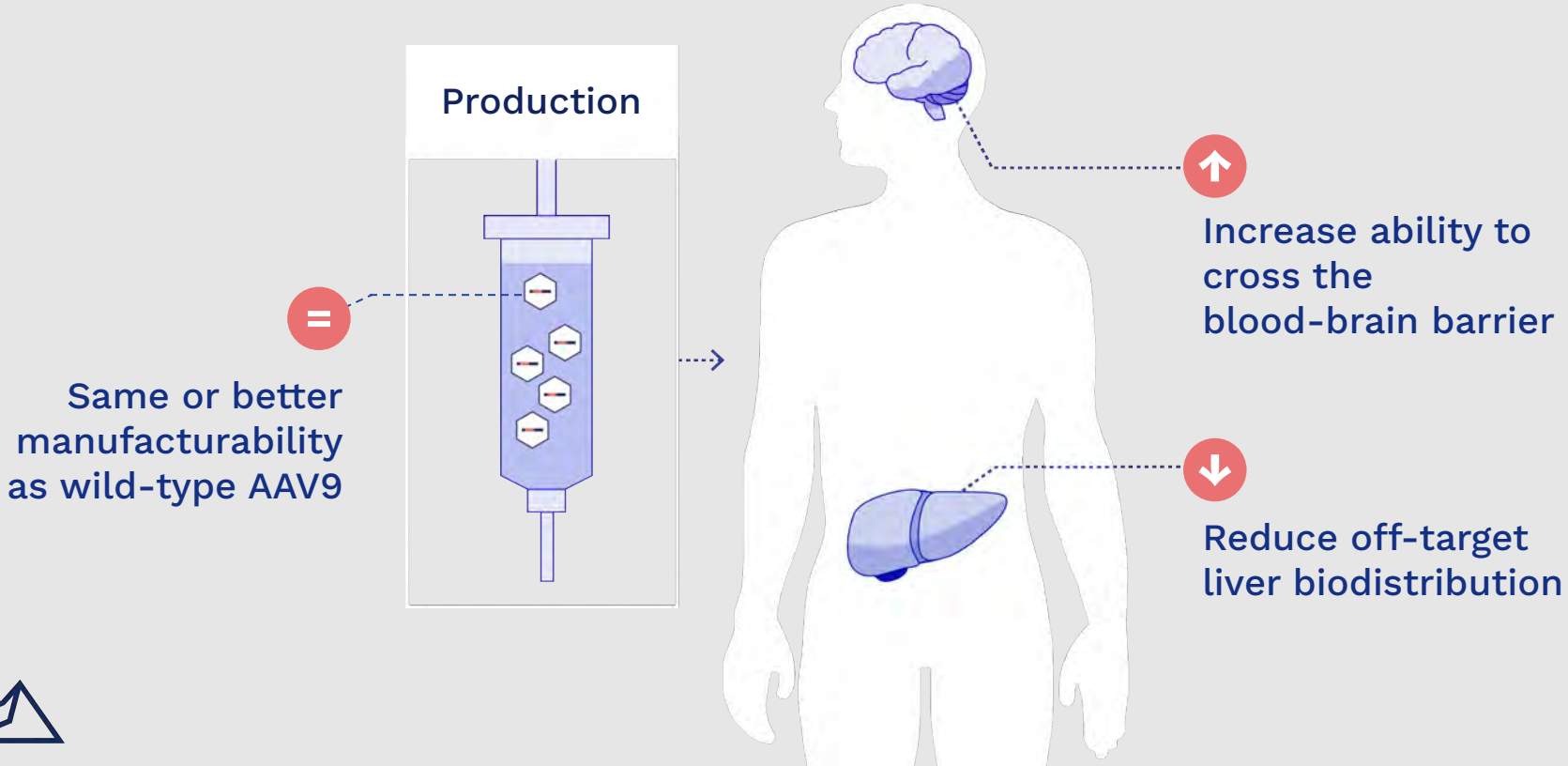
In-vitro feed mprovement

Abstract 467:

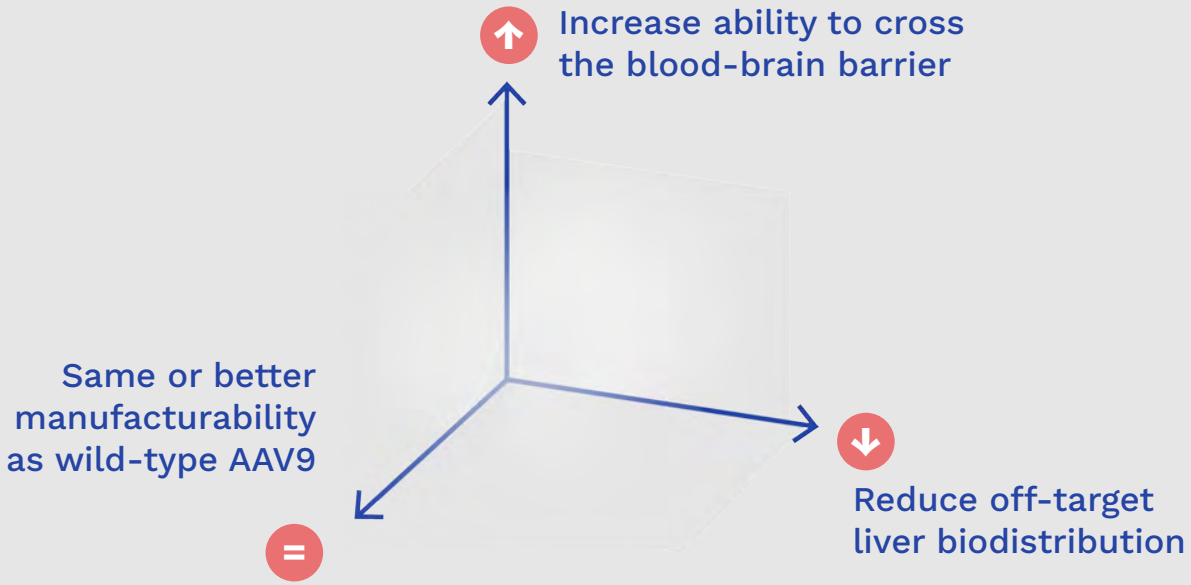
“A Robust Machine Learning Algorithm for Improving AAV Capsid Performance”



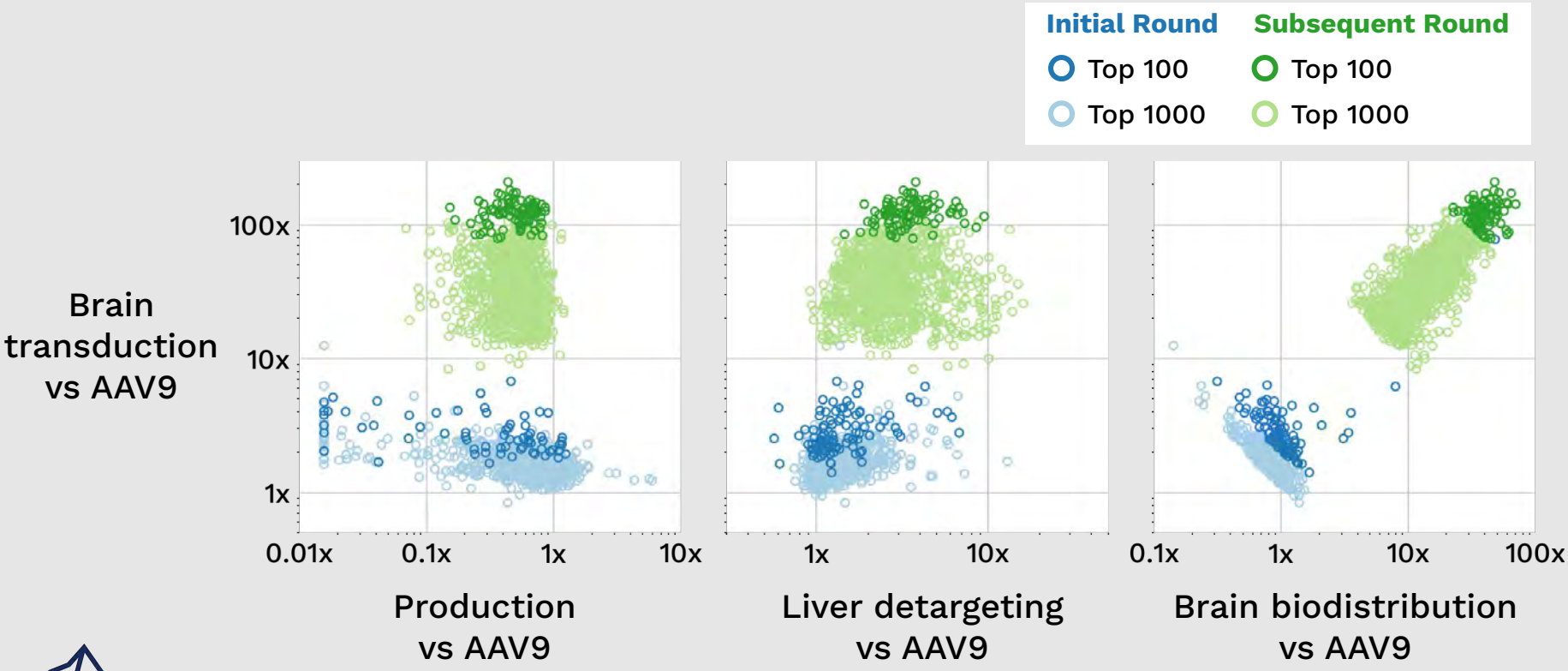
Designing capsids with improvements in multiple properties



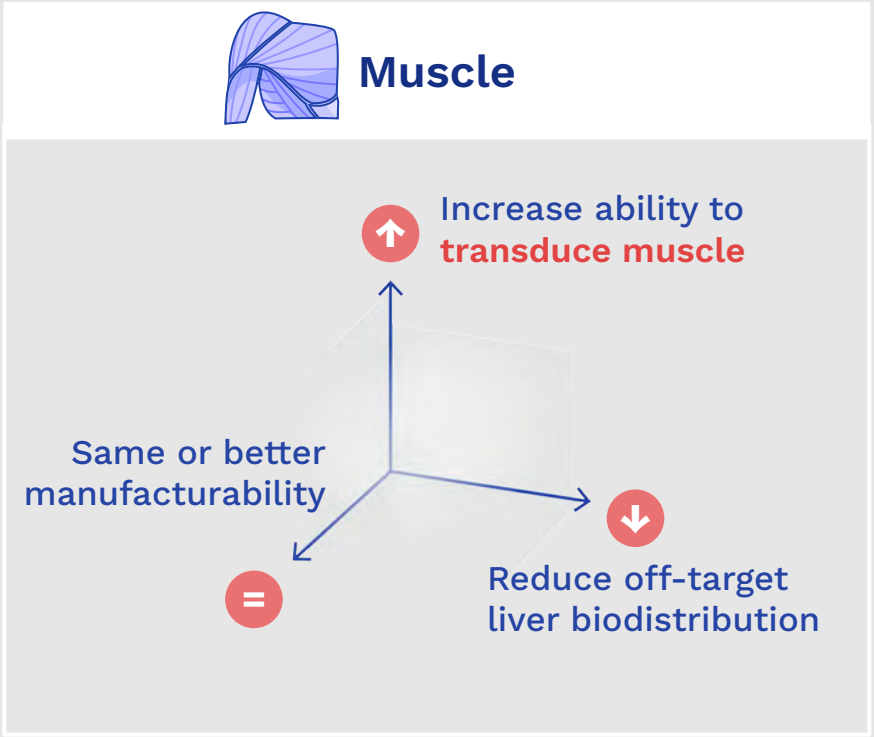
Designing capsids with improvements in multiple properties



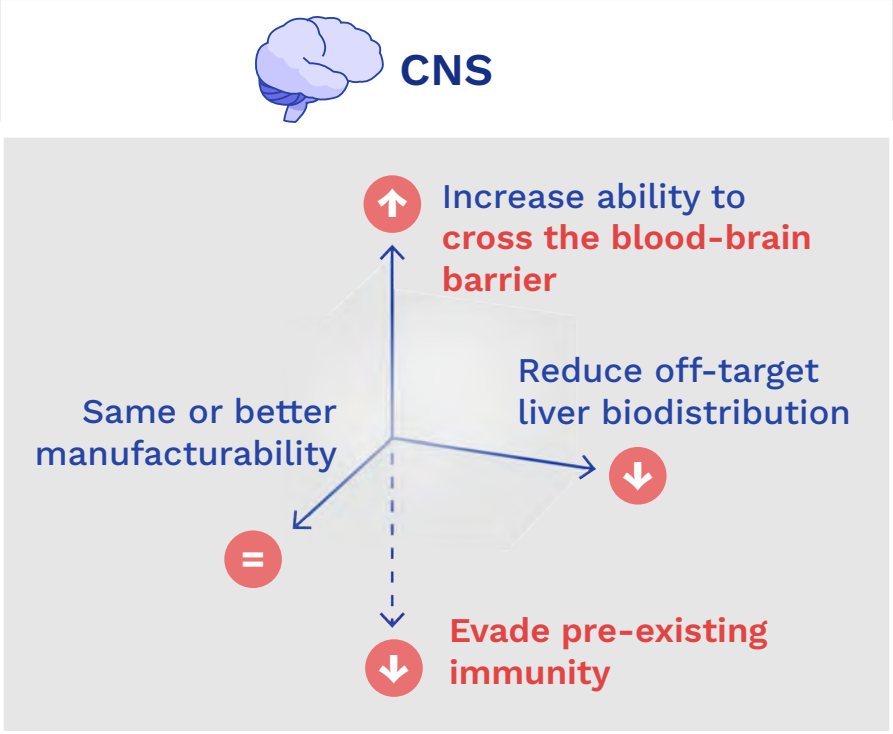
AI-powered design improves multiple AAV capsid properties relevant for CNS IV gene delivery

