Dyno Therapeutics

The Capsids You Need

NHP-validated Capsids for Best-in-class CNS and Ocular Gene Delivery

ESGCT 2023

EXCITING TIMES

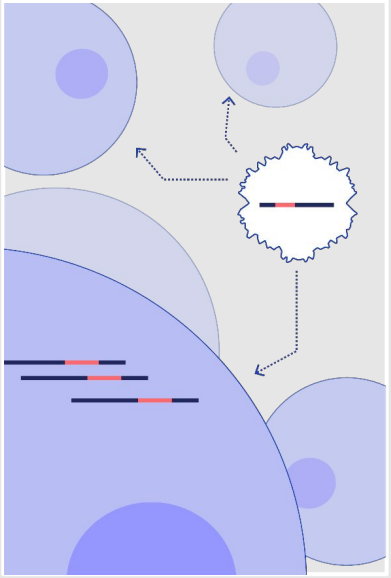
INCREDIBLE POTENTIAL

DELIVERY

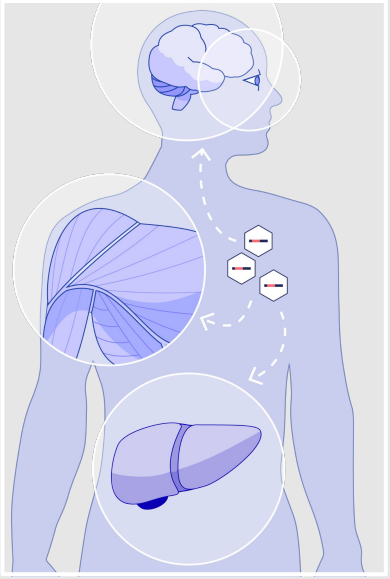
our shared challenge

Dyno’s goal is solving in vivo gene delivery

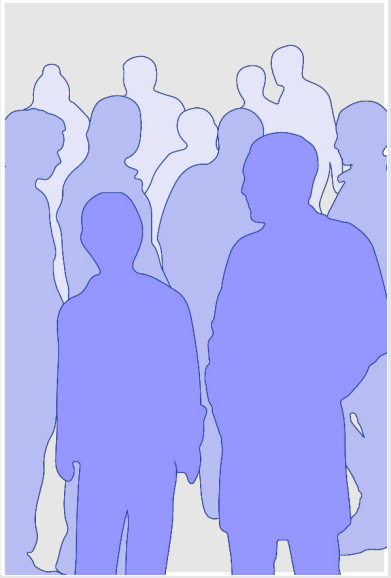
Every cell



Every organ



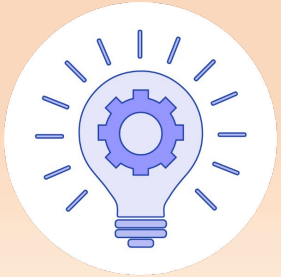
Every patient



Dyno:

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

Why partner with Dyno?