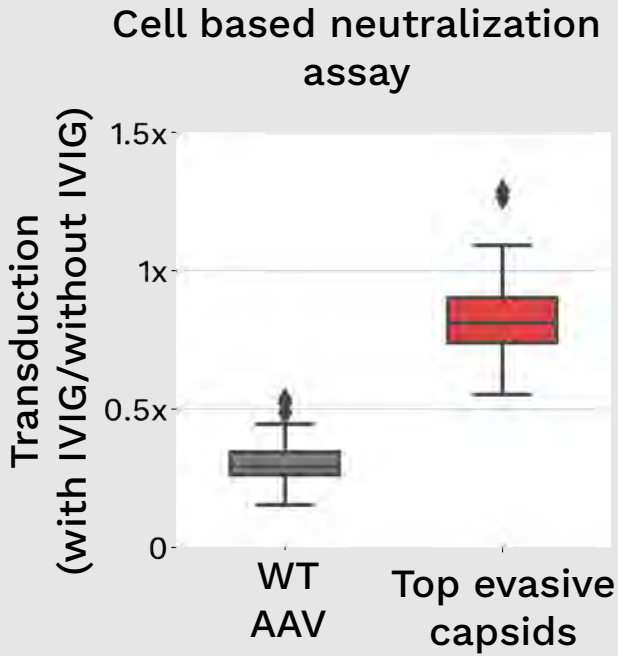


Future applications for multi-property optimization



Adding dimensions for multi-property optimization



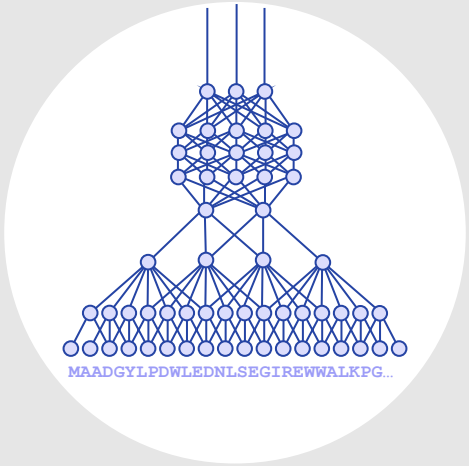


Overview of Dyno's Platform

Data excellence

Essential to power AI design

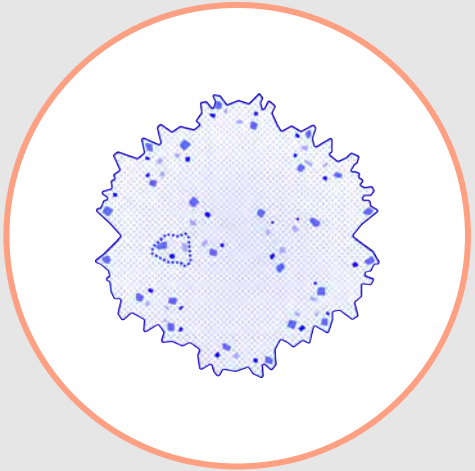
Coherence across four scales



AI excellence

Explores sequence space efficiently

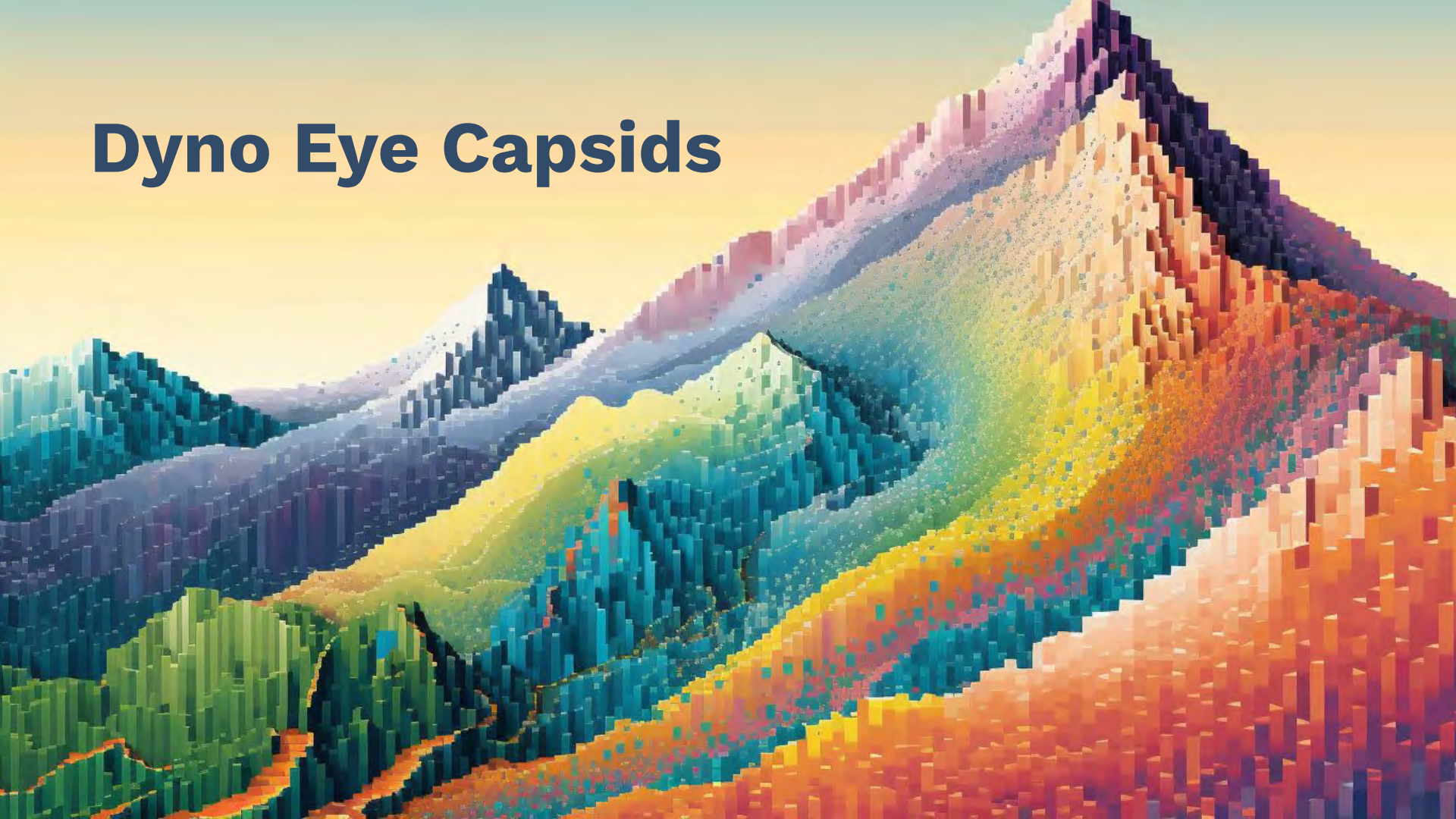
Optimizes multiple properties

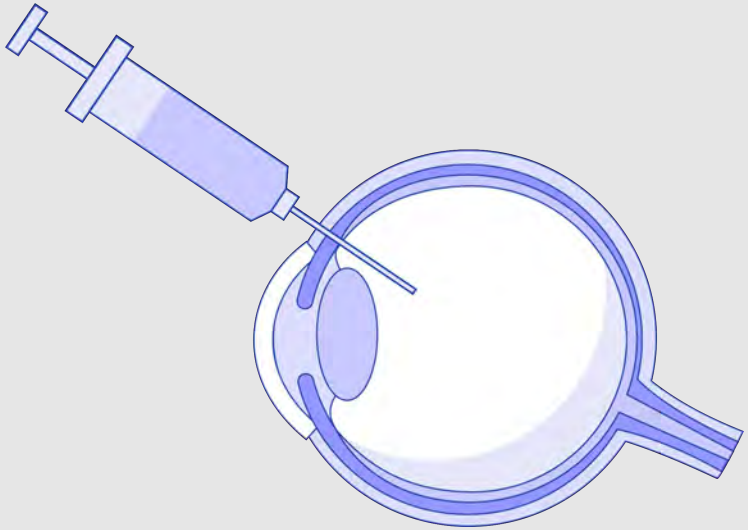


Better capsids

Confident comparisons of Dyno capsids to natural and external engineered capsids

Dyno Eye Capsids





Intravitreal (IVT) injection

Safe, non-surgical method for ocular gene therapy delivery

Minimal transduction using AAV2 intravitreal delivery

Dyno-86m

AAV capsid for **IVT delivery** designed using **Generative AI**

1x production vs AAV2

80x transduction vs AAV2

2-3x transduction vs external IVT engineered capsid

>100,000 capsids scale

Generative AI design

in vivo **NHP** measurement

>100,000 capsids scale

Generative AI design

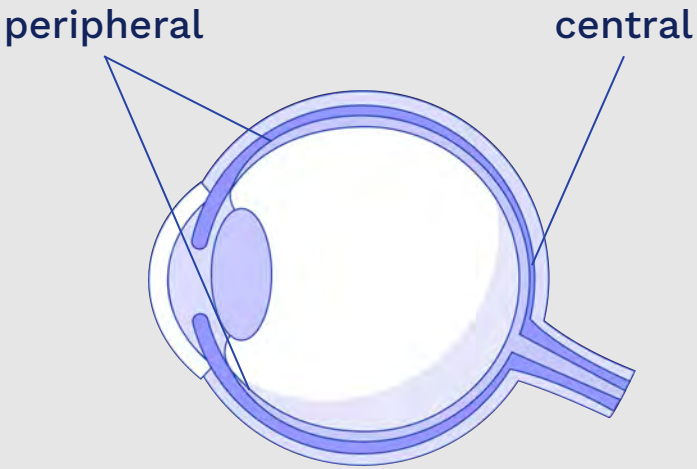
***in vivo* NHP** measurement

1-100 capsids scale

***in vivo* NHP validation**

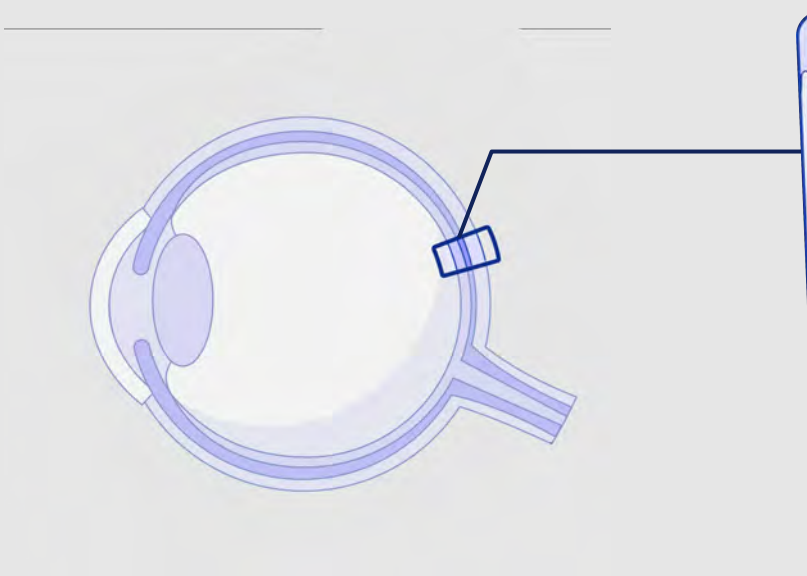
Spatial localization

Where along retina?

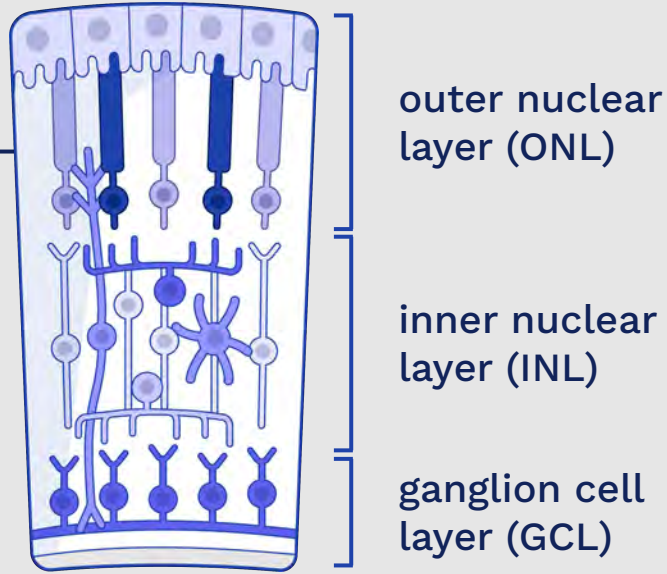


Spatial localization

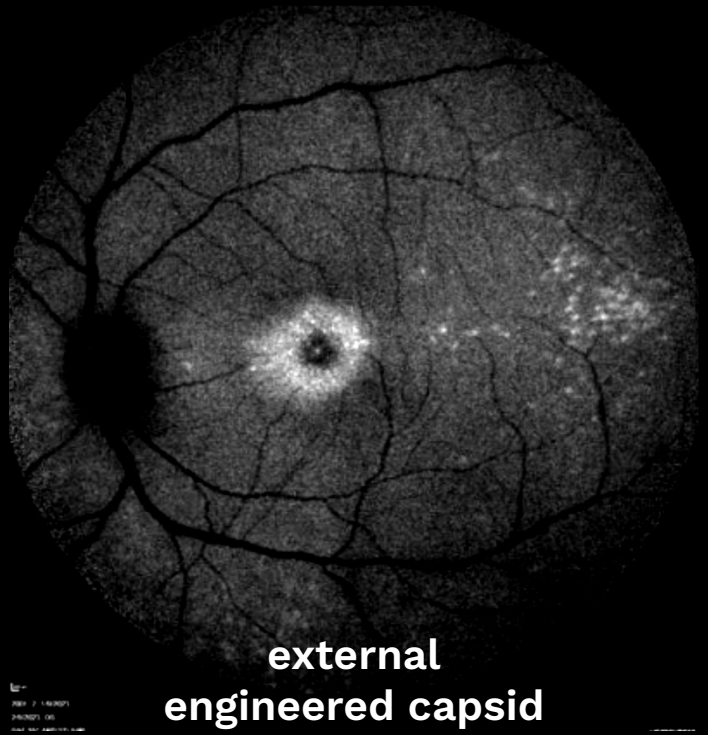
Where along retina?



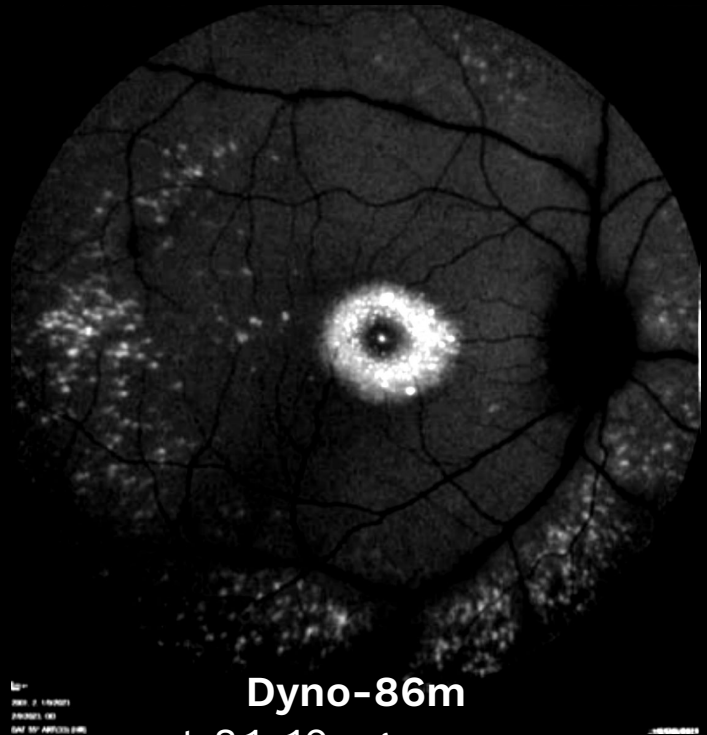
Which retinal layers?



Fluorescent fundus imaging confirms widespread delivery in single capsid delivery



external engineered capsid
at 1.1e11 vg per eye

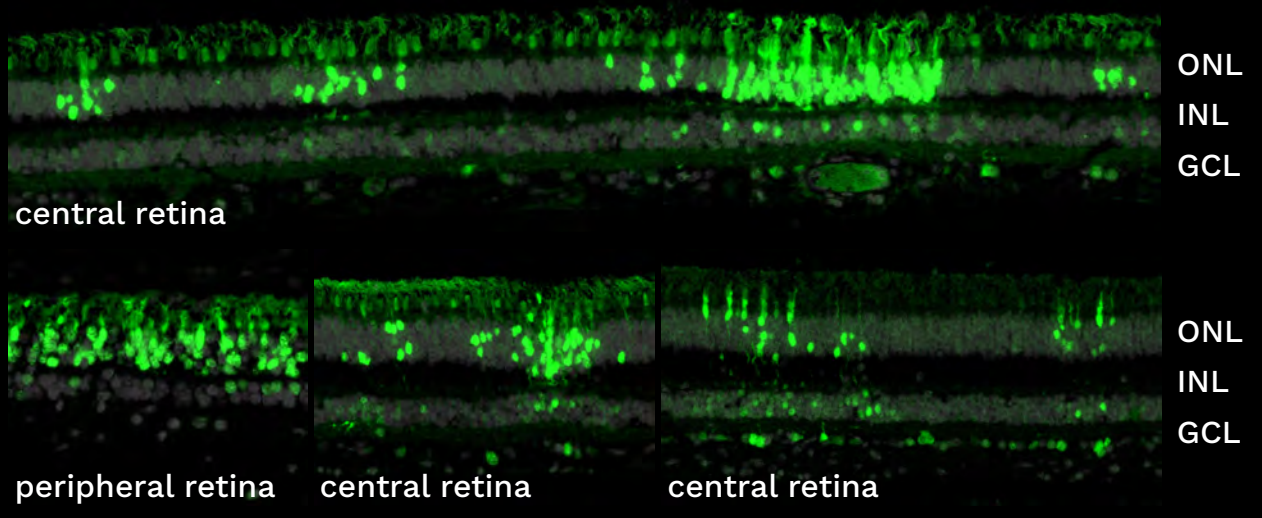


Dyno-86m
at 8.1e10 vg per eye

validation

Dyno-86m

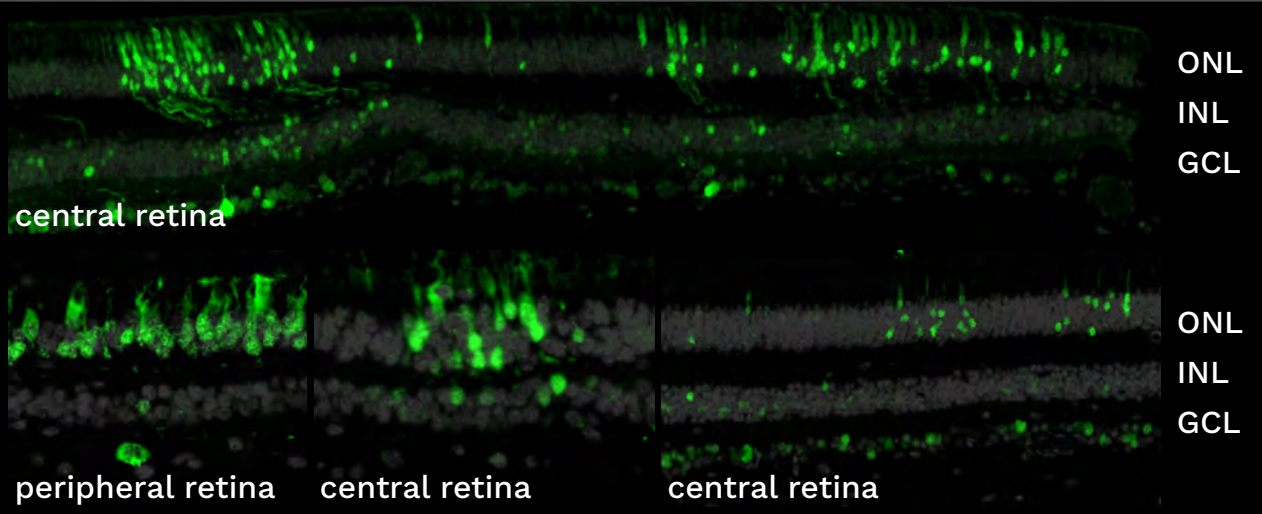
at 2.3e11 vg per eye



comparison

external engineered capsid for IVT delivery

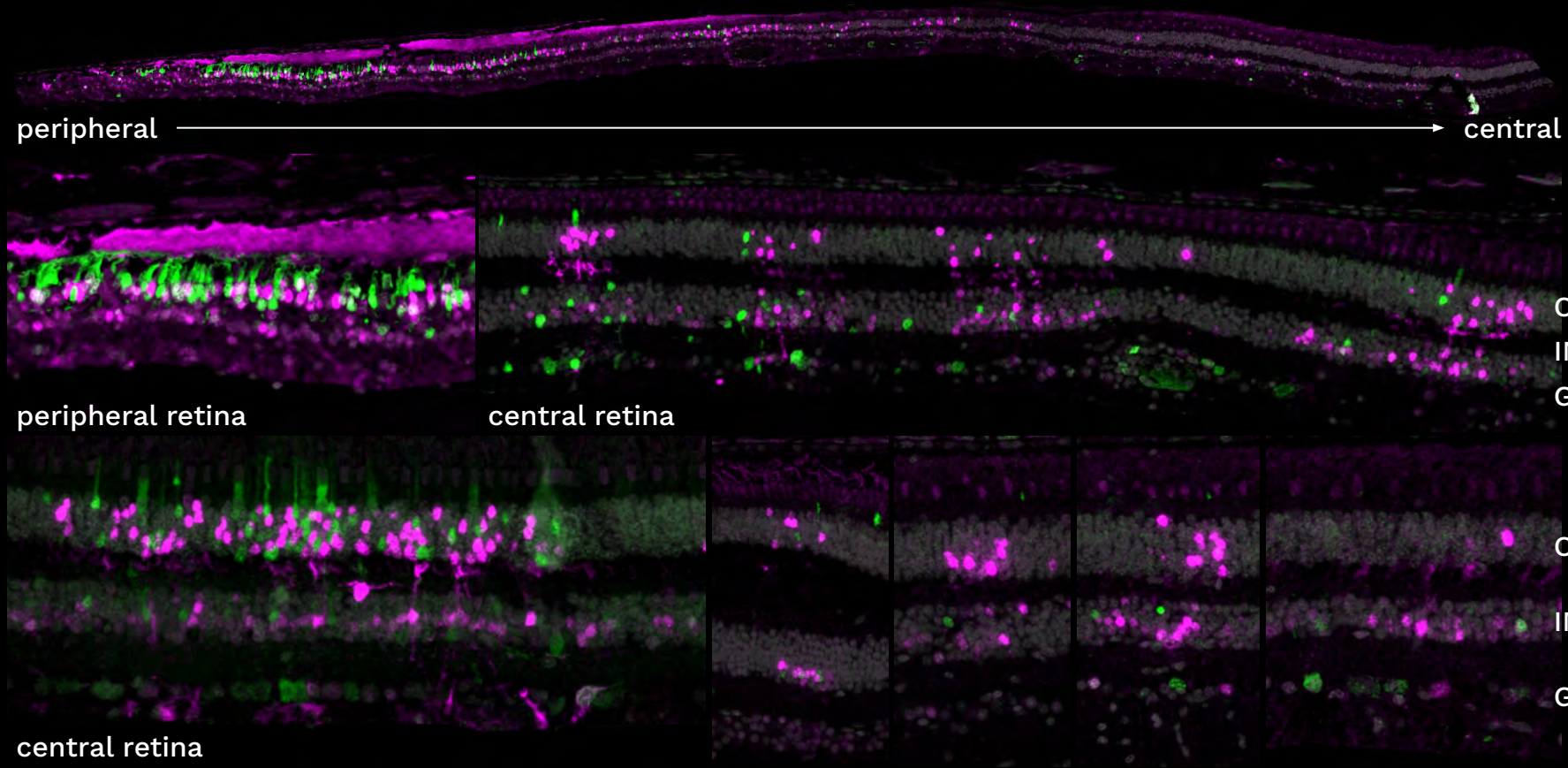
at 2.1e11 vg per eye



Head-to-head comparison

2 capsids co-injected

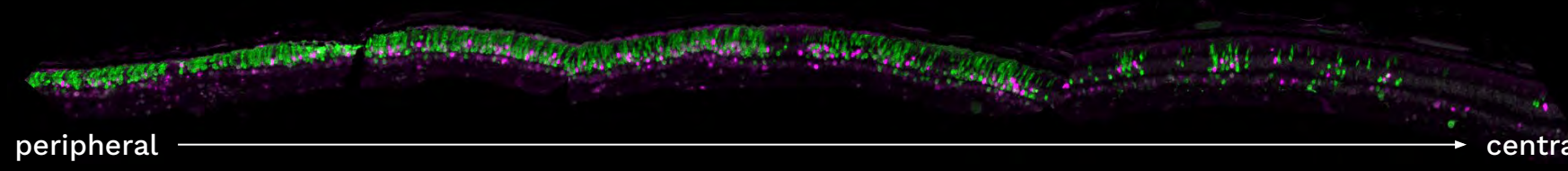
Dyno-86m capsid (8.1e10 vg) +
external engineered capsid (9.3e10 vg)