Innovation

Resilience

Drive

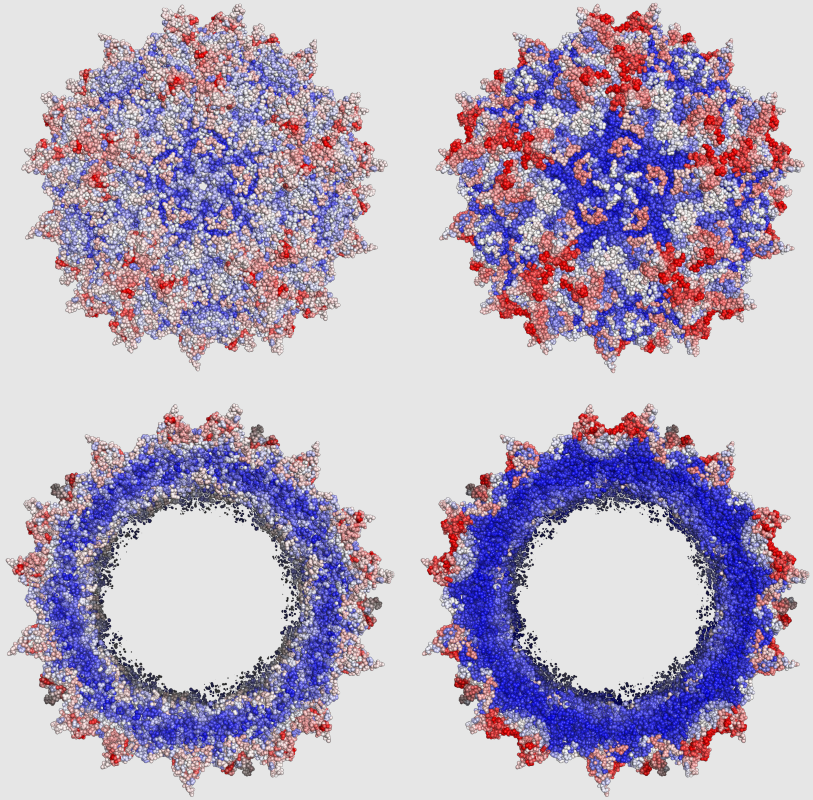
100% focused on capsid engineering

Dyno's origins

Using multiplexing, in **one experiment:**

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

Substitutions **Insertions**

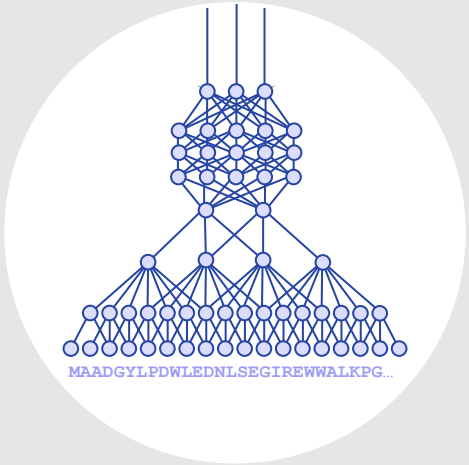


Ogden & Kelsic et al., Science 2019

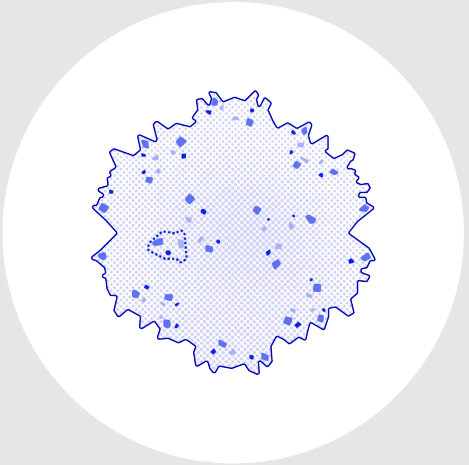
Dyno's team has grown!

Dyno’s platform

Data excellence



AI excellence



Better capsids

Why partner with Dyno?

Partnership-centric business model = 100% alignment

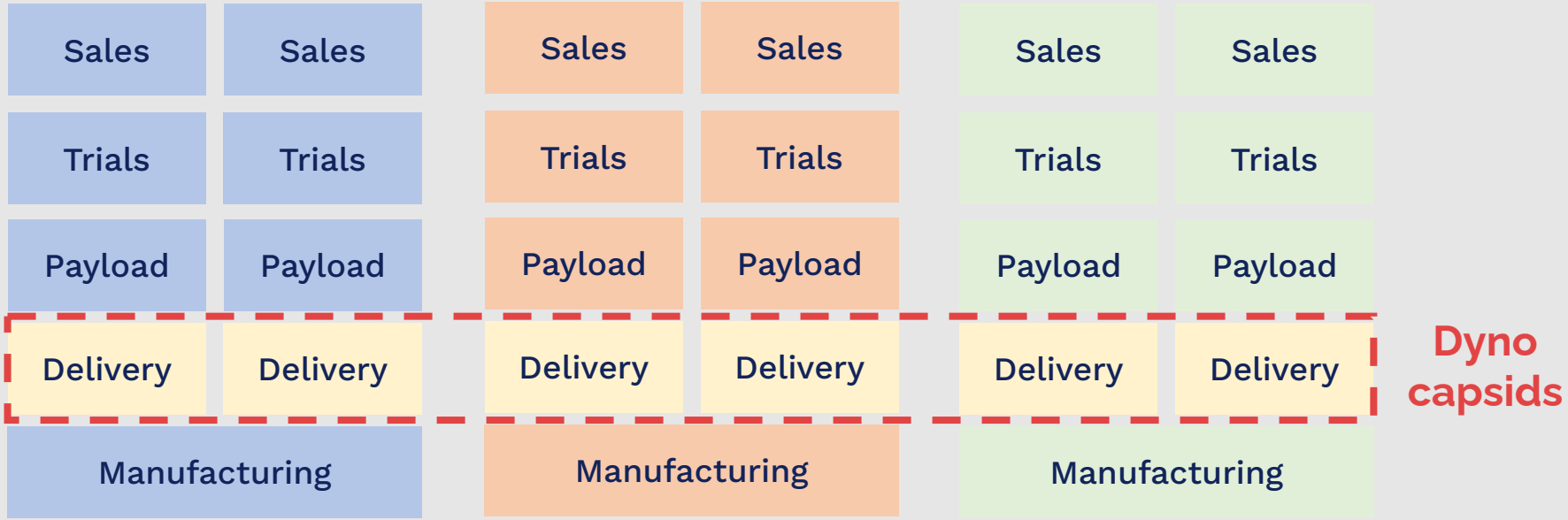
Openness

Trust

Continuous Improvement

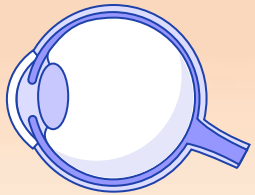
Dyno's partnerships to date...

Our ambition is solving delivery generally and broadly



We are open to partnering

Any organ



Dyno eCap 1

Any indication

Dyno bCap 1

Along with other emerging optimized capsids

Dyno Therapeutics

Engineering the world's best AAV capsids

so our partners can work

at the leading edge of gene delivery

Challenge: safe and effective gene delivery

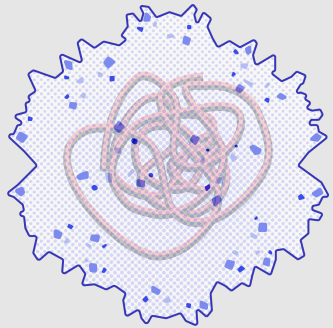
IV injection

Easily **administered**

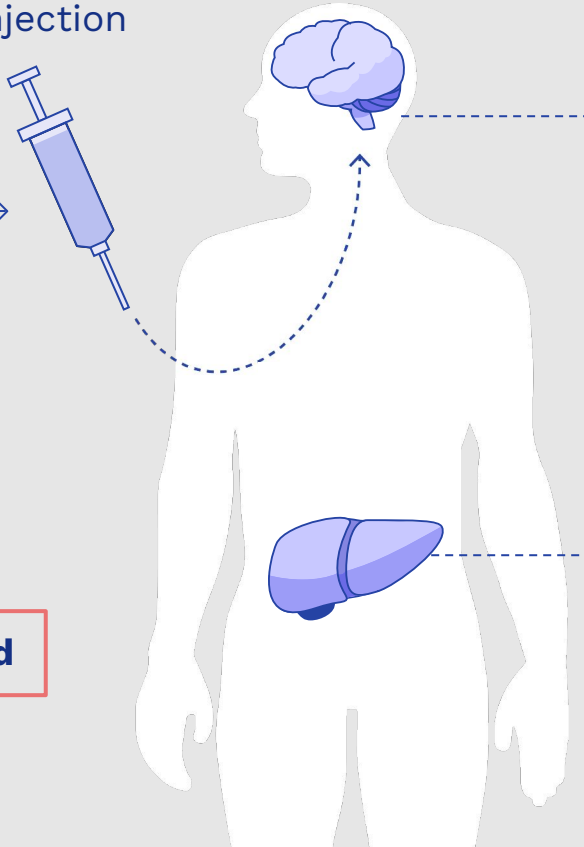
AAV capsid

+

genetic payload



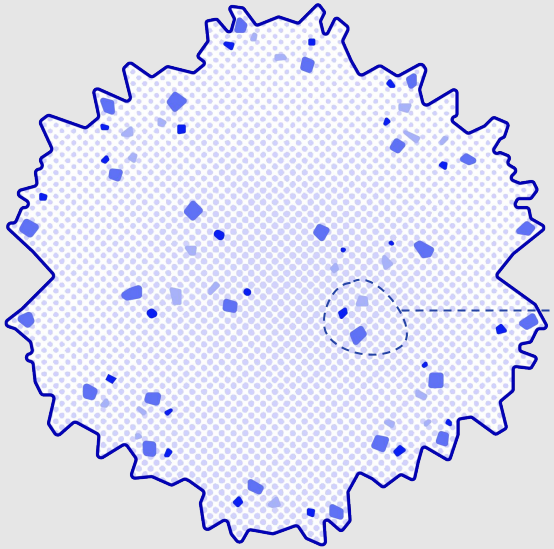
Easily **manufactured**



Transducing **target organs**

De-targeting **peripheral organs**

The AAV capsid: a ~736 letter sequence design problem



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GEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWL
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YFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTDNN
GVKTIANNLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQ
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SSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTLKFSVAGPSN
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MNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEE
EIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQGILPGMVWQDRD
VYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADP
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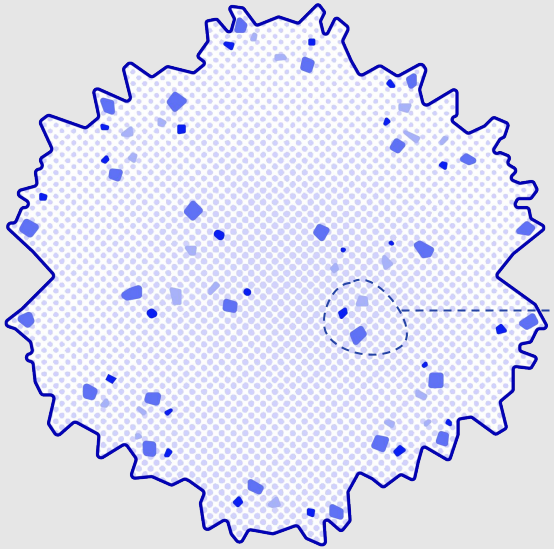
YKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL*

A better capsid: Dyno bCap 1 sequence



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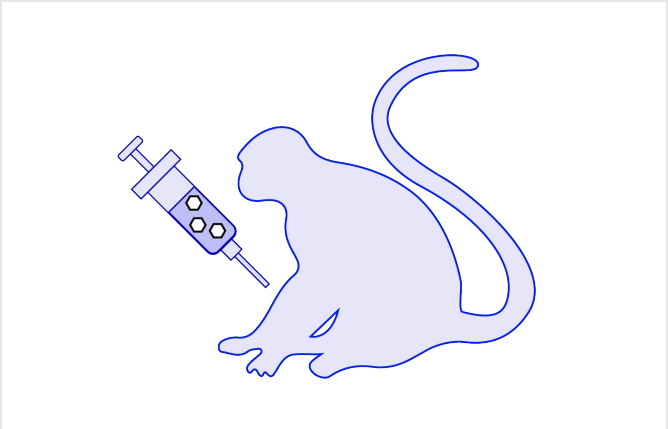