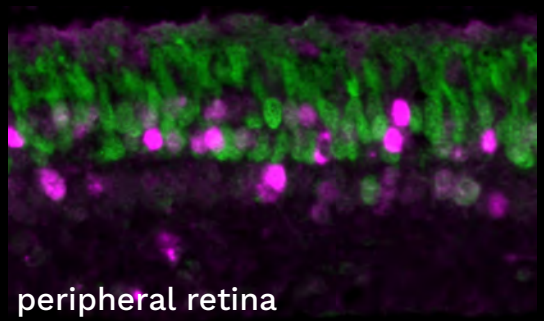


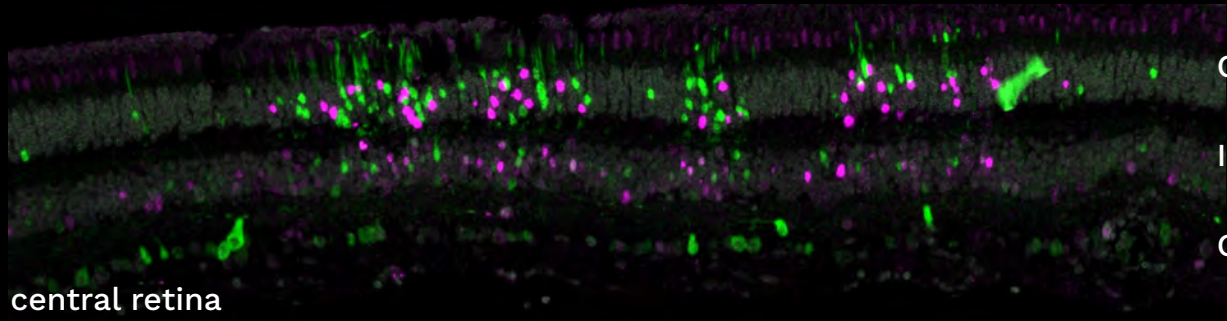
Head-to-head comparison

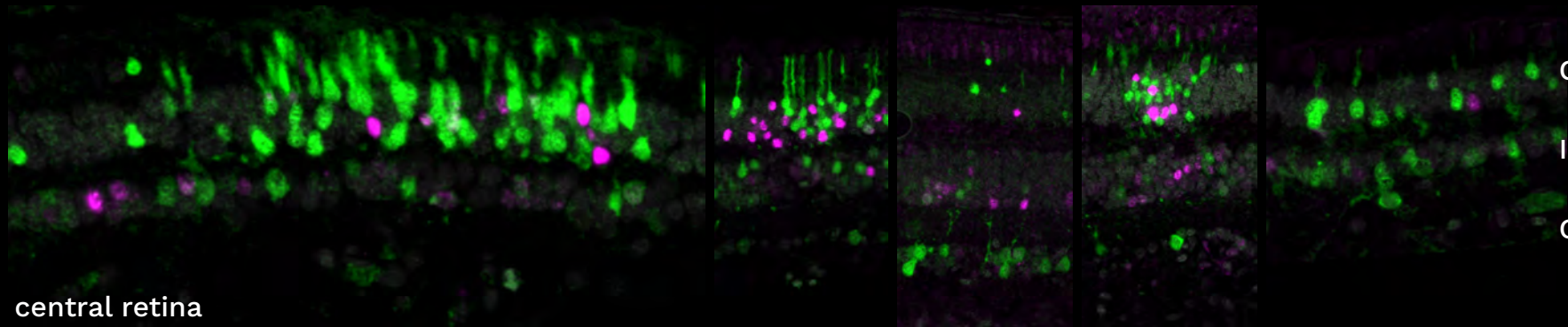
2 capsids co-injected, reporter swap

Dyno-86m capsid (7.8e10 vg) +
external engineered capsid (1.1e11 vg)





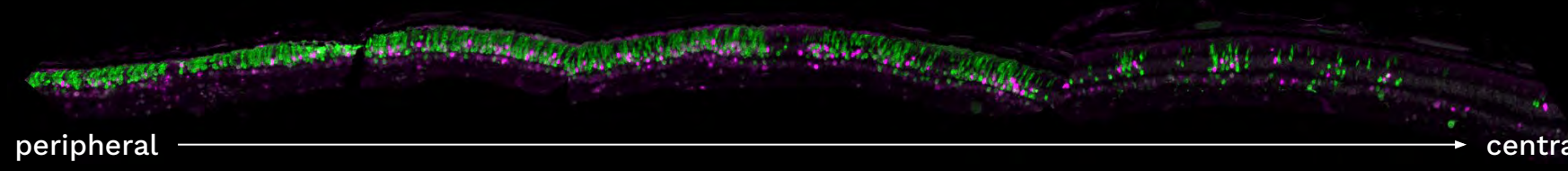


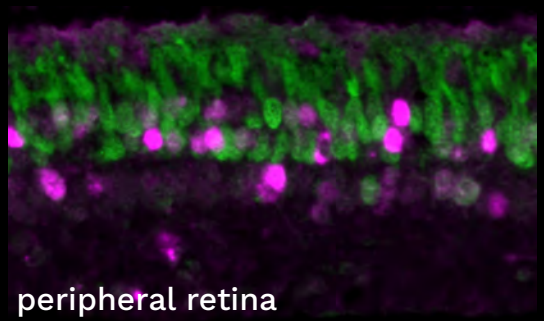


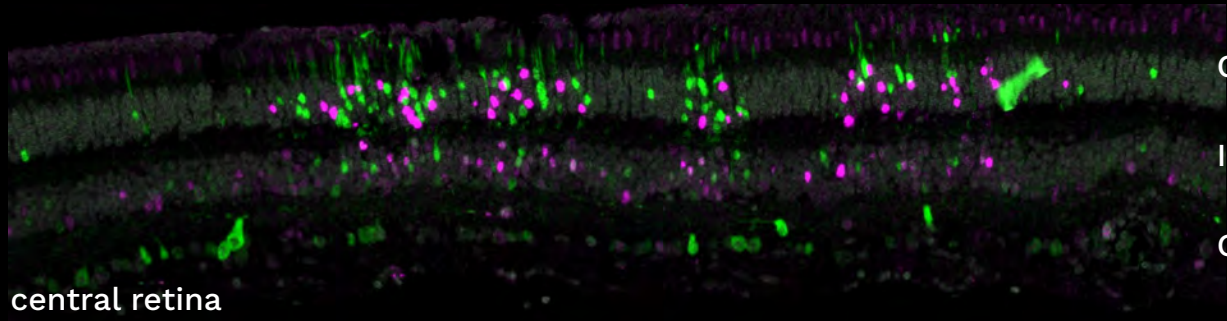
Head-to-head comparison

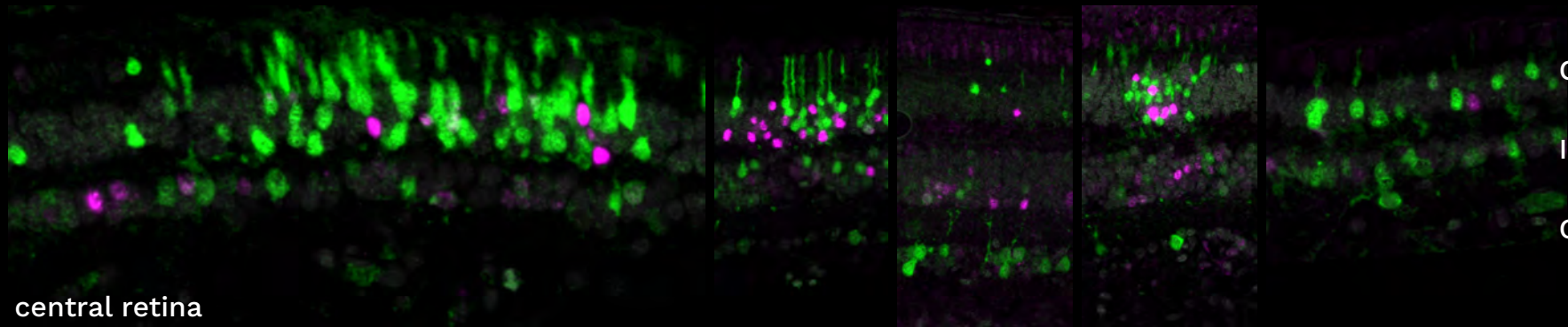
2 capsids co-injected, reporter swap

Dyno-86m capsid (7.8e10 vg) +
external engineered capsid (1.1e11 vg)





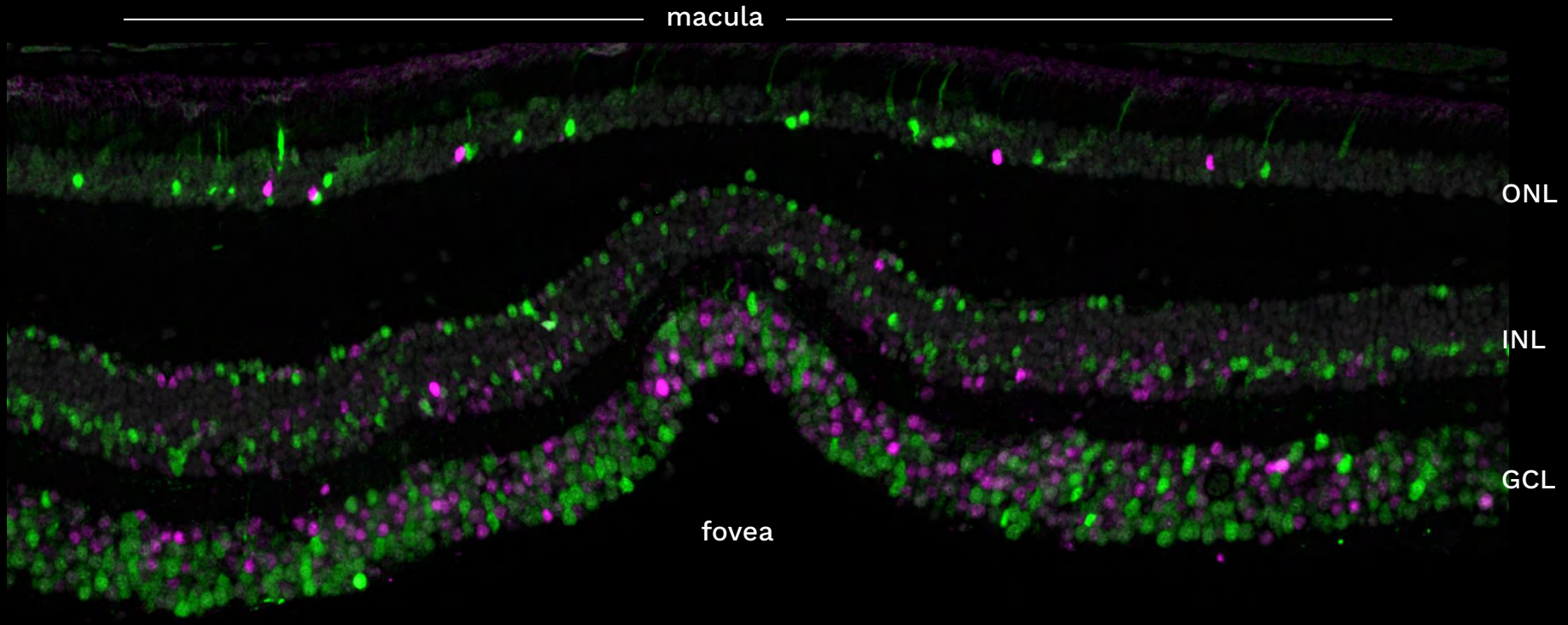




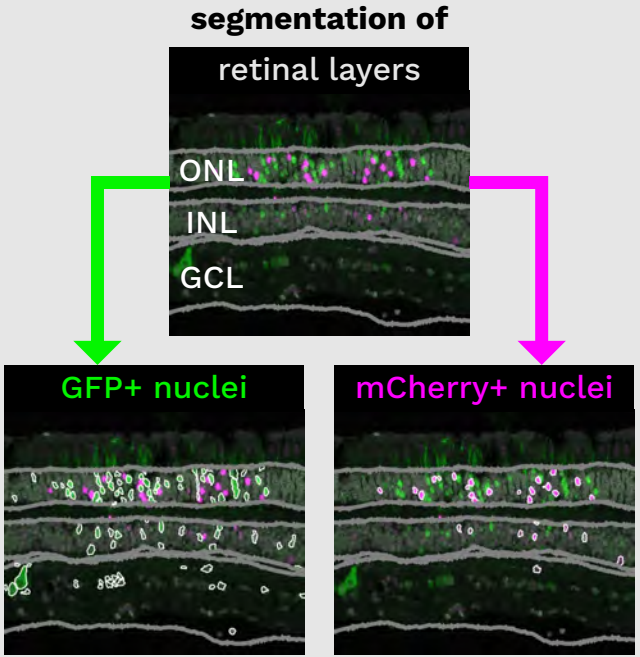
Head-to-head comparison

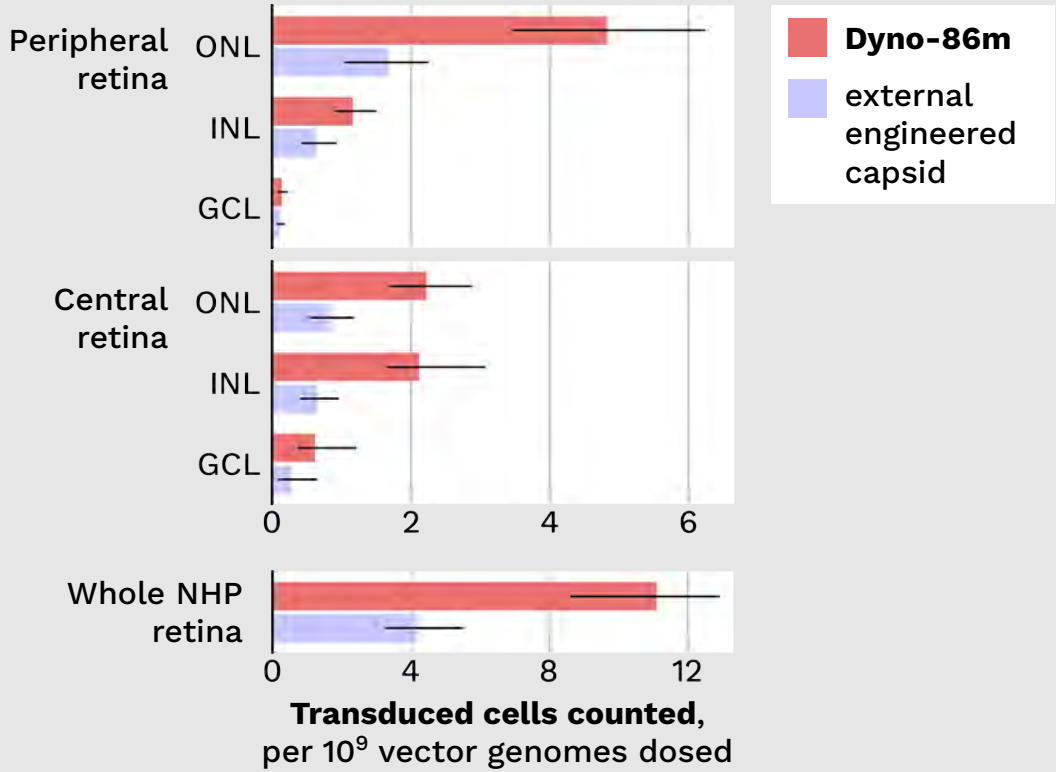
2 capsids co-injected, reporter swap

Dyno-86m capsid (7.8e10 vg) +
external engineered capsid (1.1e11 vg)



Quantifying capsid properties



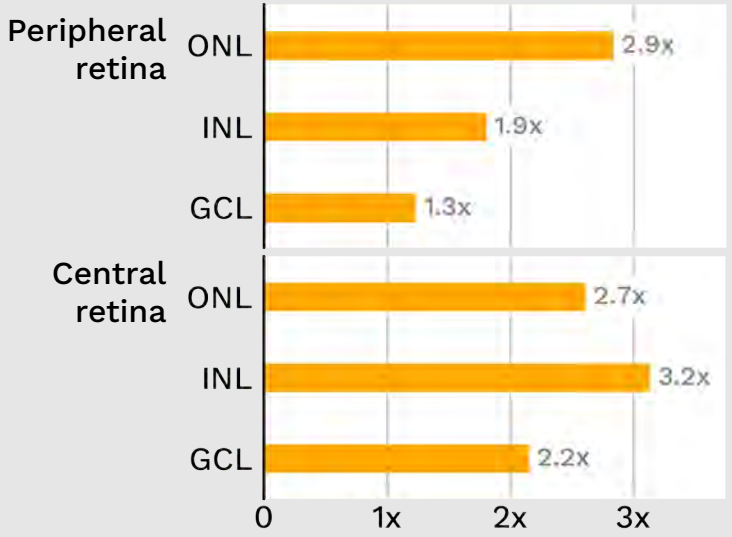


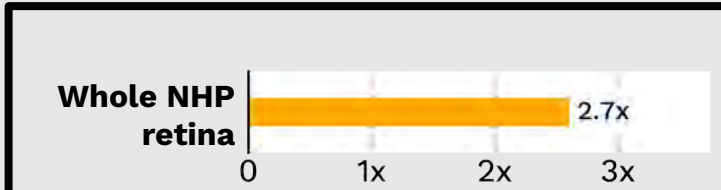
Intravitreal injection

Dyno-86m vs external engineered capsid

quantified by histology:

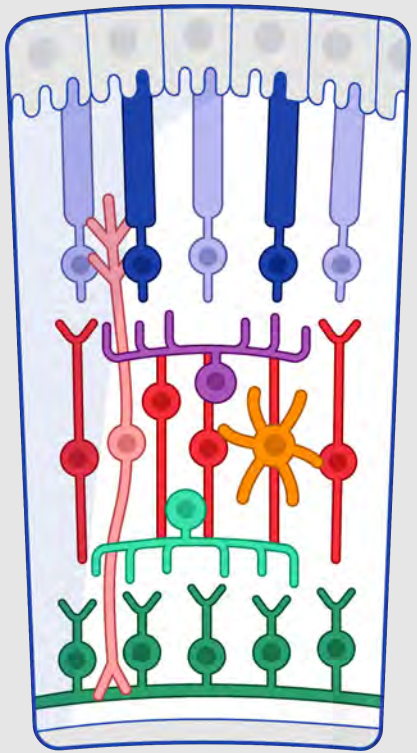
Dyno-86m transduces 2.7x more cells.





Fold-change, **Dyno-86m** vs external engineered capsid

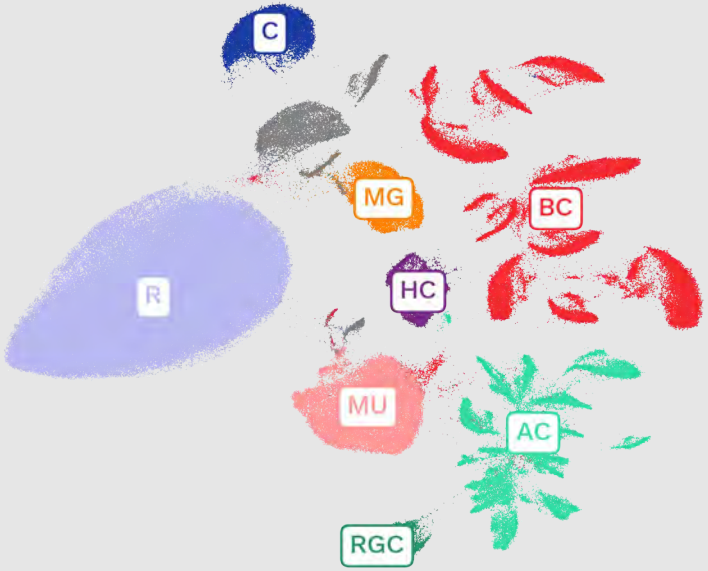
Cell type tropism



Outer nuclear layer
- Rods
- Cones

Inner nuclear layer
- Horizontal cells
- Bipolar cells
- Müller glia
- Microglia
- Amacrine cells

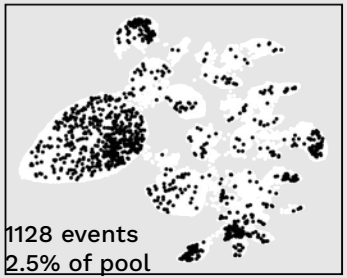
Ganglion cell layer
- Retinal ganglion cells

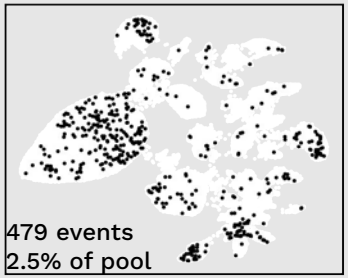


Single-nuclei RNA sequencing of NHP retina

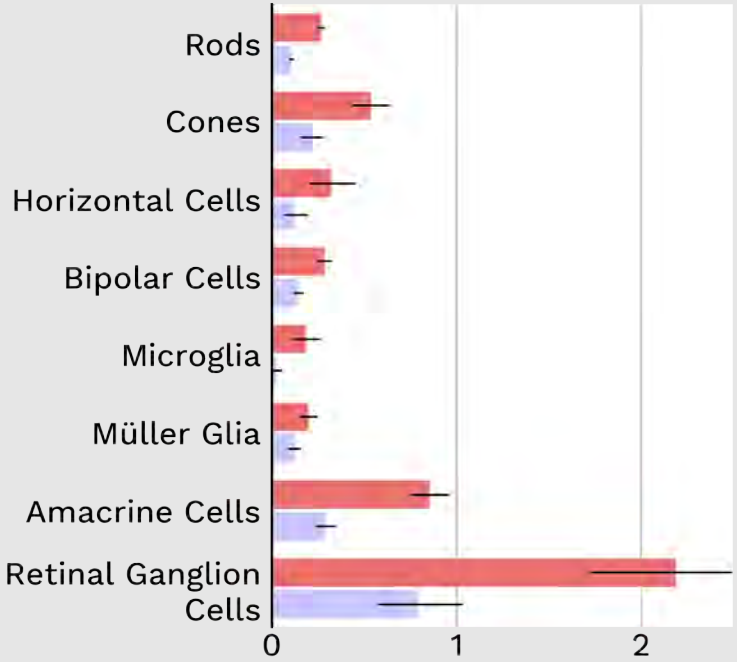
Cell type tropism

Dyno-86m





External engineered capsid



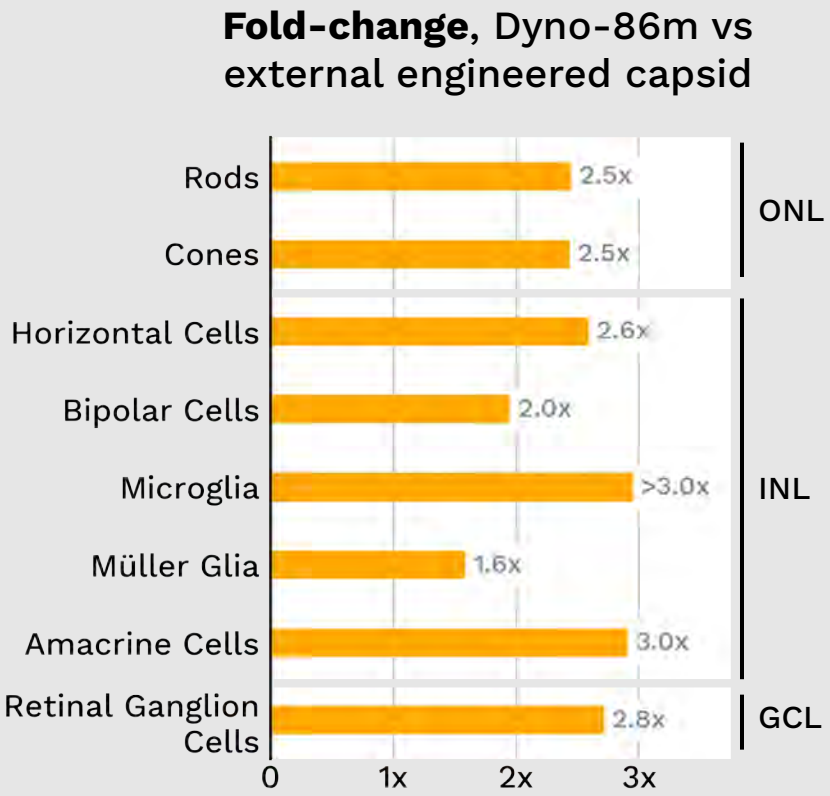
Transduced cells, normalized to injected capsid dose

Intravitreal injection

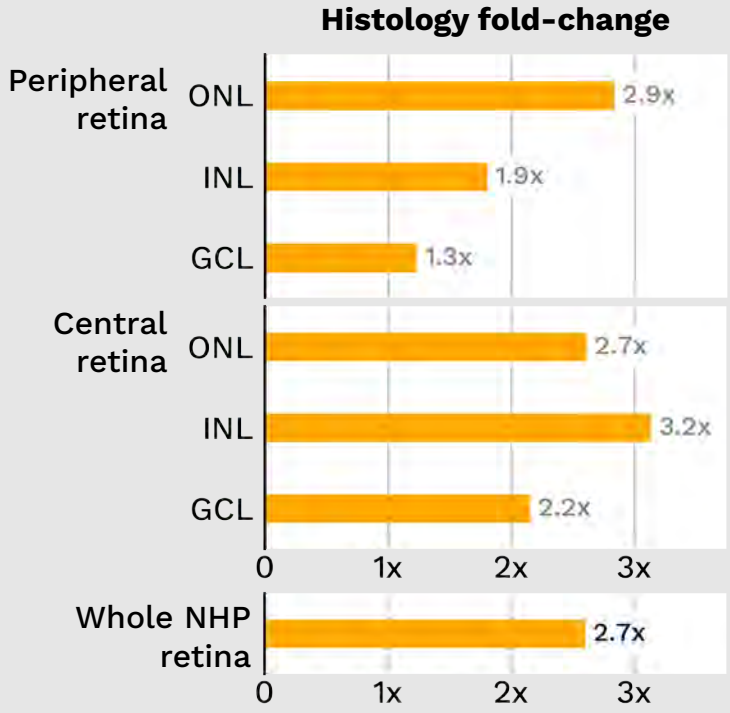
Dyno-86m vs external engineered capsid

quantified by single-nuclei RNA sequencing:

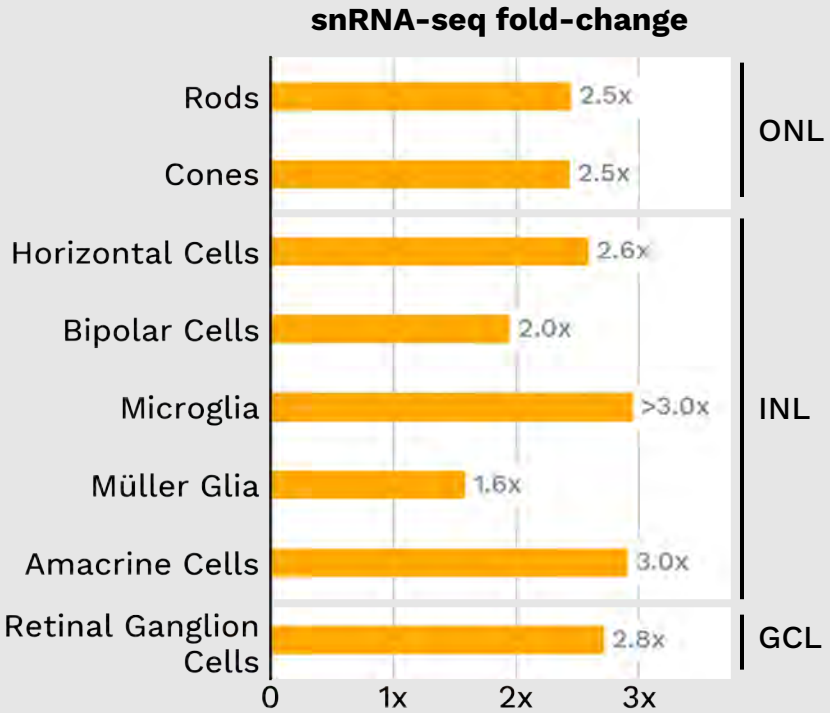
Dyno-86m transduces 2-3x more cells.



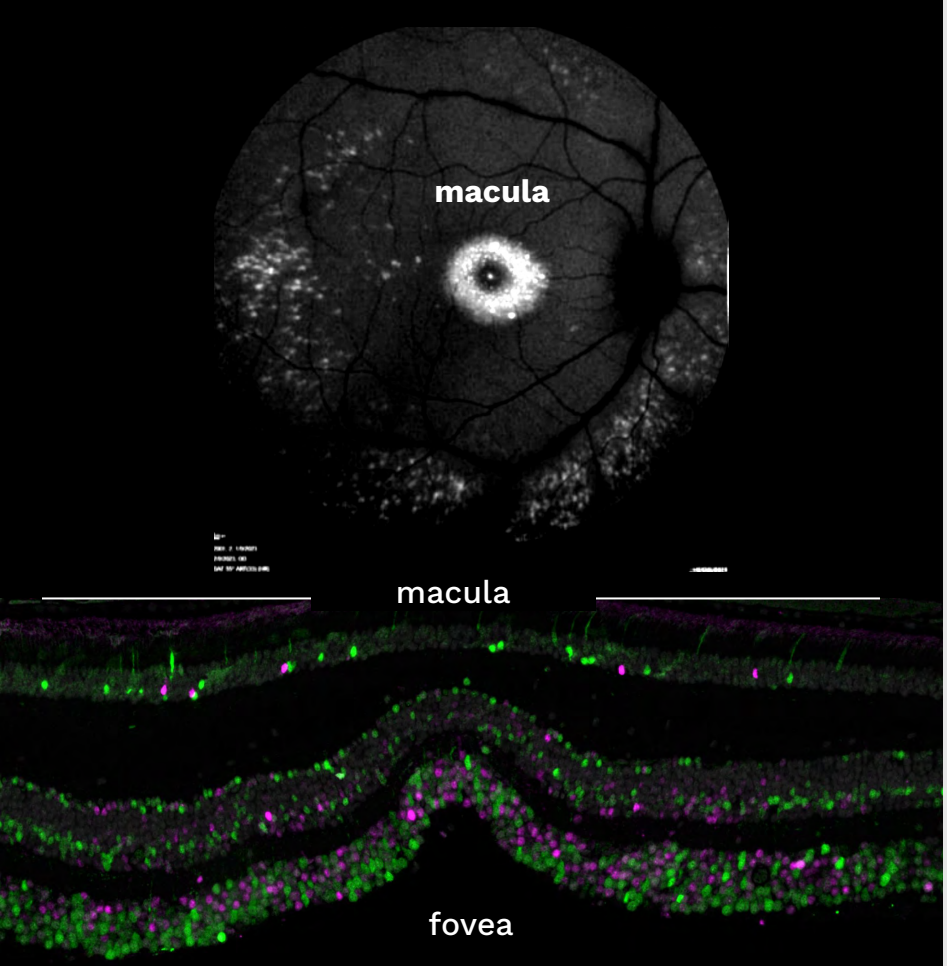
Histology and snRNA-seq closely match



Dyno-86m vs
external engineered capsid

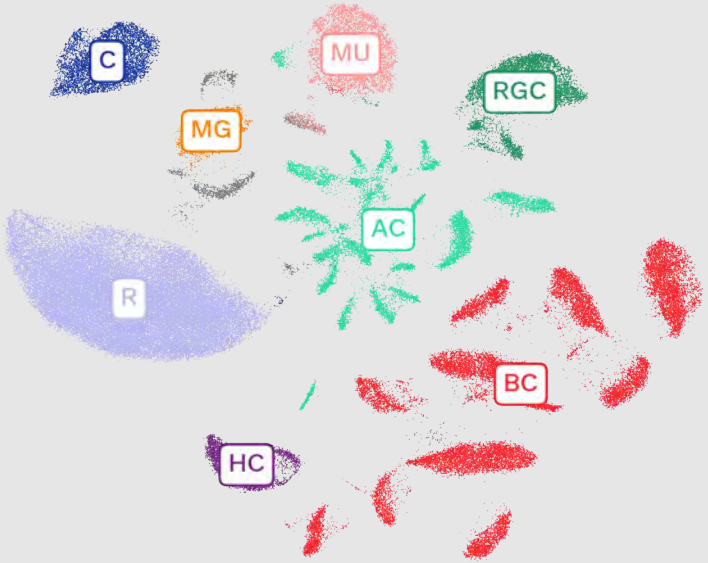


Dyno-86m vs
external engineered capsid



Dyno-86m capsid +
external engineered capsid

snRNA-seq of macular retina



94,000 total cells sequenced

R : Rods	MU : Müller glia
C : Cones	MG : Microglia
HC : Horizontal cells	AC : Amacrine cells
BC : Bipolar cells	RGC: Retinal ganglion cells

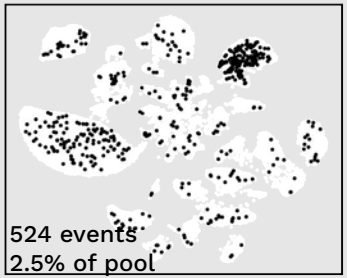
snRNA-seq of macular retina

70 capsid pool

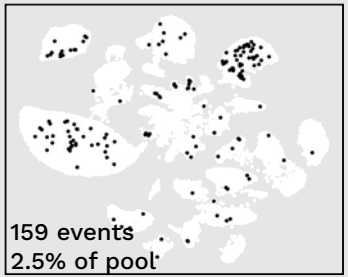
intravitreal co-injection in Cyno NHPs

4.3e11 vg total dose per eye

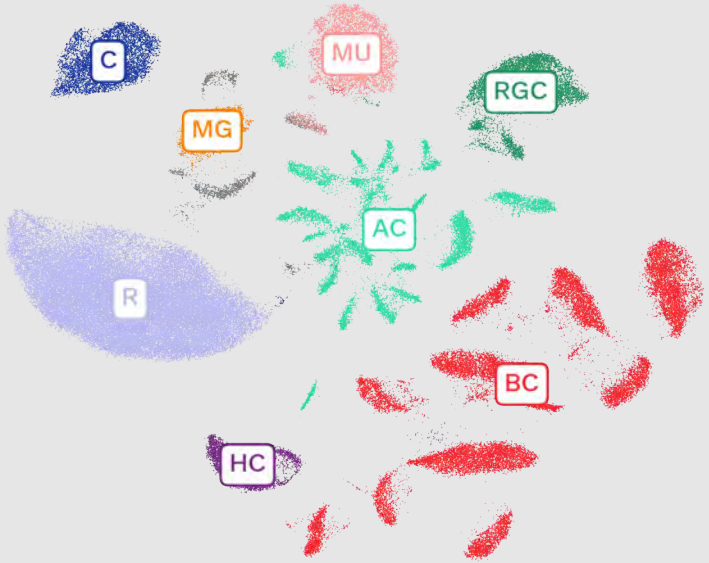
Dyno-86m



AAV2



External engineered capsid



94,000 total cells sequenced

R : Rods	MU : Müller glia
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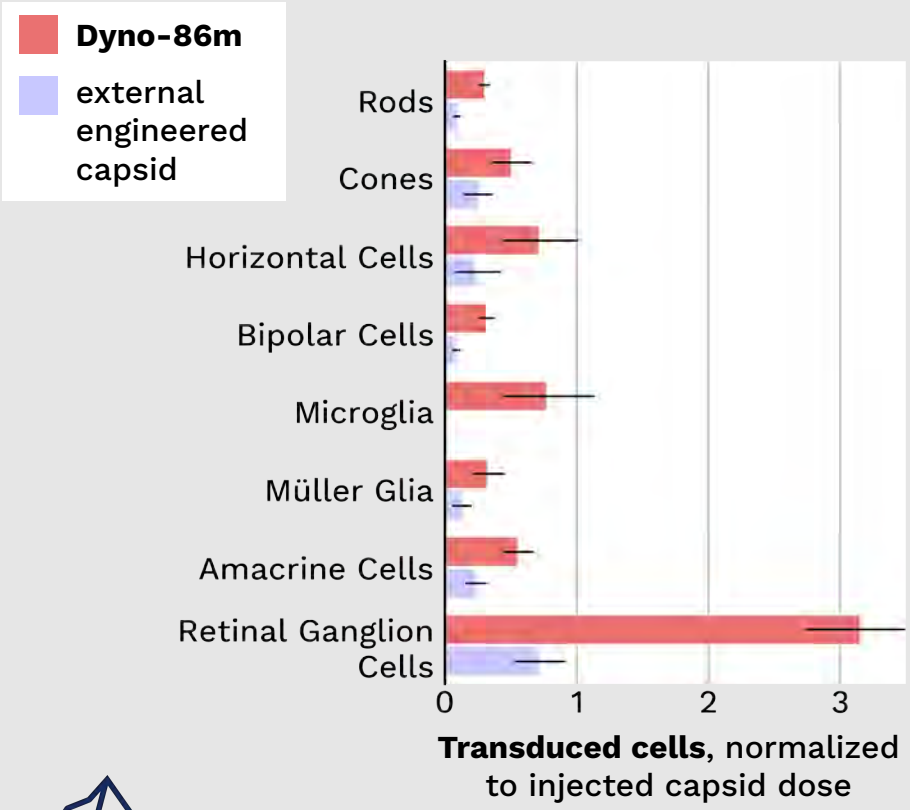
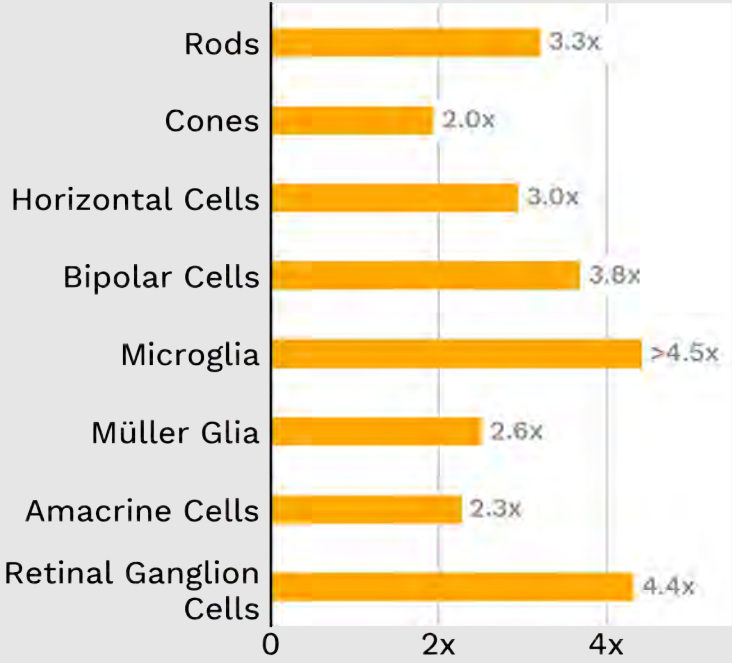
HC : Horizontal cells	AC : Amacrine cells
BC : Bipolar cells	RGC: Retinal ganglion cells

Dyno-86m outperforms external engineered capsid in macular region



Transduced cells, normalized to injected capsid dose



Dyno-86m vs external engineered capsid

Dyno-86m

Designed by Dyno
using **proprietary data** and **generative AI**

Thoroughly validated in NHPs
for human translatability

Dyno's best capsid for intravitreal delivery
assessed by histology, bulk NGS and snRNA-seq

Available for **immediate licensing**

usi e AI

Thorc NHPs

Dyn very

assesse NA-seq

Abstract 1284:

“Dyno-86m: Optimizing Intravitreal Delivery to the Non-Human Primate Retina with Machine-Guided AAV Capsid Design”

>100,000 capsids scale

next iteration using **all proprietary data** and **generative AI**

Dyno-gvk

2x transduction vs Dyno-86m

4-6x transduction vs external IVT engineered capsid

>100,000 capsids scale

Dyno-86m designed and tested

1-100 capsids scale

Dyno-86m validated

Dyno's best capsids for IVT eye delivery

Dyno-86m

Dyno-gvk

Dyno-86m

1x production vs AAV2

80x transduction vs AAV2

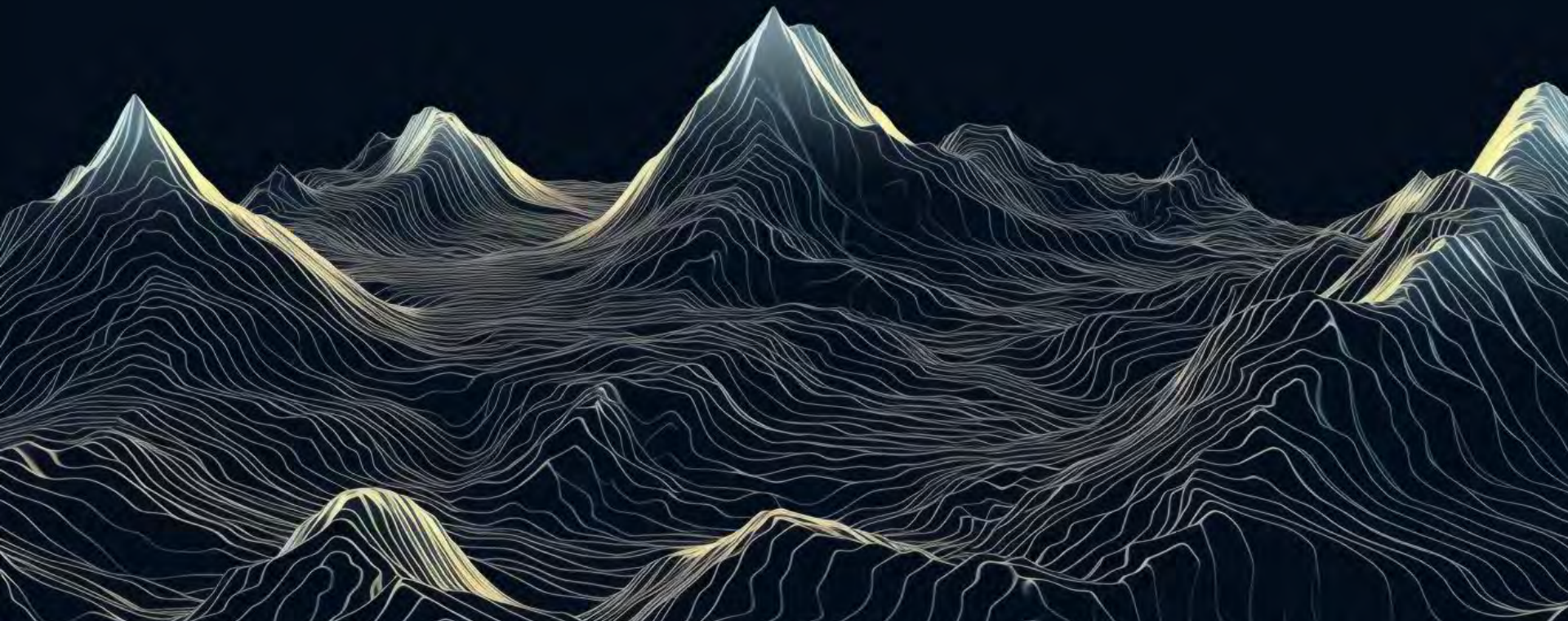
2-3x transduction vs external IVT engineered capsid