Dyno bCap 1 delivery

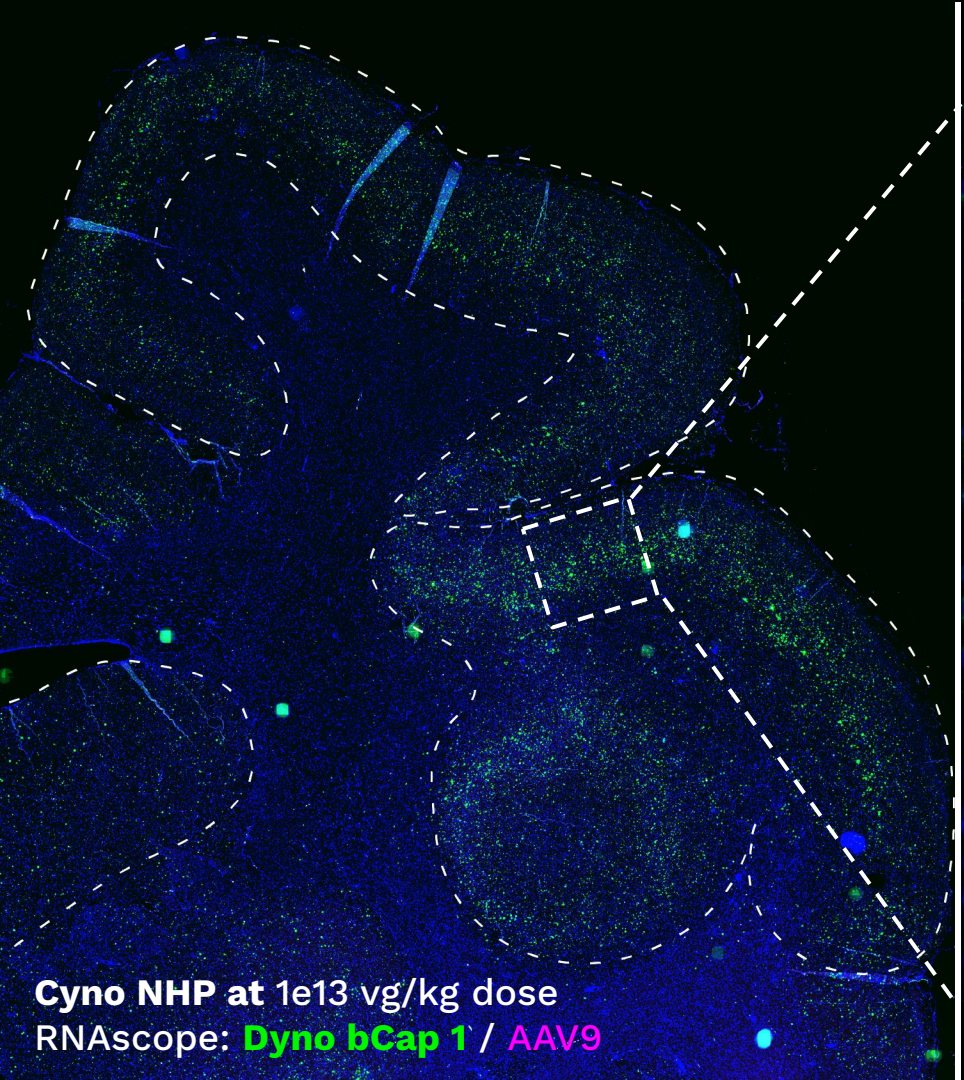
Delivers **pan-brain** and across the **CNS,** crossing the **blood-brain-barrier** after IV administration