Dyno-gvk

2x transduction vs Dyno-86m

4-6x transduction vs external IVT engineered capsid

Dyno Brain Capsids



Dyno bCap 1 delivery

Designed using **Generative AI** for IV delivery **pan-brain** and across the **CNS**

1x production vs AAV9

10x liver detargeting vs AAV9

100x brain transduction vs AAV9

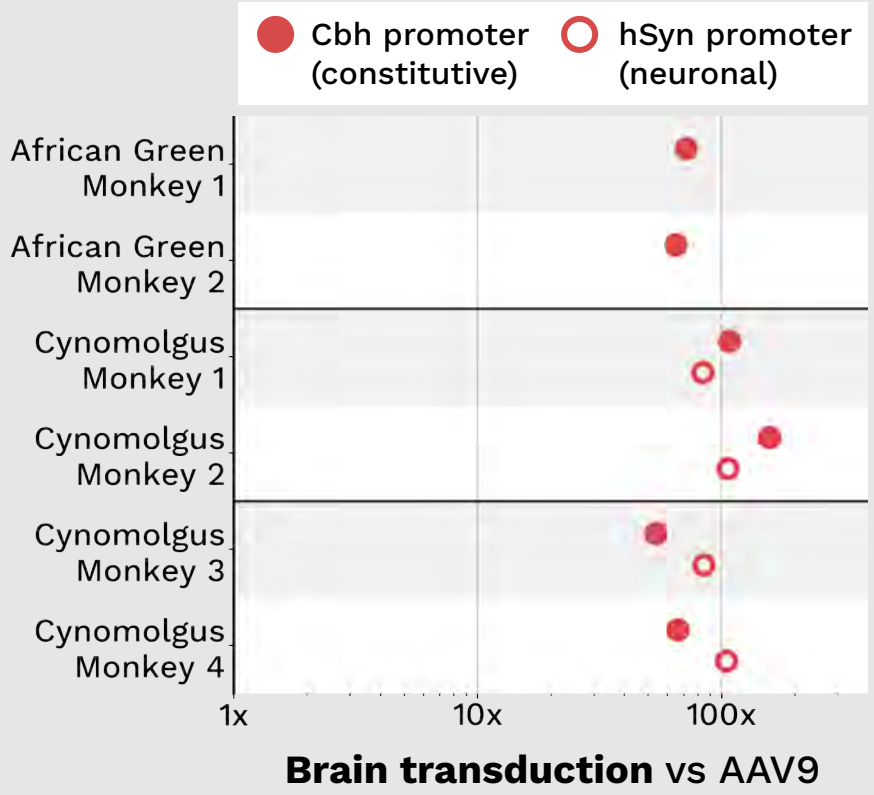
Field-leading potential
in **head-to-head** comparison with external engineered capsids

Consistent 100x improved CNS transduction

>100,000 capsids scale

Generative AI design

***in vivo* NHP** measurement



Brain transduction vs AAV9

>100,000 capsids scale

Generative AI design

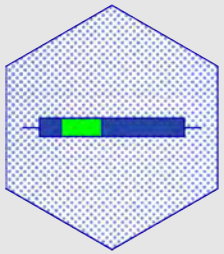
***in vivo* NHP** measurement

1-100 capsids scale

***in vivo* NHP validation**

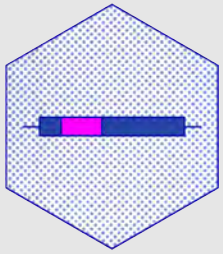
Validating Dyno bCap 1 delivery

50%



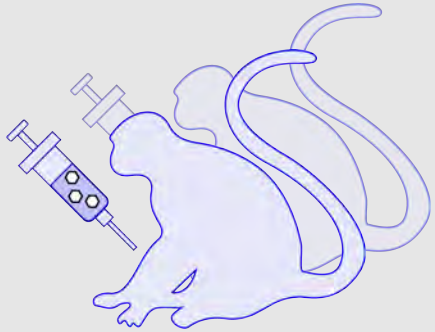
Dyno bCap 1 capsid with CBh-**GFP**

50%



AAV9 capsid with CBh-**mCherry**

2 Cyno NHPs

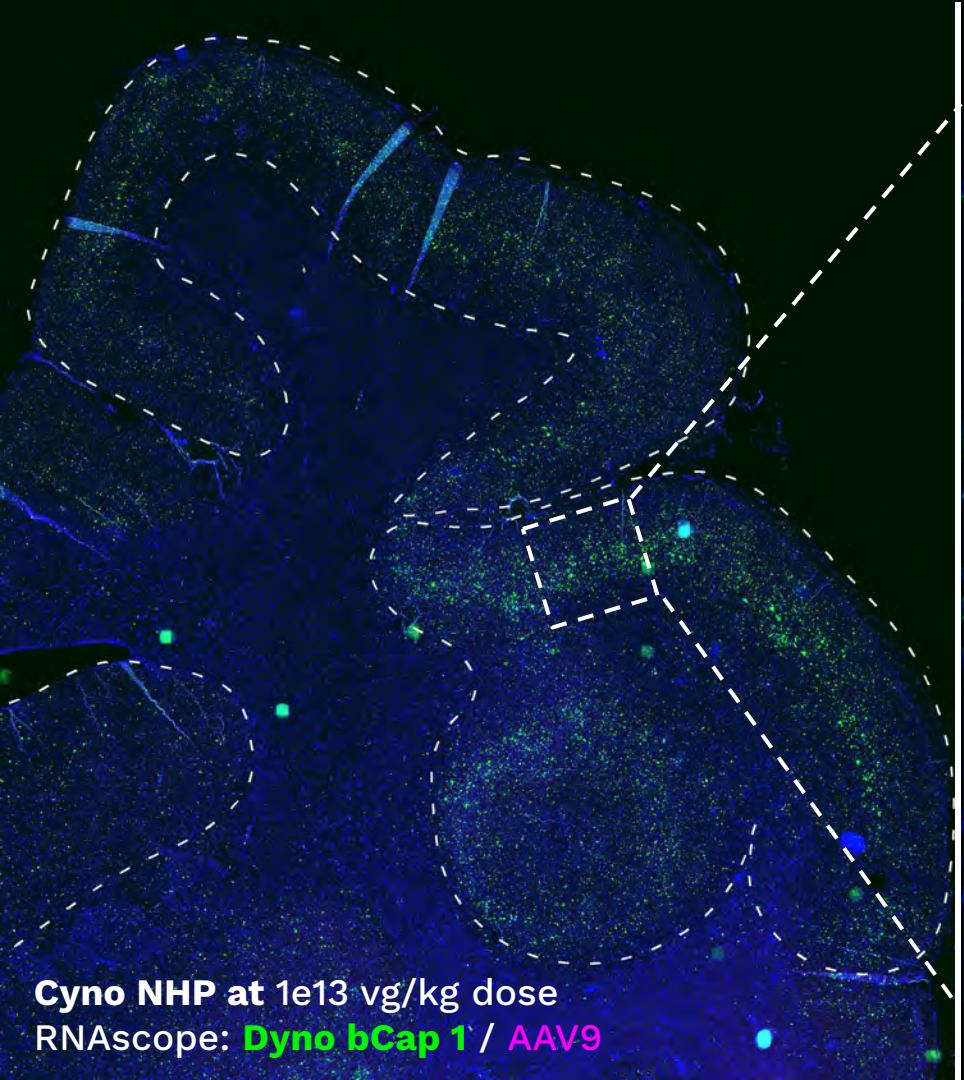


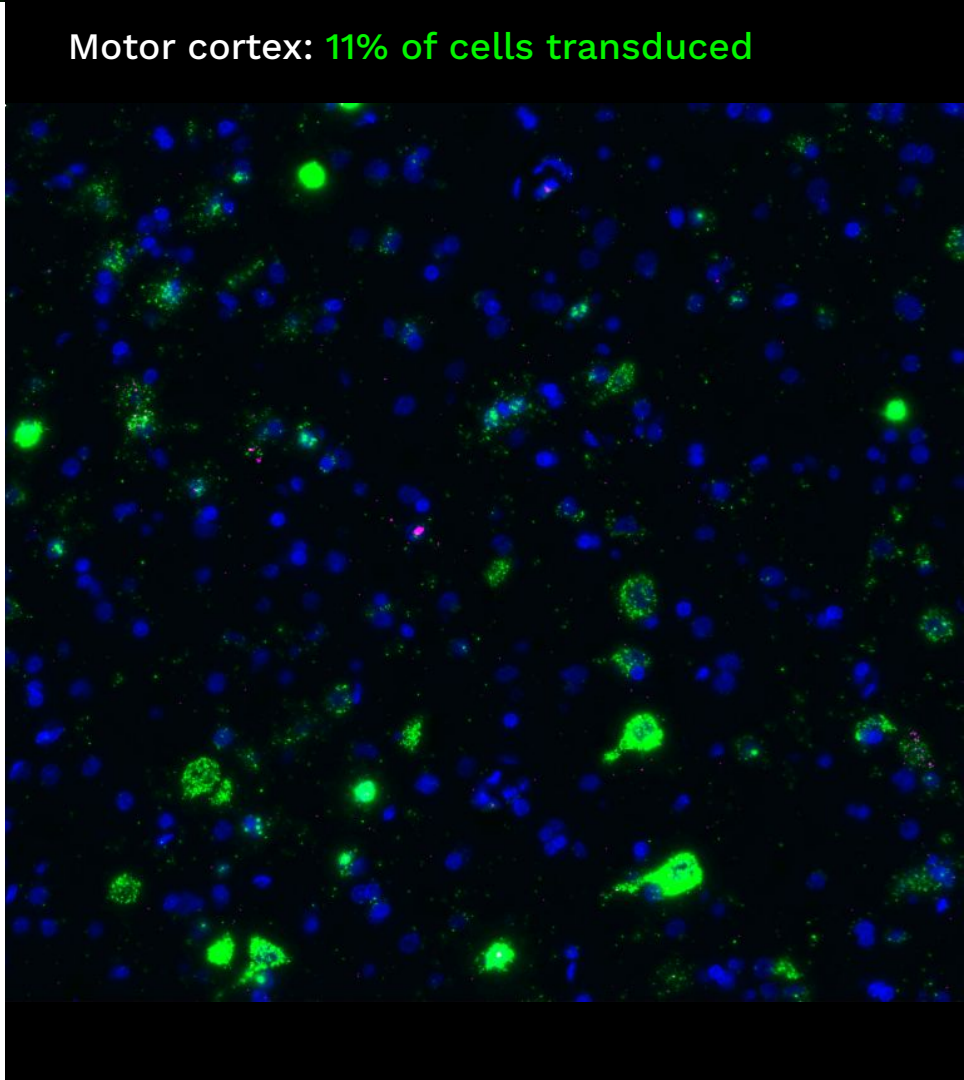
1e13 vg/kg/capsid

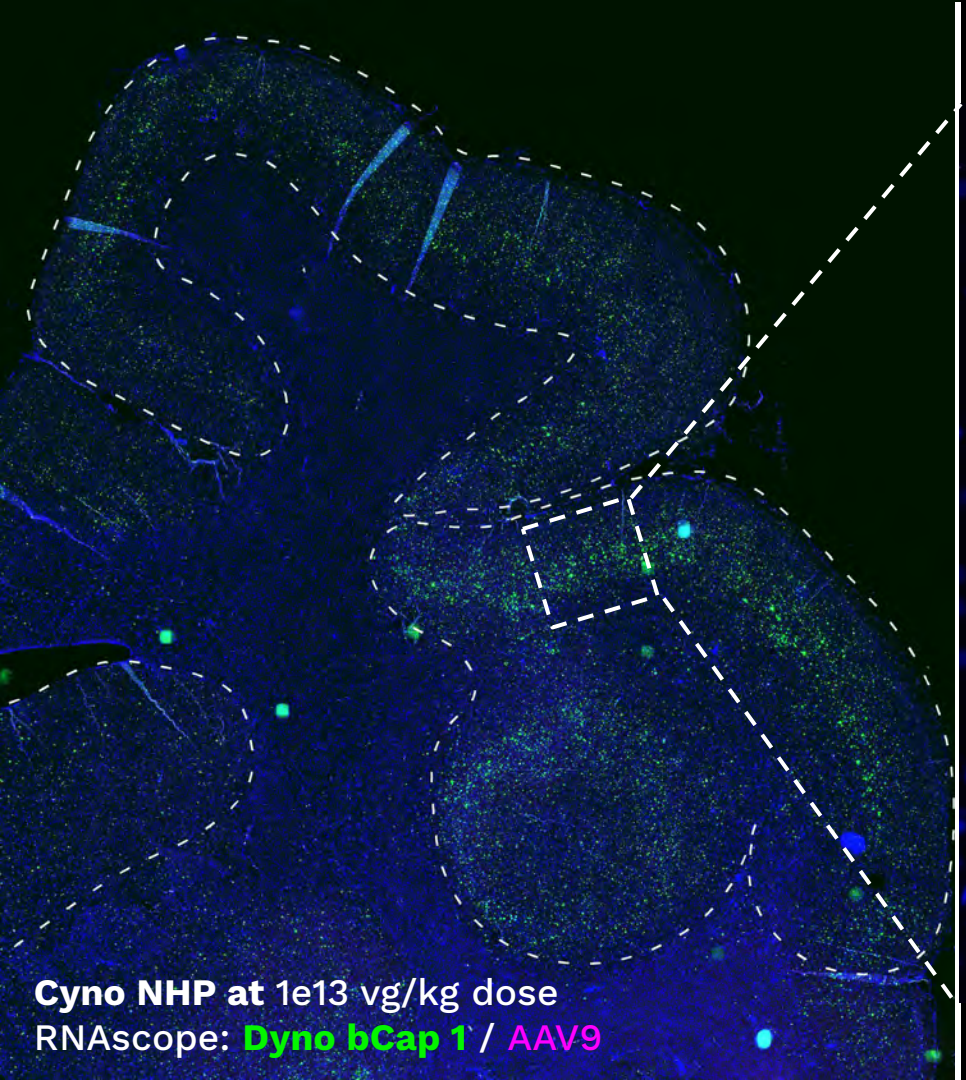
5e12 vg/kg/capsid

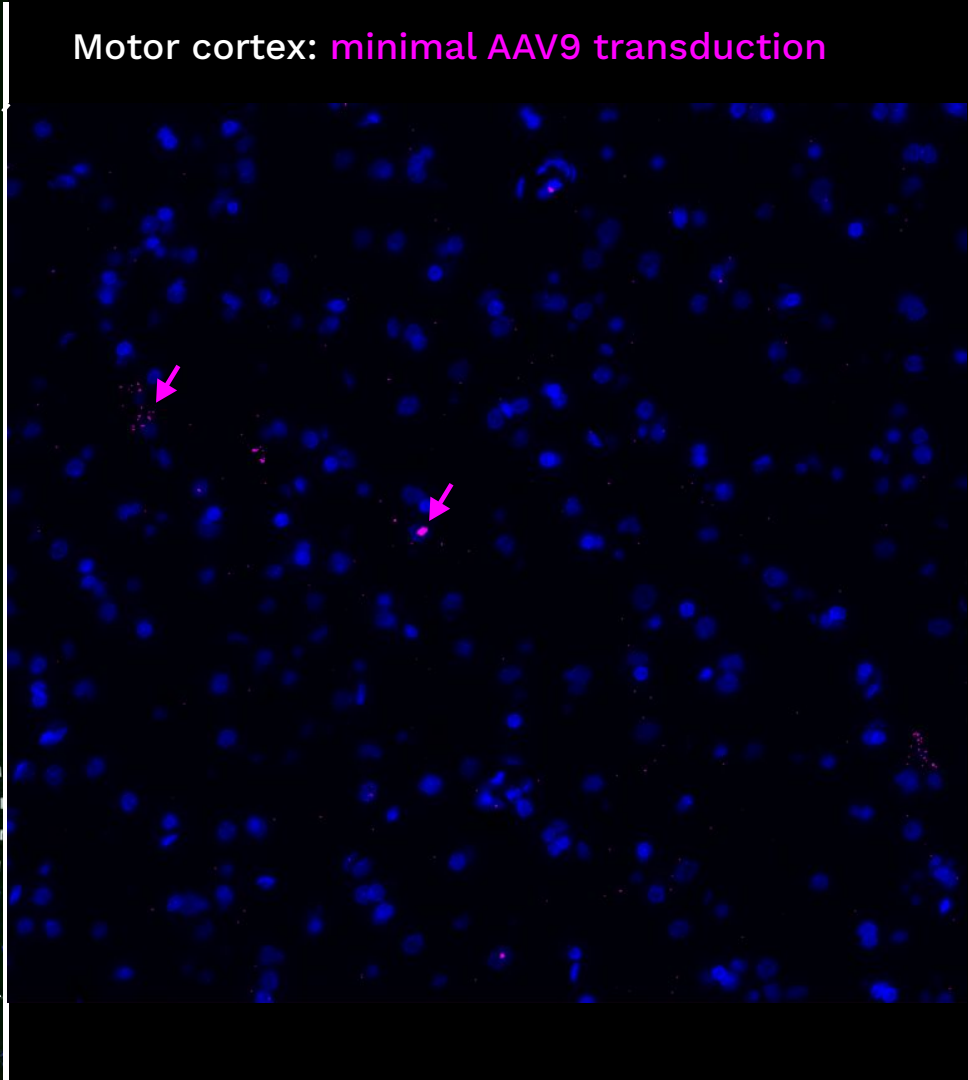
28 days in life

- **Transduction (NGS)**
- **Biodistribution (NGS)**
- **RNAscope histology**
- **IF and IHC histology**