1x production vs AAV9

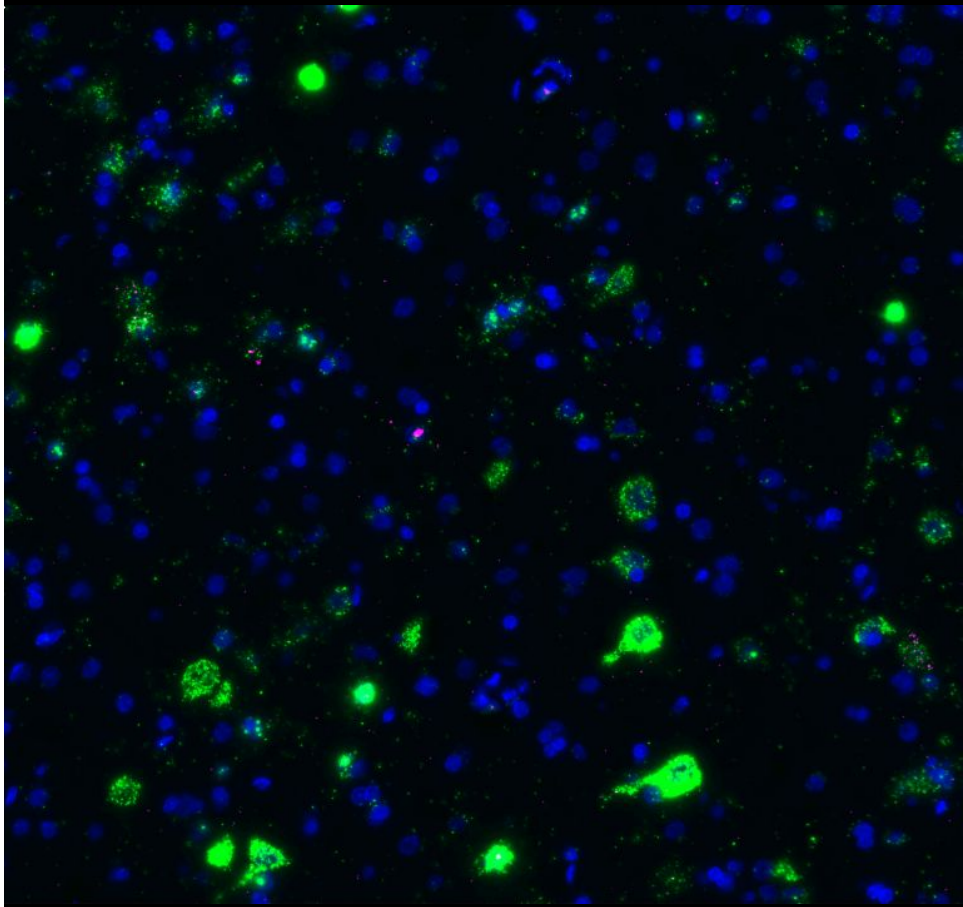
10x liver detargeting vs AAV9

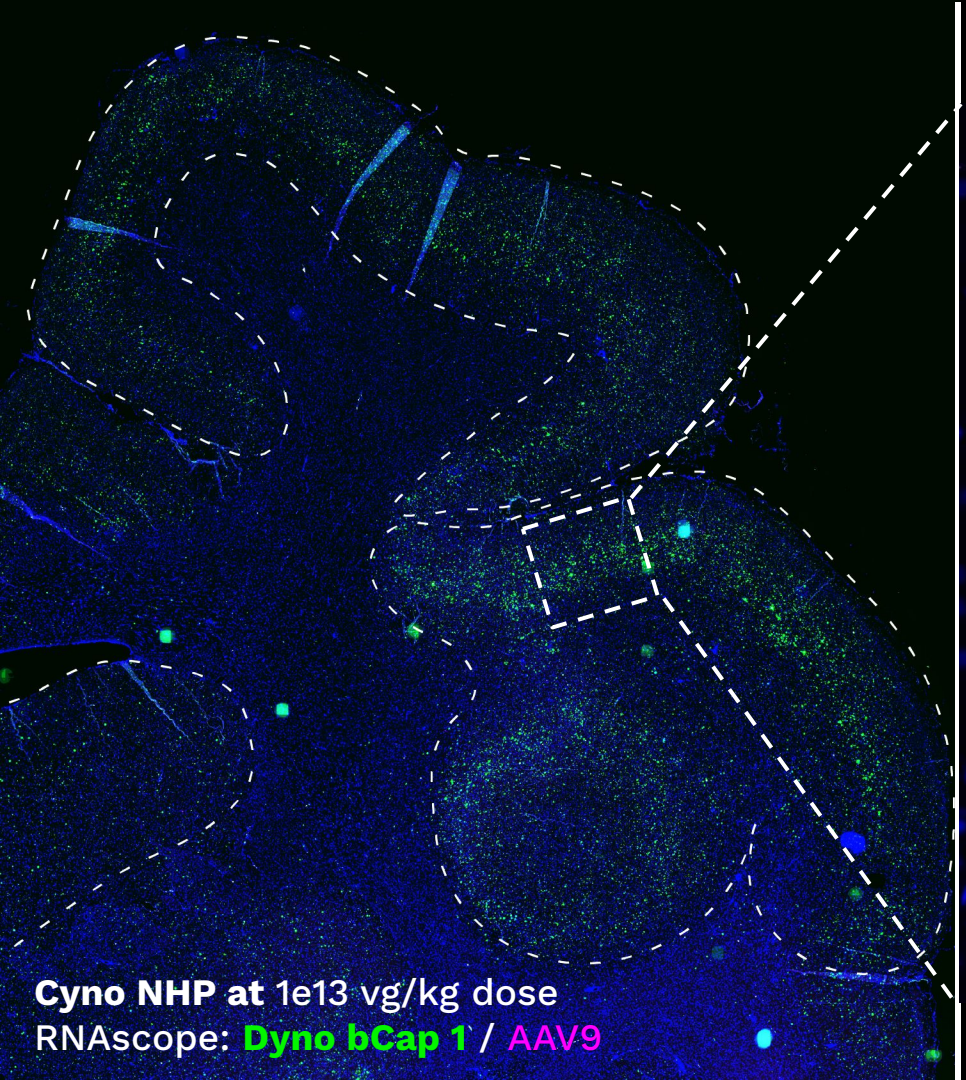
100x brain transduction vs AAV9

Transduces neurons and other therapeutically relevant cell-types

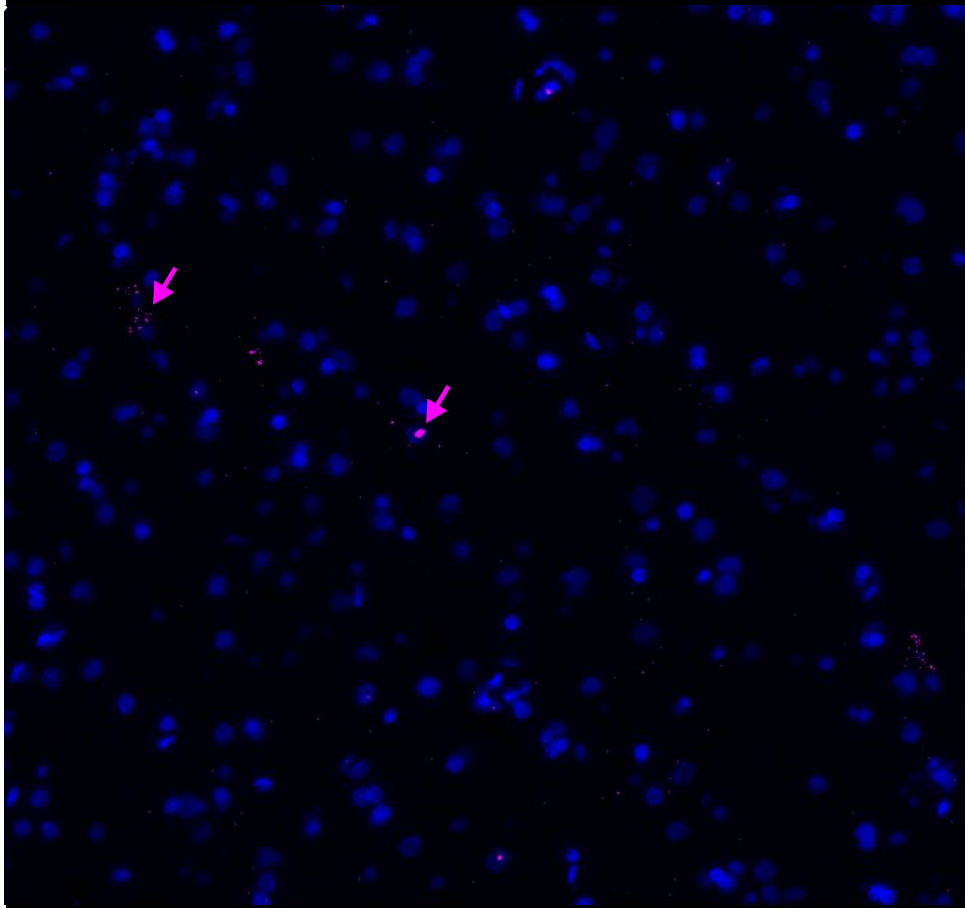


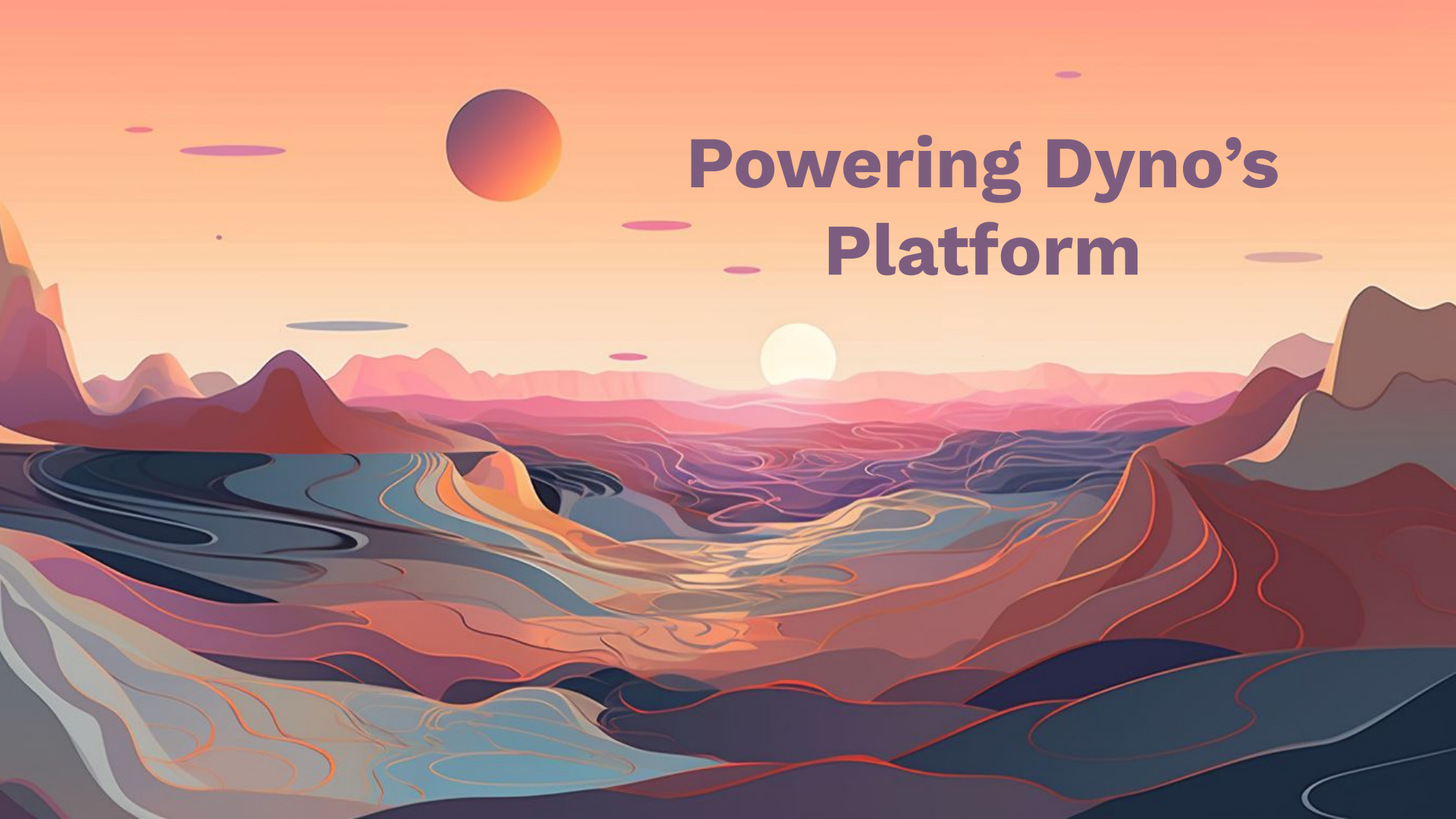
Motor cortex: 11% of cells transduced



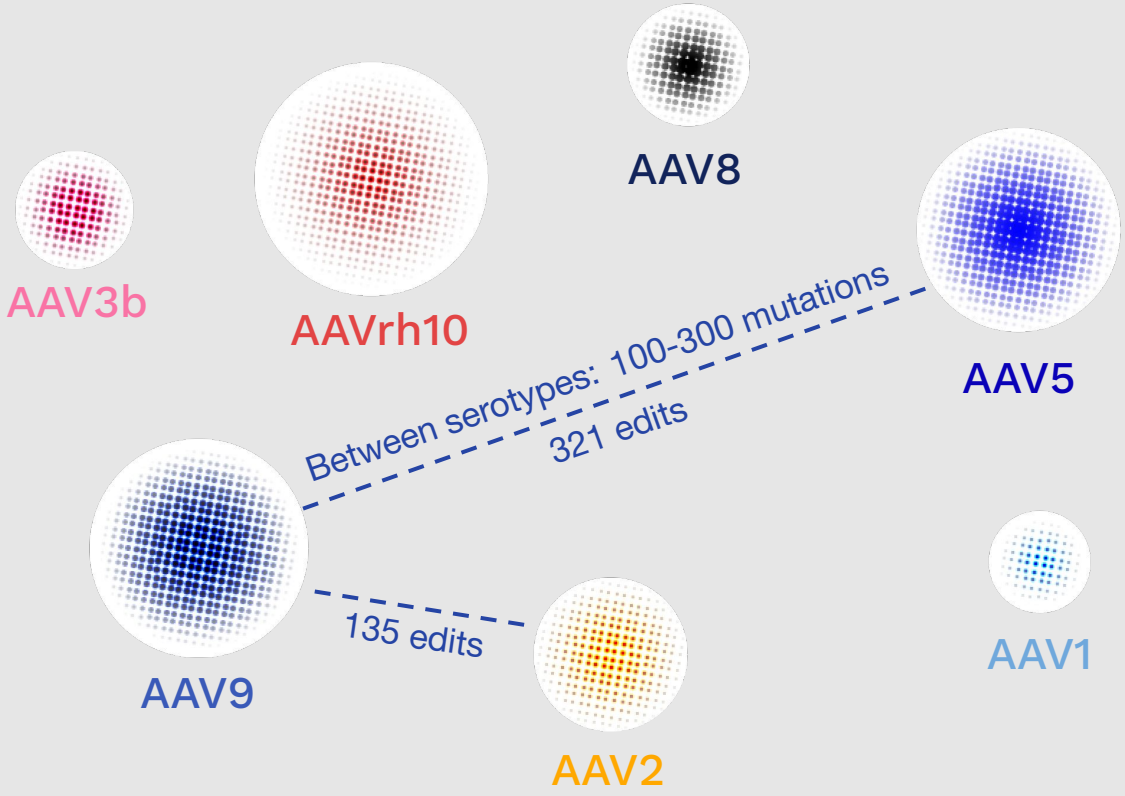


Motor cortex: minimal AAV9 transduction



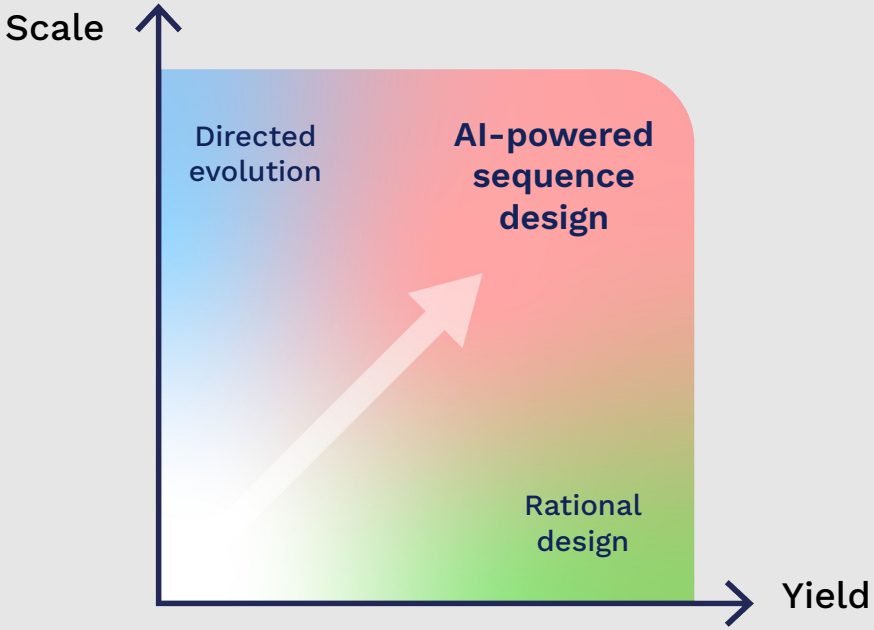

Powering Dyno’s