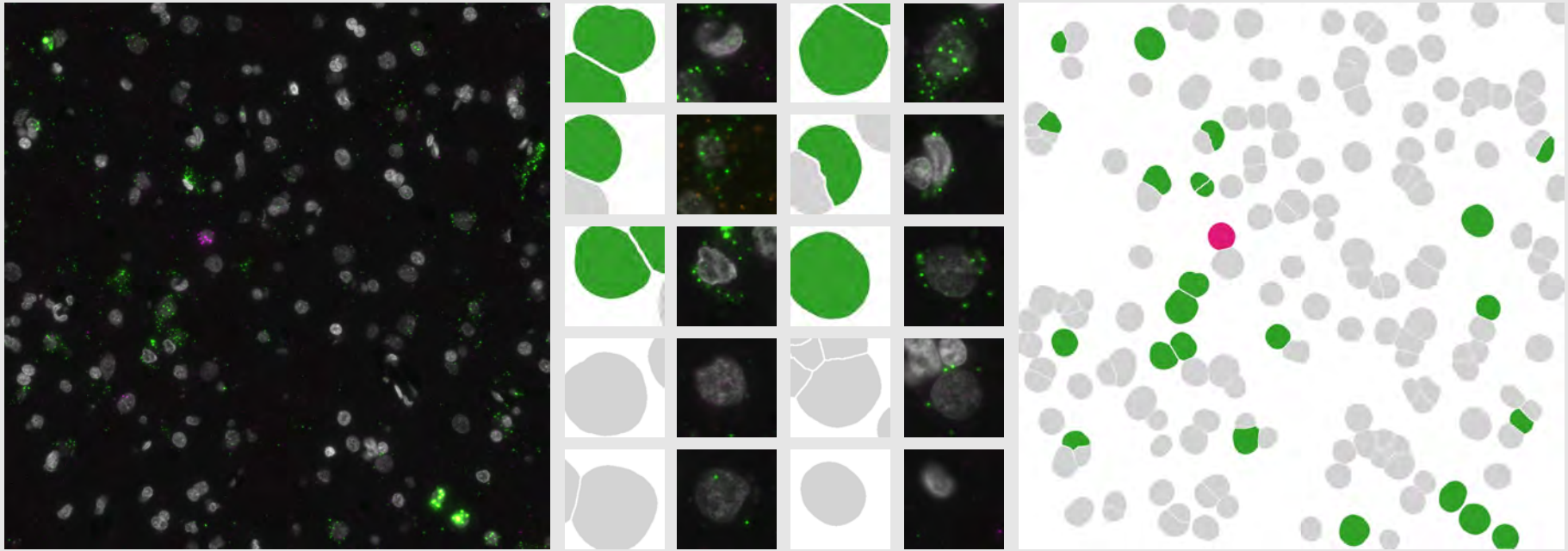

Cyno NHP at 1e13 vg/kg dose
RNAscope: **Dyno bCap 1** / AAV9


Motor cortex: 11% of cells transduced


Cyno NHP at 1e13 vg/kg dose
RNAscope: **Dyno bCap 1** / AAV9


Motor cortex: minimal AAV9 transduction

Quantification of % total cells transduced

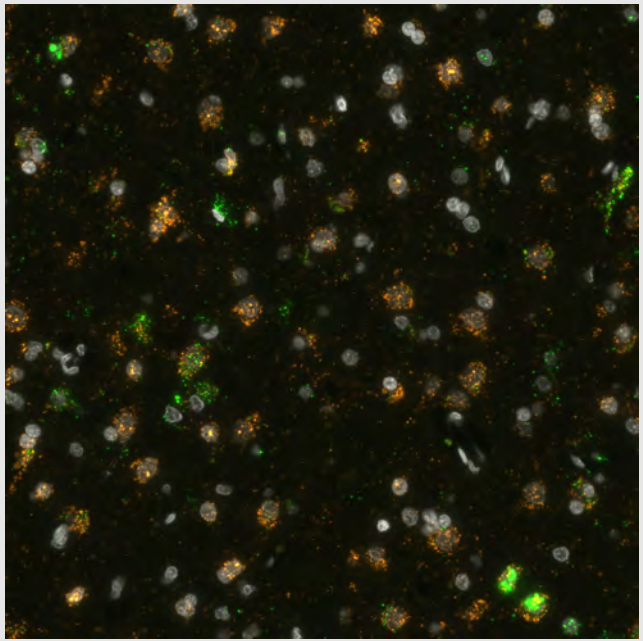


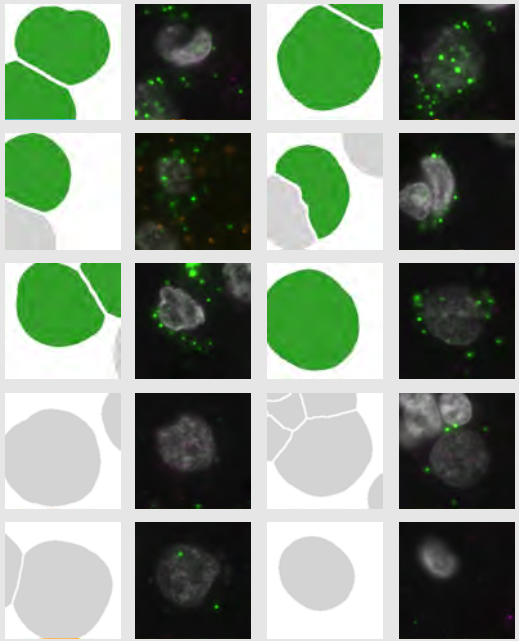
Dyno bCap 1 transduction

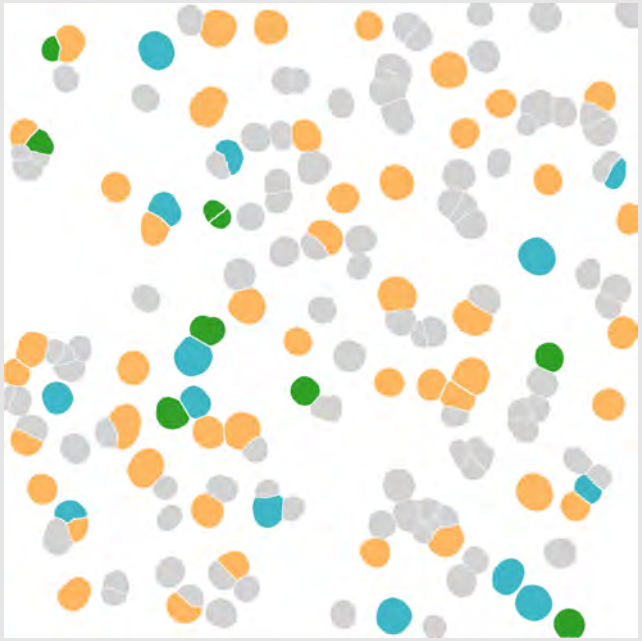
Cell without transduction

AAV9 transduction

Quantification of % Fox3+ neurons transduced







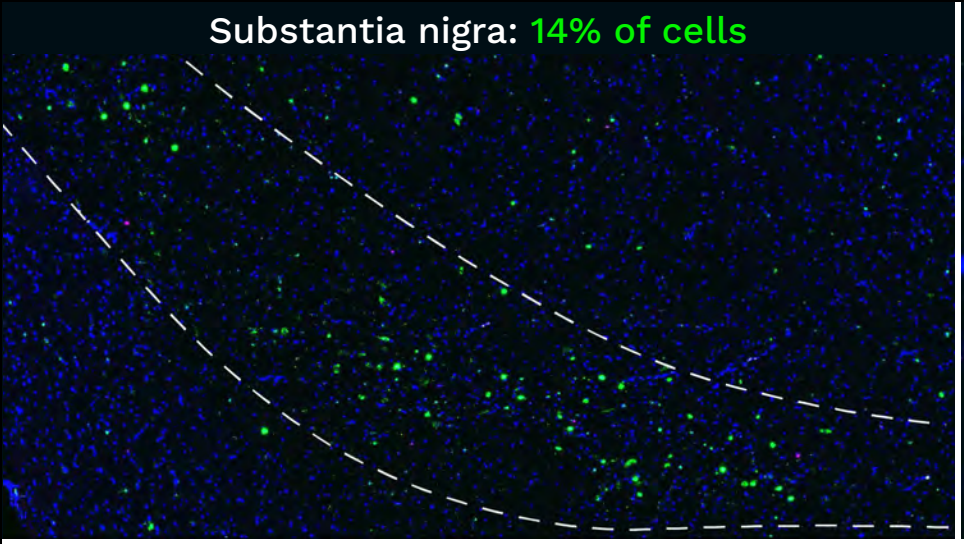
Fox3 transcript encodes NeuN protein

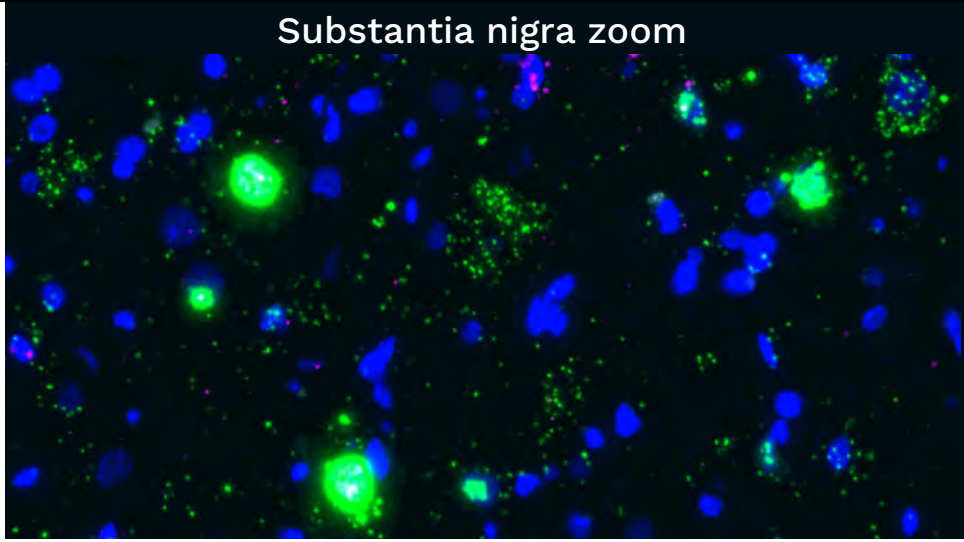
Dyno bCap 1 transduction in Fox3- cell

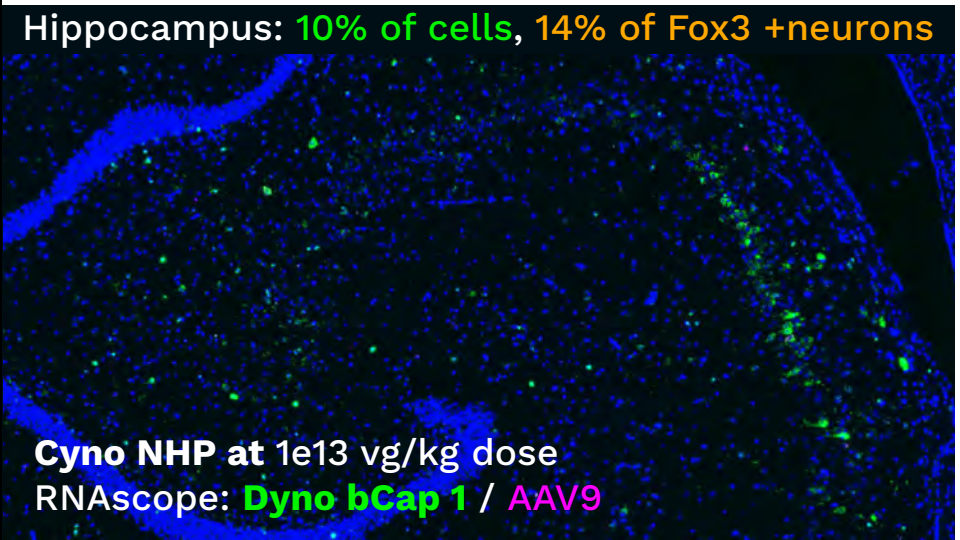
Dyno bCap 1 transduction Fox3+ neuron

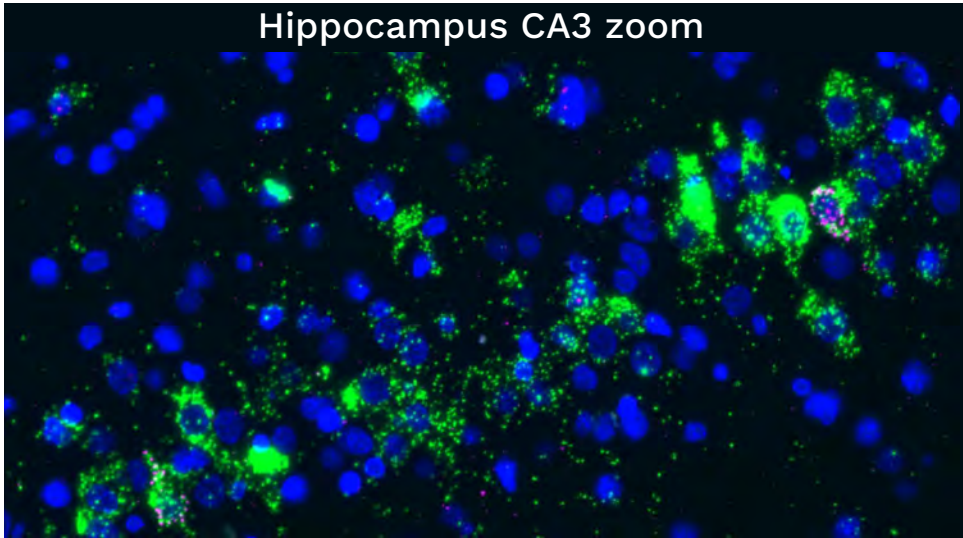
Fox3- neuron without transduction

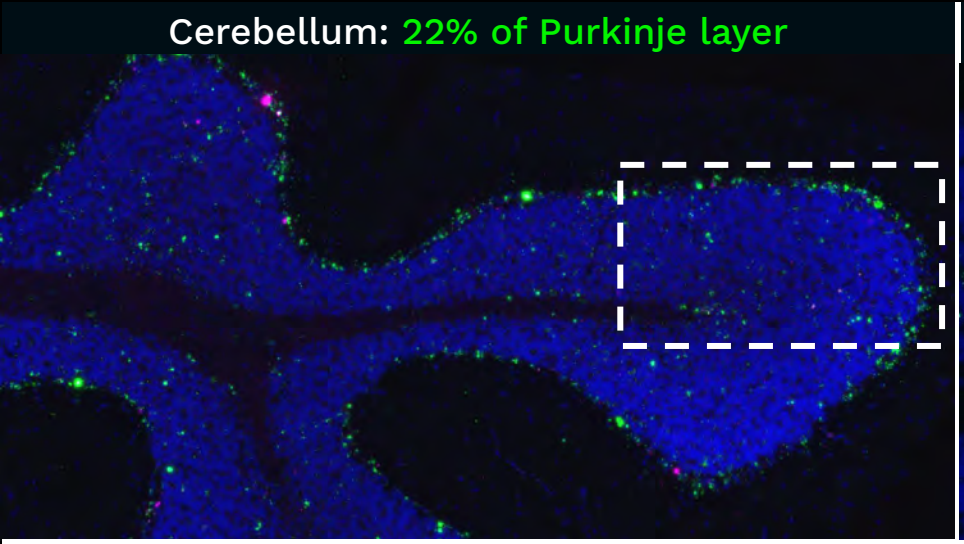
Fox3- cell without transduction

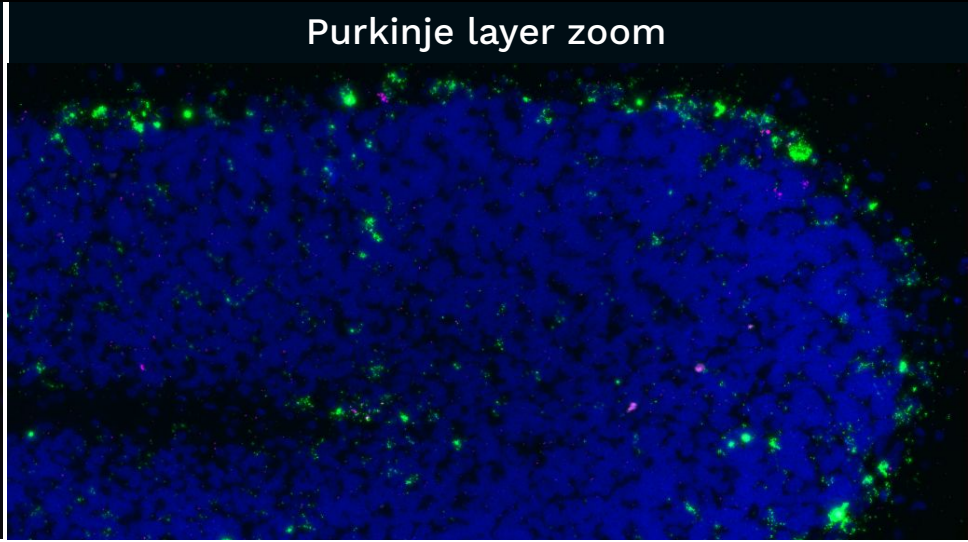

Substantia nigra: 14% of cells

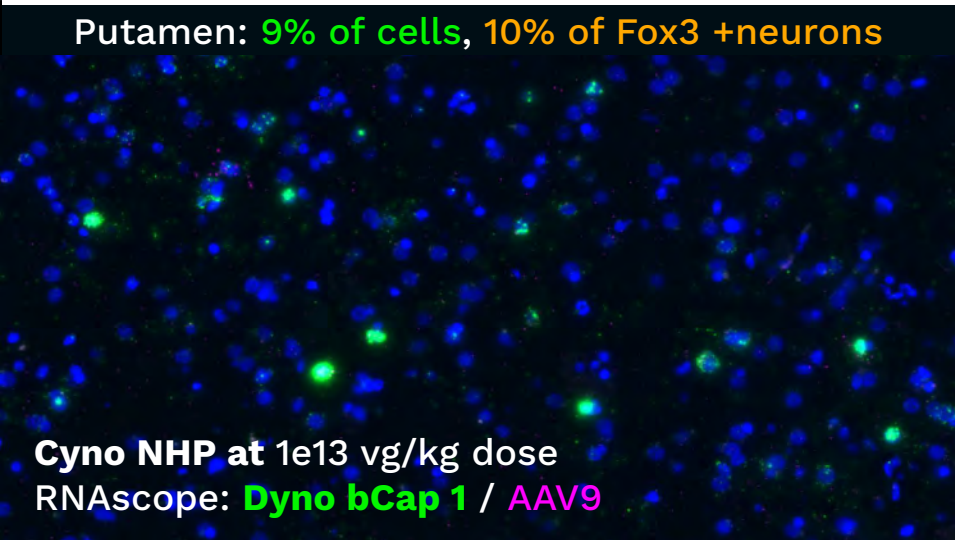

Substantia nigra zoom

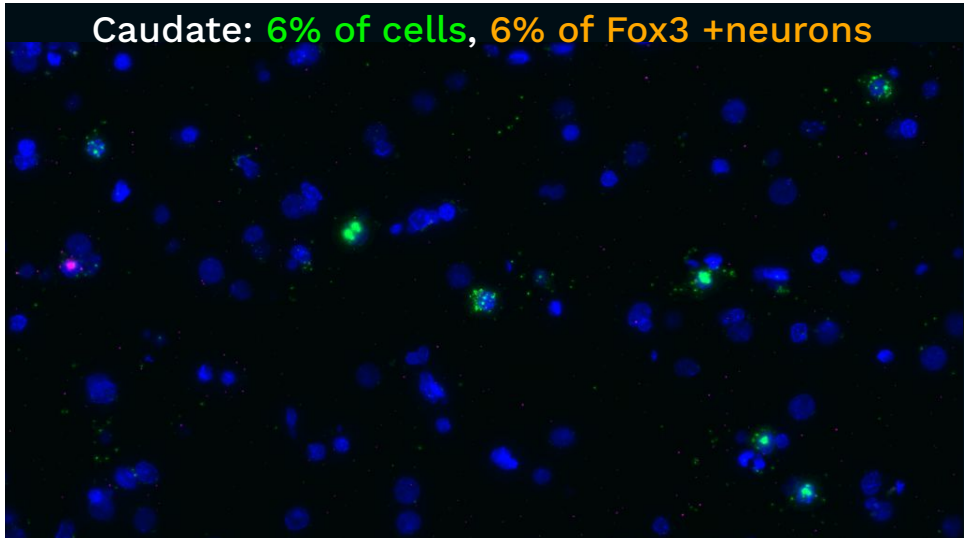

Hippocampus: 10% of cells, 14% of Fox3 +neurons
Cyno NHP at 1e13 vg/kg dose
RNAscope: Dyno bCap 1 / AAV9


Hippocampus CA3 zoom


Cerebellum: 22% of Purkinje layer

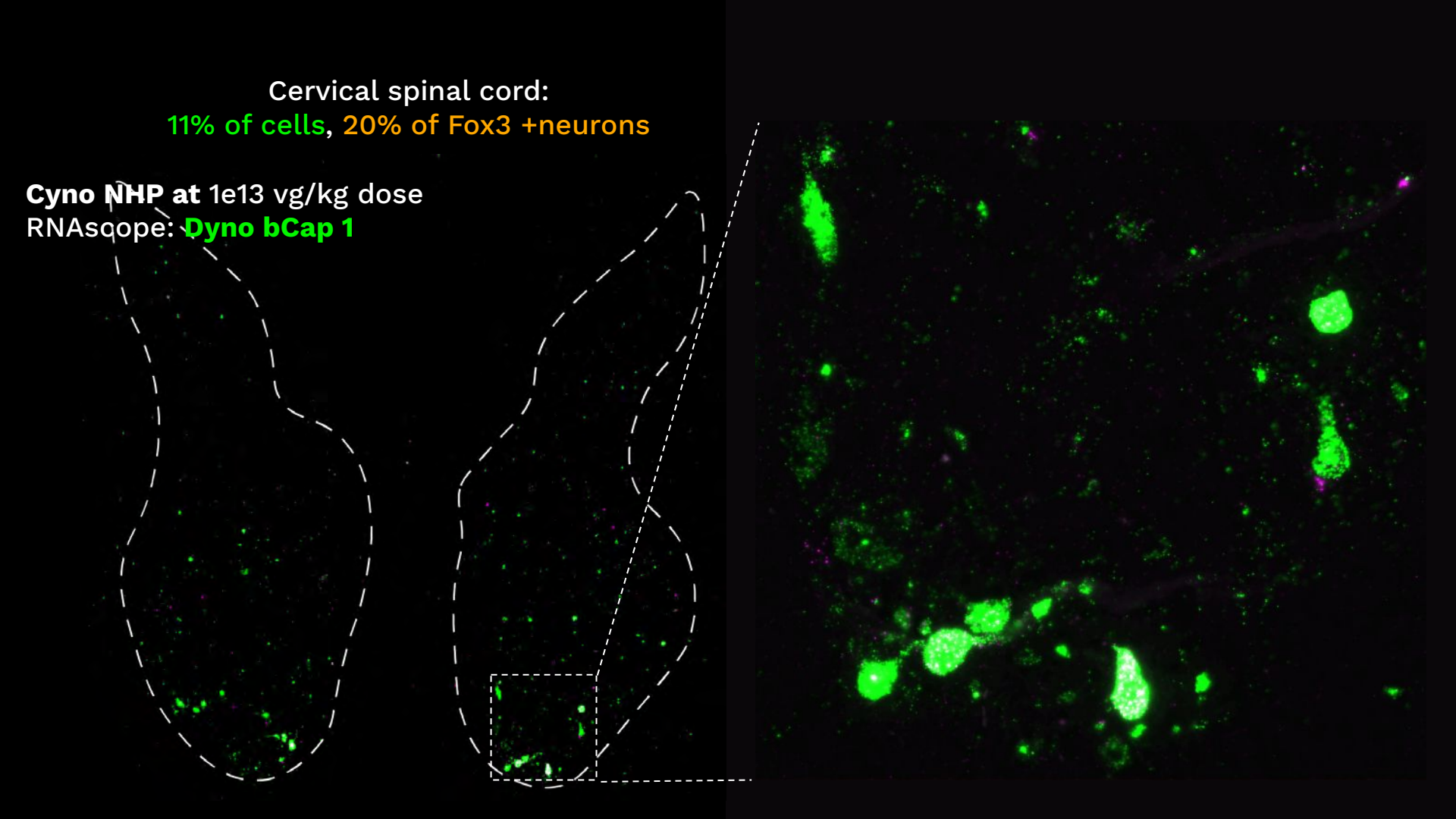

Purkinje layer zoom

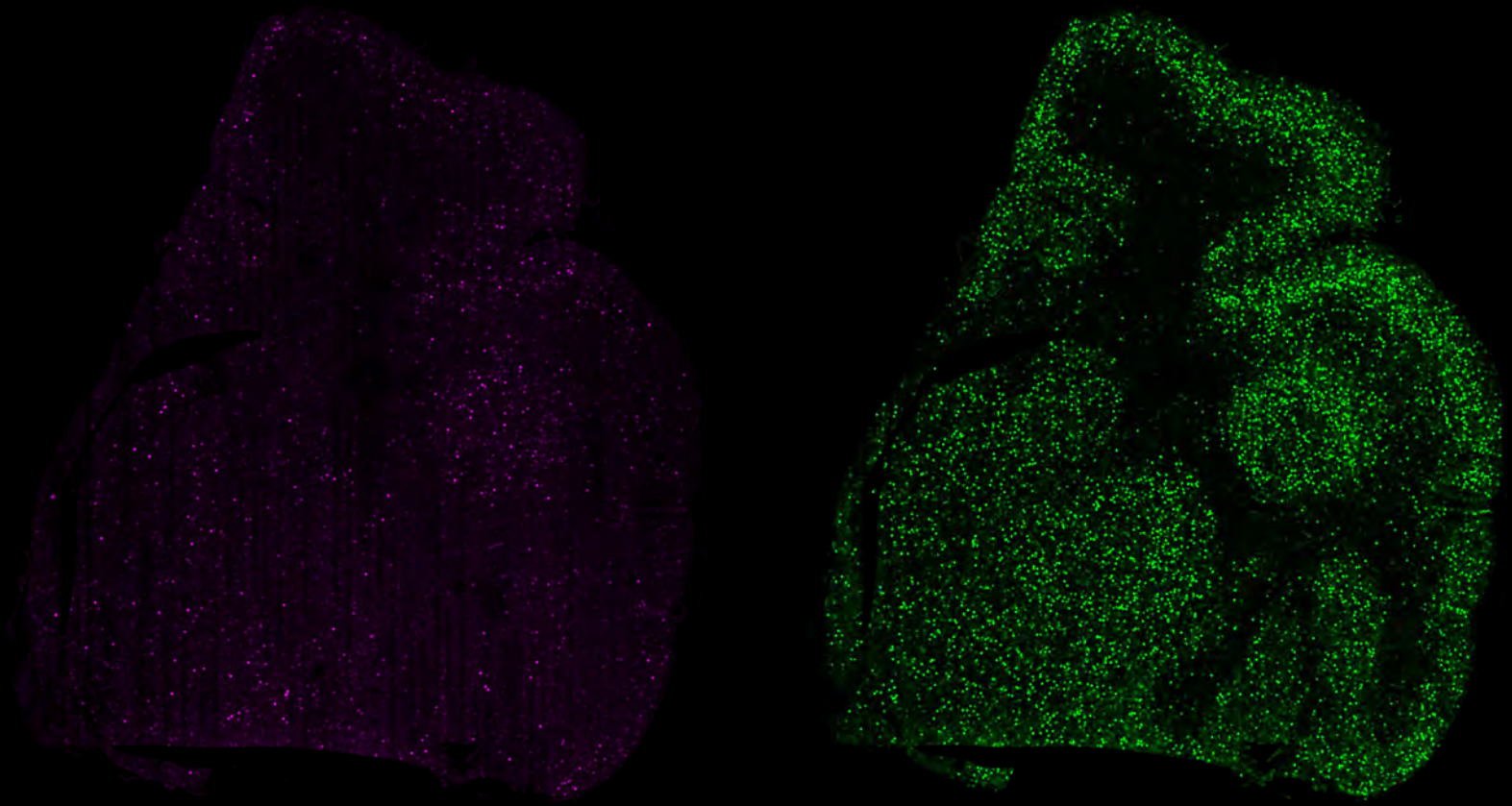

Putamen: 9% of cells, 10% of Fox3 +neurons
Cyno NHP at 1e13 vg/kg dose
RNAscope: Dyno bCap 1 / AAV9


Caudate: 6% of cells, 6% of Fox3 +neurons

Cervical spinal cord:
11% of cells, 20% of Fox3 +neurons

Cyno NHP at 1e13 vg/kg dose
RNAscope: **Dyno bCap 1**

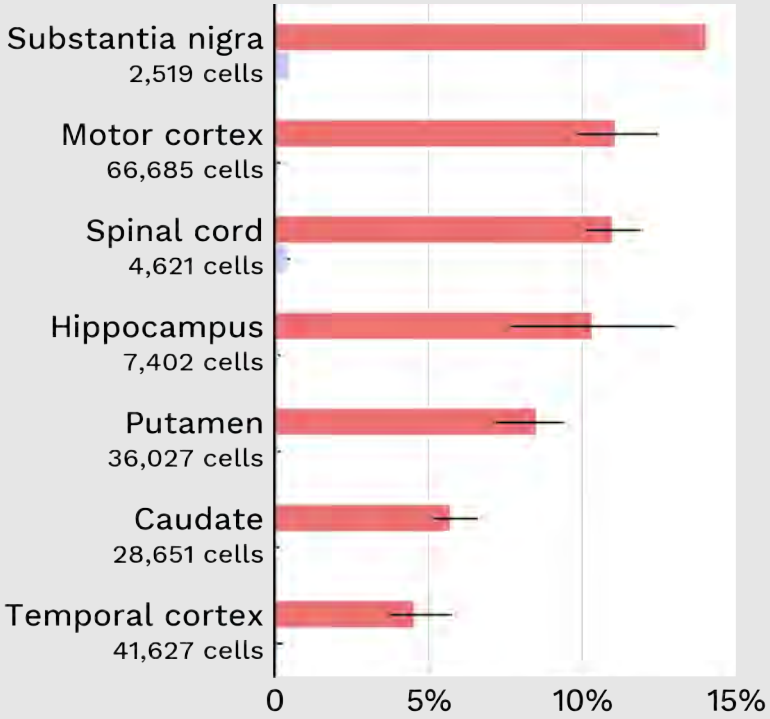


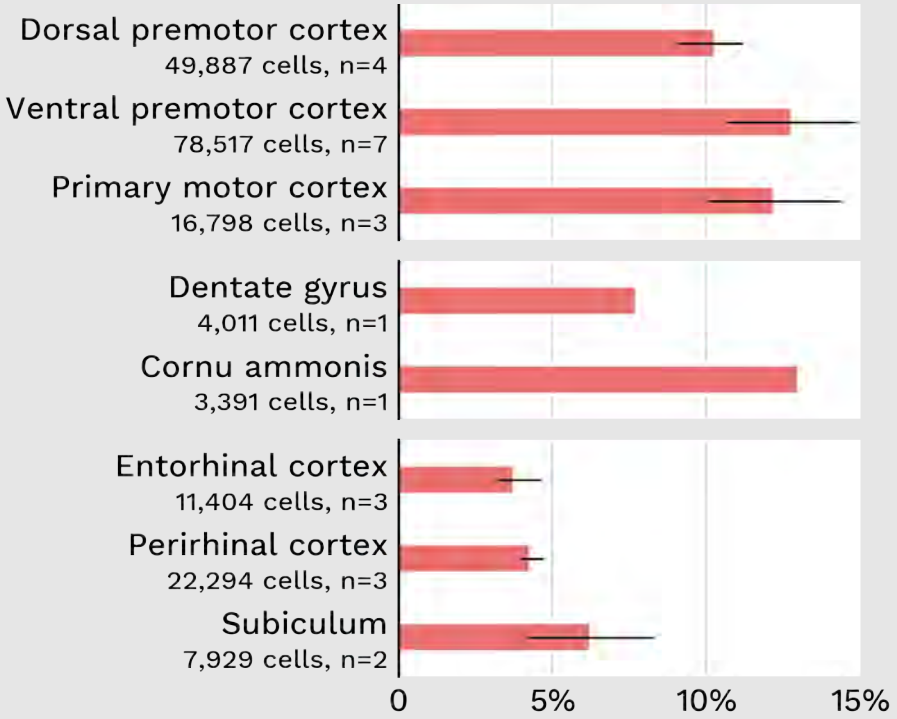


AAV9 delivery

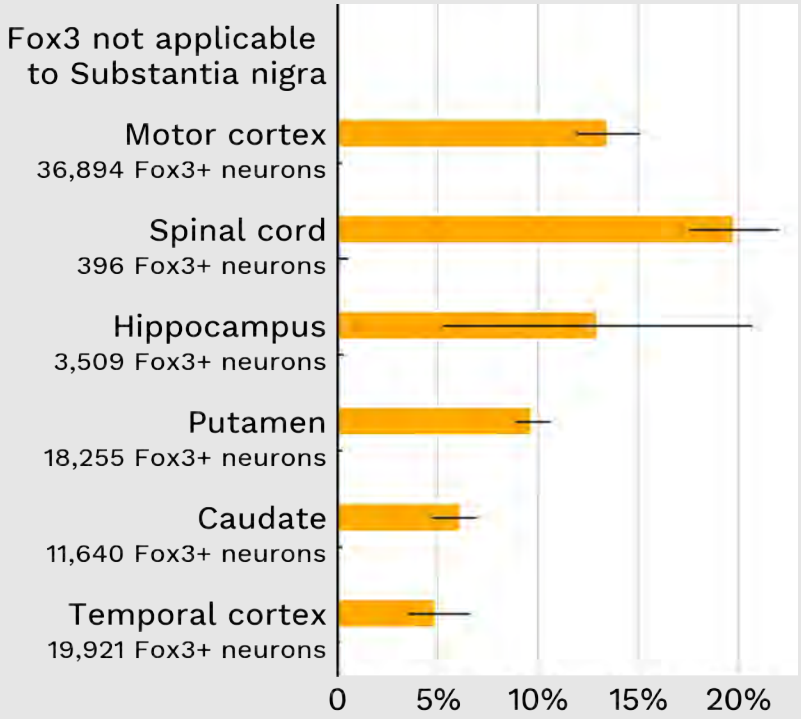
Dyno bCap 1 delivery

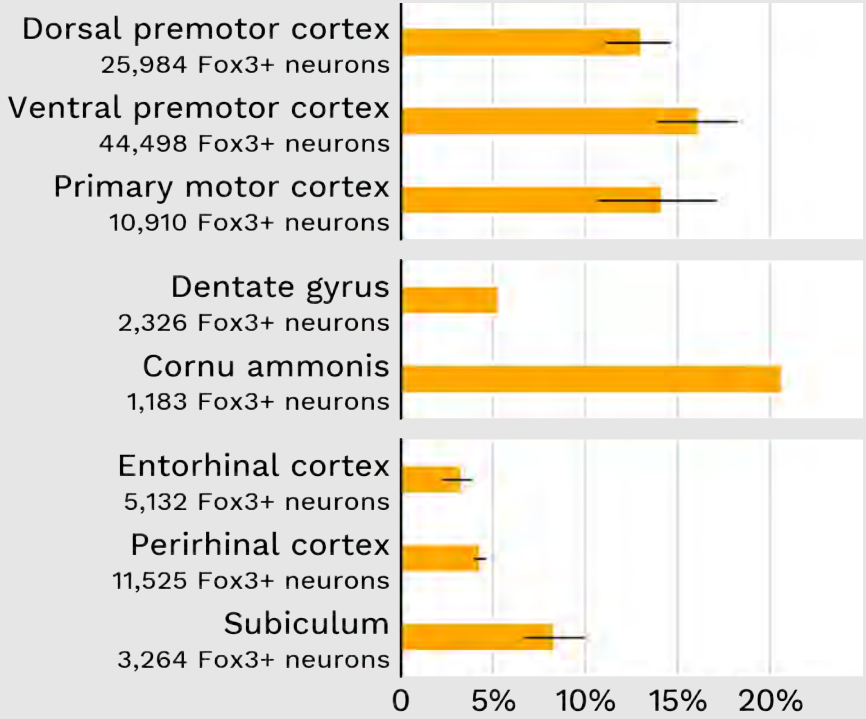
Pan-brain transduction quantified from histology



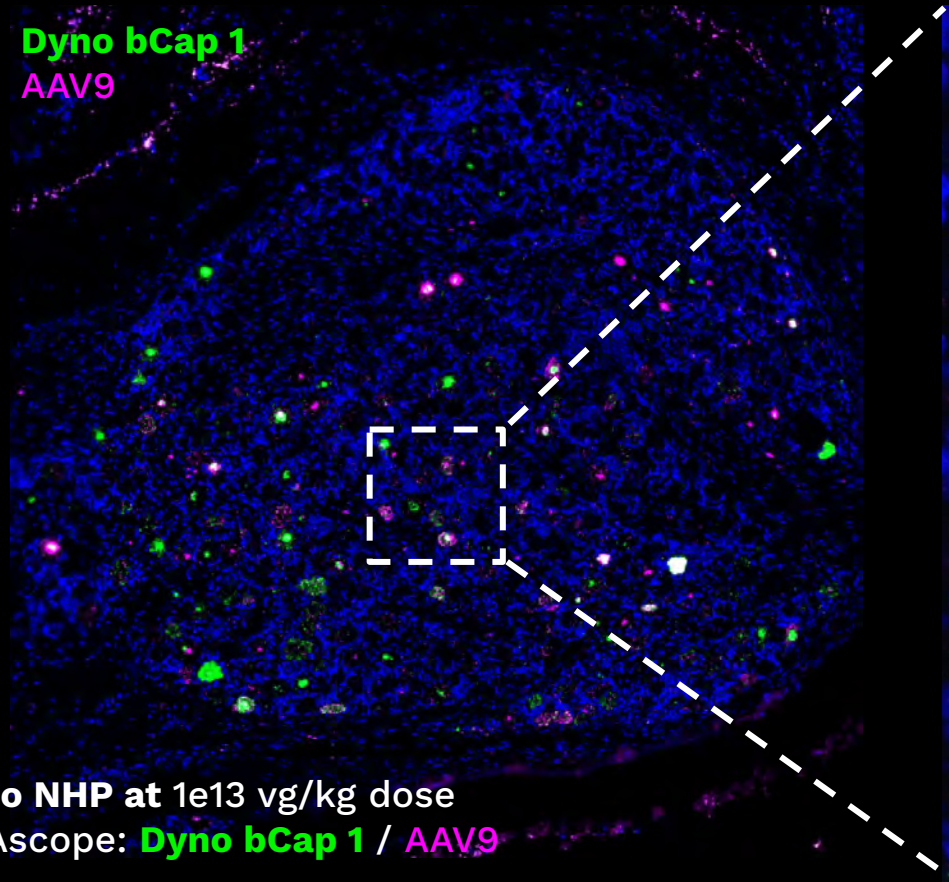


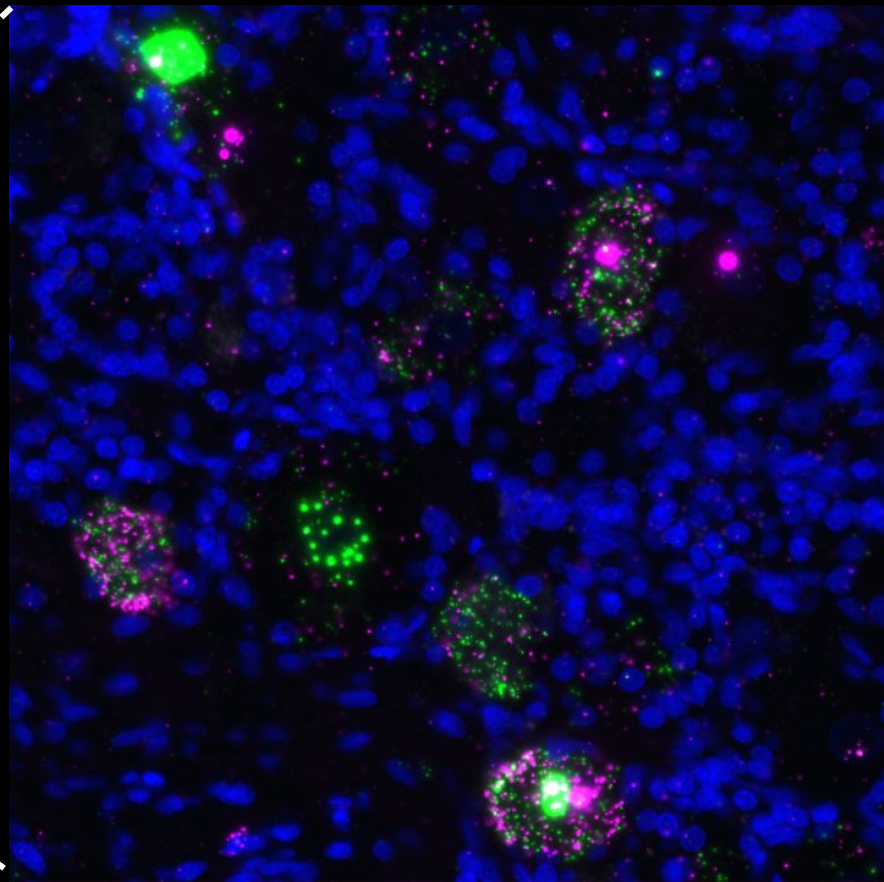
Pan-brain neuronal transduction quantified from histology (in Fox3+ cells)





Off-target DRG transduction similar to AAV9

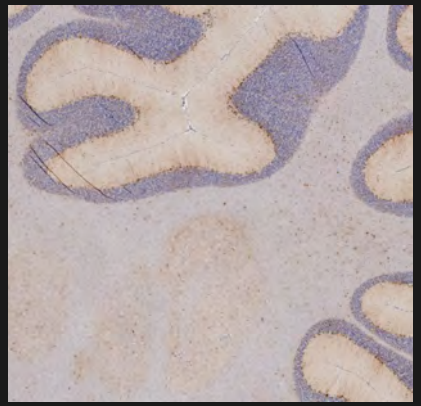




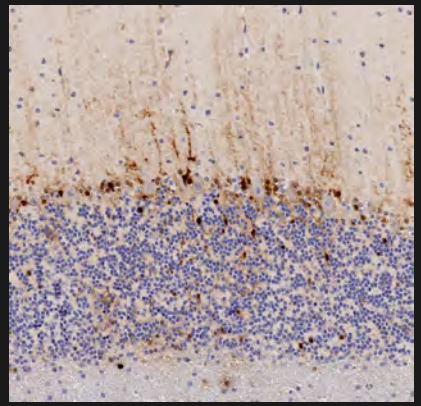
Cyno NHP at 1e13 vg/kg dose
RNAscope: **Dyno bCap 1** / AAV9

IHC confirms RNAscope quantification

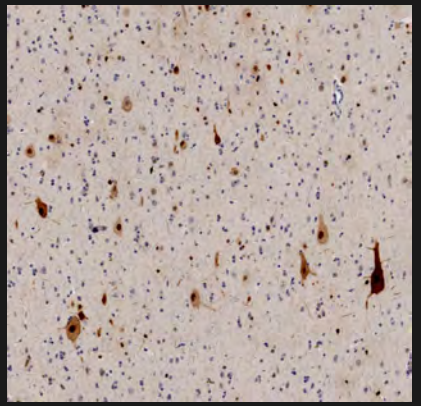
Dyno bCap 1
(nuclear)



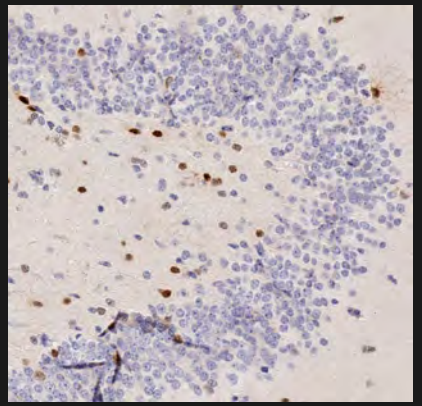
Cerebellum



Purkinje Layer

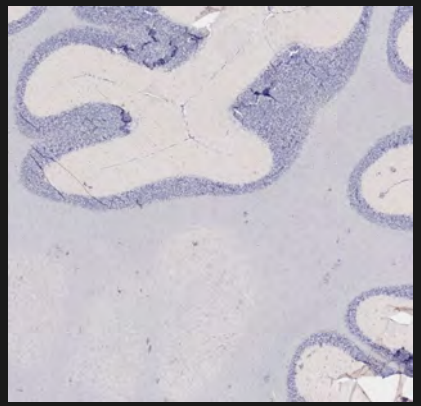


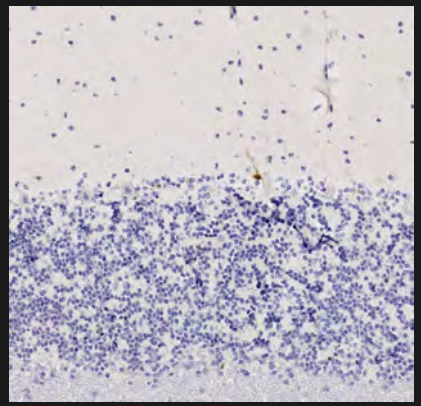
Motor Cortex

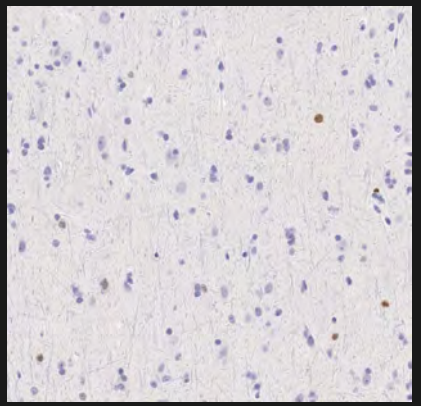


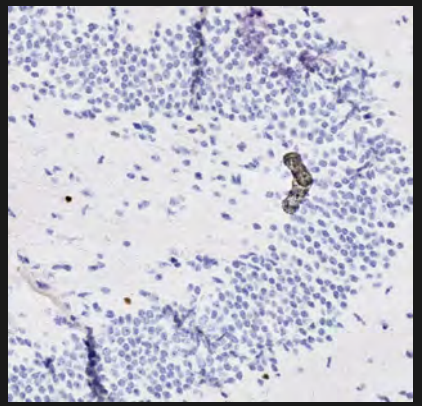
Dentate Gyrus

AAV9
(nuclear)

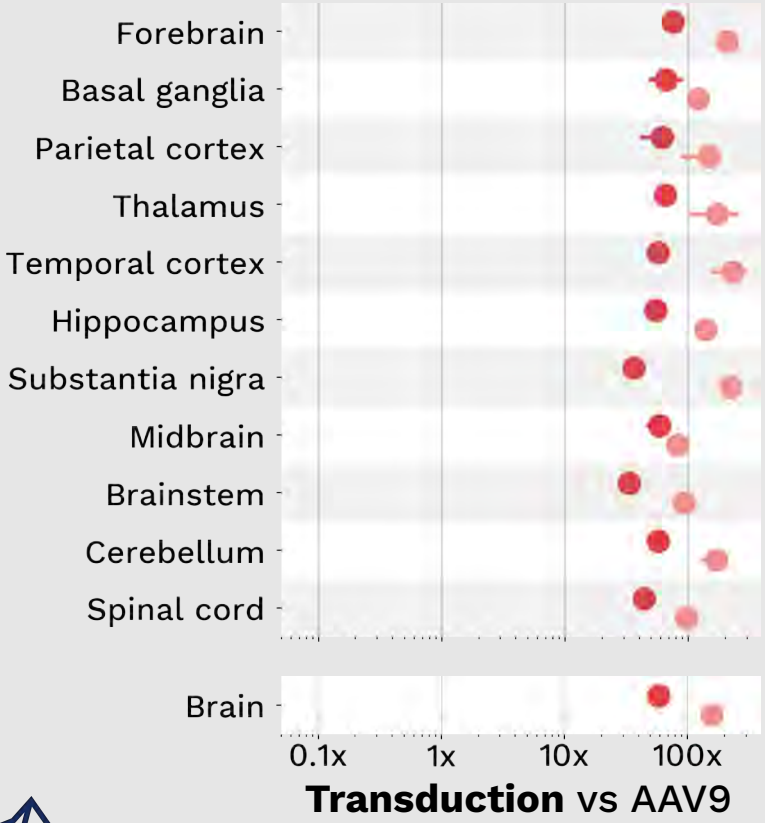




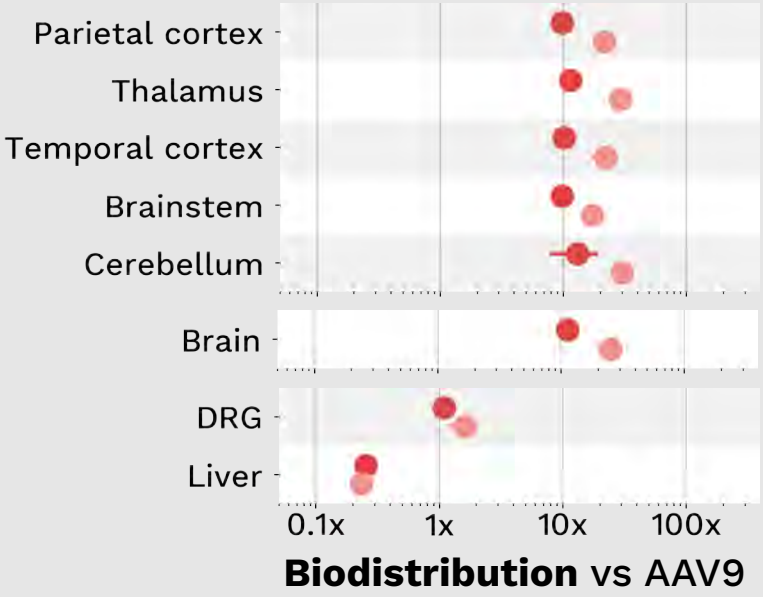




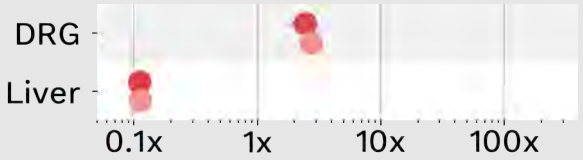
Pan-brain transduction quantified from NGS



Transduction vs AAV9



Biodistribution vs AAV9



Transduction vs AAV9

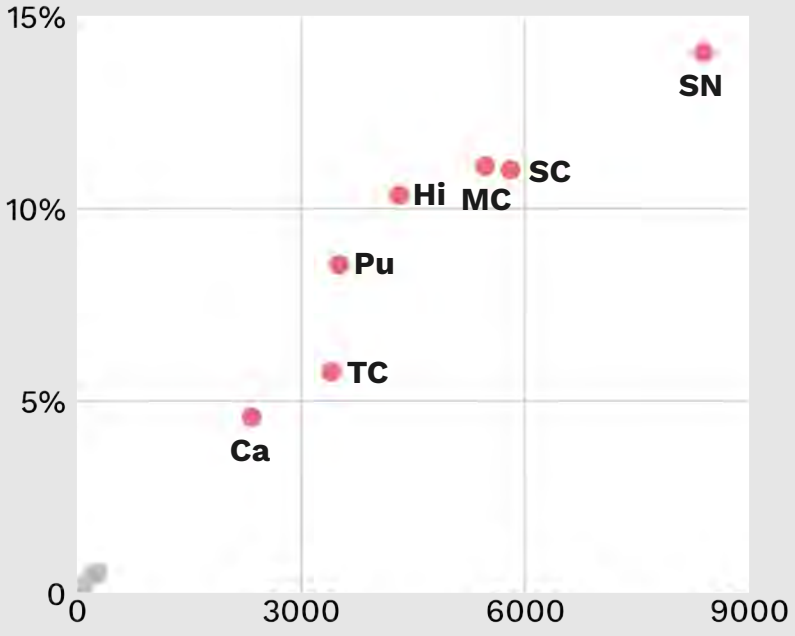
Cyno 1
1e13 vg/kg

Cyno 2
5e12 vg/kg

same dose per capsid

Transduction by NGS closely matches histology

% cells transduced, quantified from histology

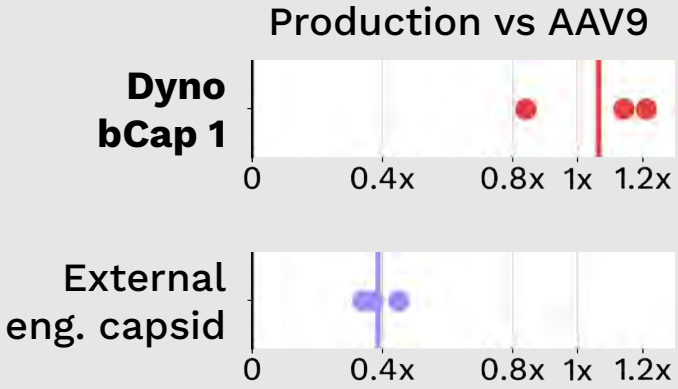


Barcode-ids counted per µg of tissue RNA

Dyno bCap 1
AAV9

SN : Substantia nigra
MC : Motor cortex
SC : Spinal Cord
Hi : Hippocampus
Pu : Putamen
TC : Temporal cortex
Ca : Caudate

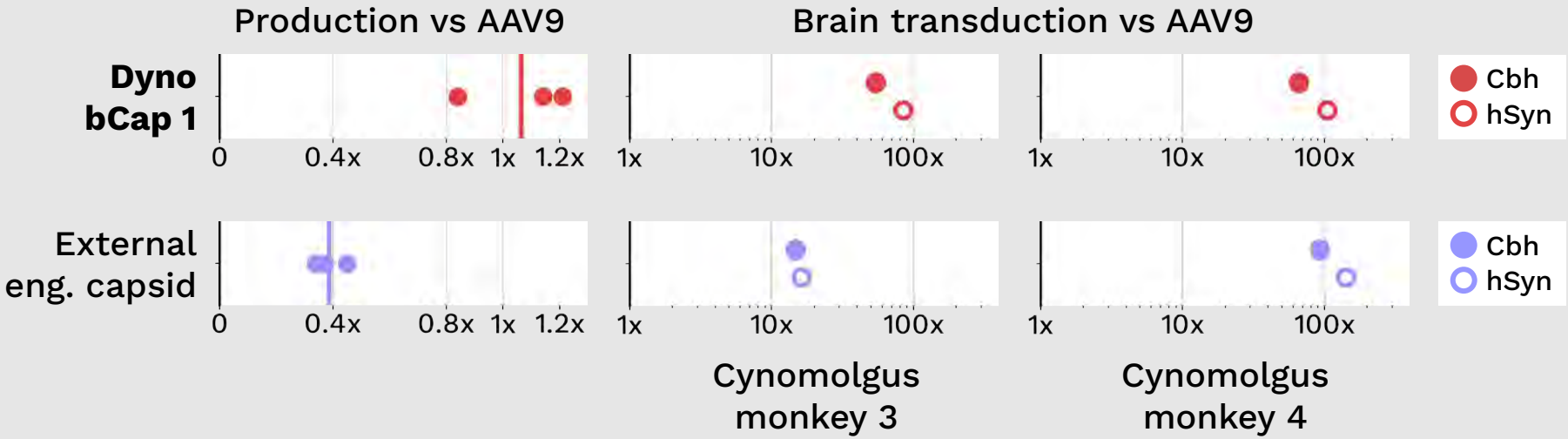
Dyno bCap 1 capsid has the greatest all-around potential for CNS IV delivery



Dyno bCap 1 produces with **same efficiency** as WT AAV9

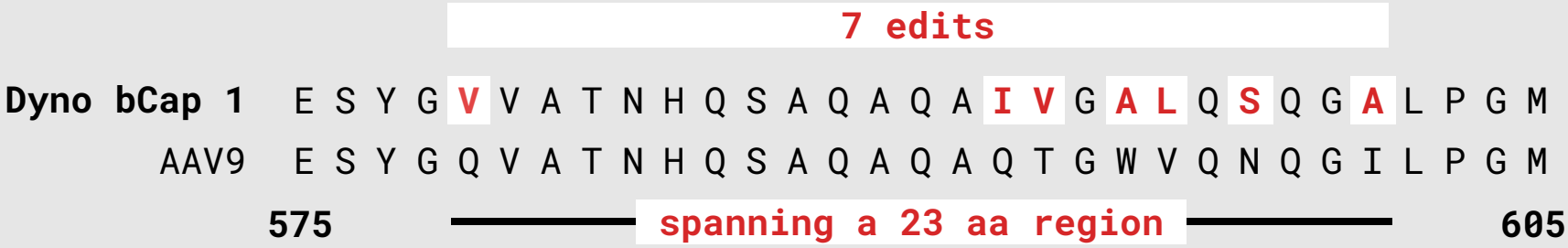
External engineered capsid produces with **0.4x efficiency** compared to WT AAV9

Dyno bCap 1 capsid has the greatest all-around potential for CNS IV delivery

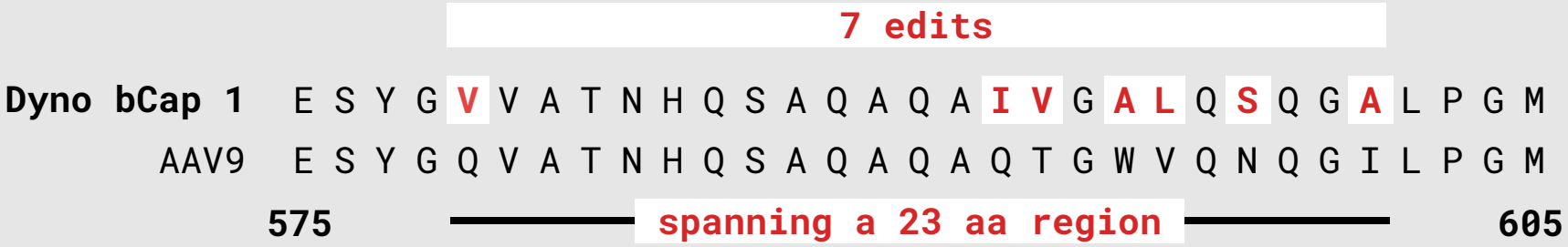


Dyno bCap 1 shows better consistency across the same NHPs in multiplexed studies

The power of Dyno's platform.



The power of Dyno's platform.



>100,000 capsids scale	2 capsids scale
Dyno bCap 1 capsid designed and tested	**Dyno bCap 1 capsid validated**

Reaching greater heights

>100,000 capsids scale

next iteration using **all proprietary data** and **generative AI**

>100,000 capsids scale

Dyno bCap 1 capsid designed and tested

2 capsids scale

Dyno bCap 1 capsid validated

>100,000 capsids scale

next iteration using **all proprietary data** and **generative AI**

>100,000 capsids scale

Dyno bCap 1 capsid designed and tested

2 capsids scale

Dyno bCap 1 capsid validated

Dyno-st2

2x transduction vs Dyno bCap 1

Dyno’s best capsids for CNS delivery

Dyno bCap 1

1x production vs AAV9

10x liver detargeting vs AAV9

100x CNS transduction vs AAV9

5-20% neurons transduced, pan-brain at **low dose of 1e13vg/kg**

Dyno-st2

2x transduction vs Dyno bCap 1

Dyno bCap 1

Dyno-st2

apsids for
very

Dync

1x produ

10x liver det

100x CNS tra

5-20% neurons t
at **low dos**

Dyr

2x transducti

ap 1

st2

Abstract 382:

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

Introducing Dyno’s best capsids in eye and CNS

Dyno-86m

Dyno-gvk

Dyno bCap 1

Dyno-st2

Partner with Dyno...

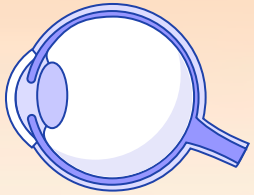
to work at the

leading edge of gene delivery

Dyno is open to partnering

Any organ	Any indication

Dyno's NHP-validated capsids are available for licensing



Dyno-86m

Dyno-gvk

Dyno bCap 1

Dyno-st2

Along with other emerging optimized capsids

Dyno: your partner of choice

- Reach out to bd@dynotx.com:
  - Learn more about our validation of **Dyno-86m**, **Dyno bCap 1** and other emerging capsids
  - Inquire about licensing in the eye, CNS and other organs

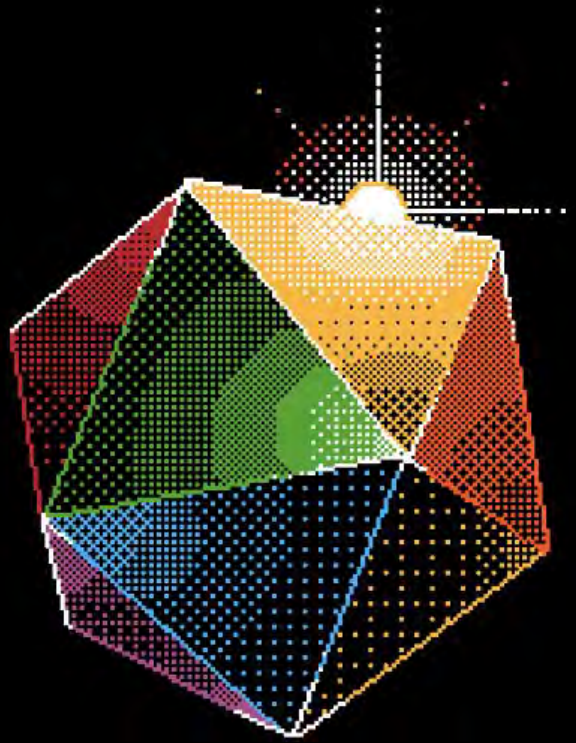
We look forward to supporting your gene therapy development needs!

Reaching greater heights

- **Dyno's mission:** Empowering diverse teams of high-potential problem solvers to transform patient lives with cutting edge science
- **Dyno's business model:** We're partnership-centric: Dyno succeeds only with partners and patients win
- **Dyno's platform works!** **Dyno-86m** and **Dyno bCap 1** sequence created with generative AI

- **Dyno has NHP-validated capsids with field leading potential:**
  - **Dyno-86m** for eye
  - **Dyno bCap 1** for brain
- **Emerging Dyno capsids are already in NHP-validation:**
  - **Dyno-gvk** for eye
  - **Dyno-st2** for brain
- **All of these capsids are available for licensing today:** contact bd@dynotx.com

To celebrate the arrival of Dyno bCap 1



DYNO Therapeutics

DYNO_bCAP_1>MAADGYLPDWLEDNLS
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AFNKDKLNSFITQYSTGQVSVEIEWELQ
KENSKRWNPEIQYTSNYYKSNNVEFAVN
TEGVYSEPRPIGTRYLTRNL

To celebrate the arrival of Dyno bCap 1

This is just the beginning

of how AI and optimized capsids like Dyno bCap 1

will solve gene delivery and unlock new therapeutic potential,

leading to transformative gene therapy products

that help more partners and many more patients.

Thank you for joining us!