Platform

AAV capsid sequence space is vast and unexplored



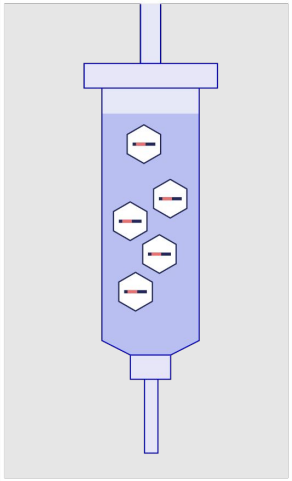
Most mutations yield **non-functional capsids**

Solving the sequence design challenge with AI-powered design



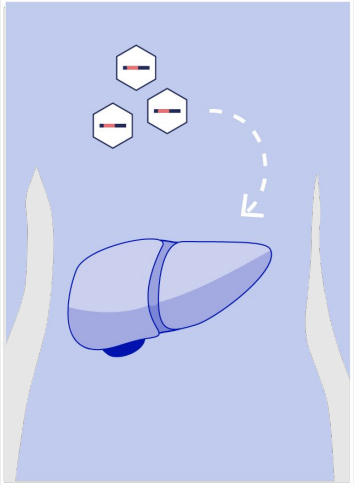
We directly measure key capsid properties

Production



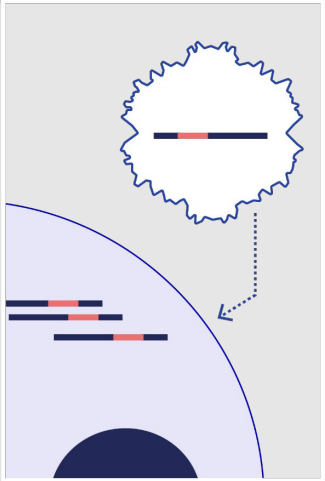
Vector genomes post purification

Biodistribution



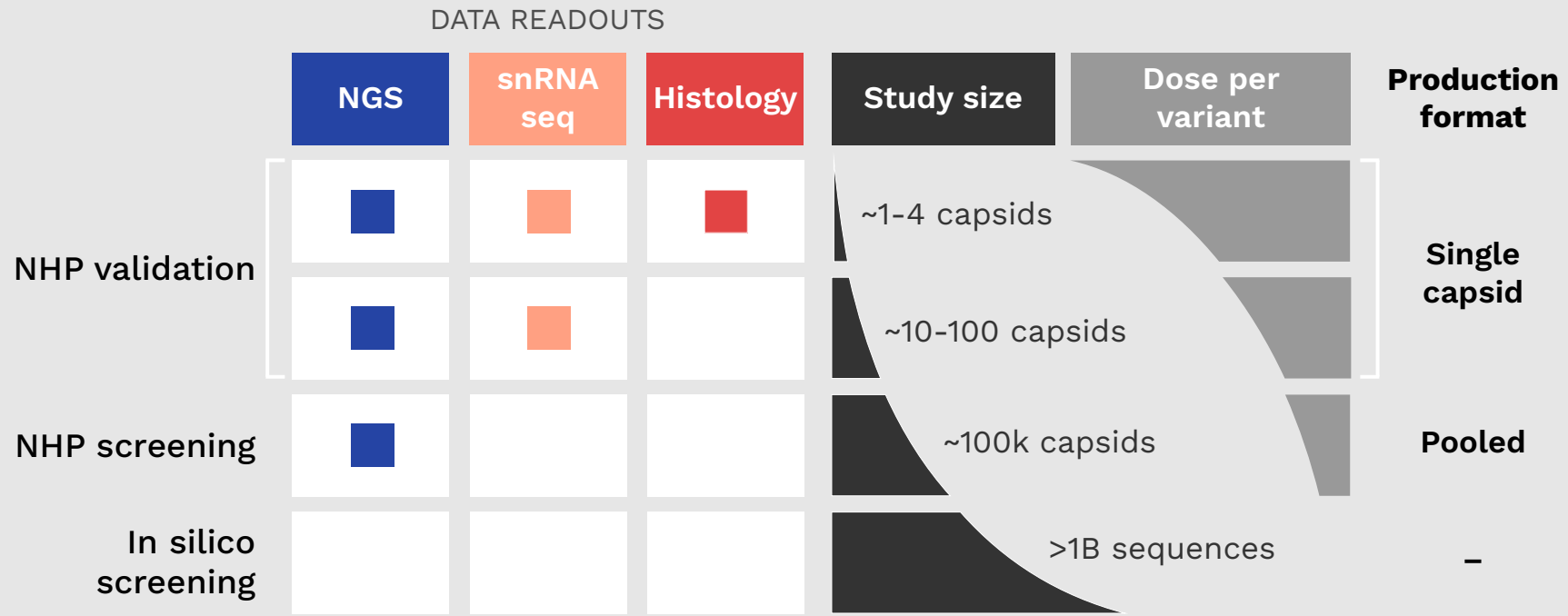
Vector genomes across organs

Transduction

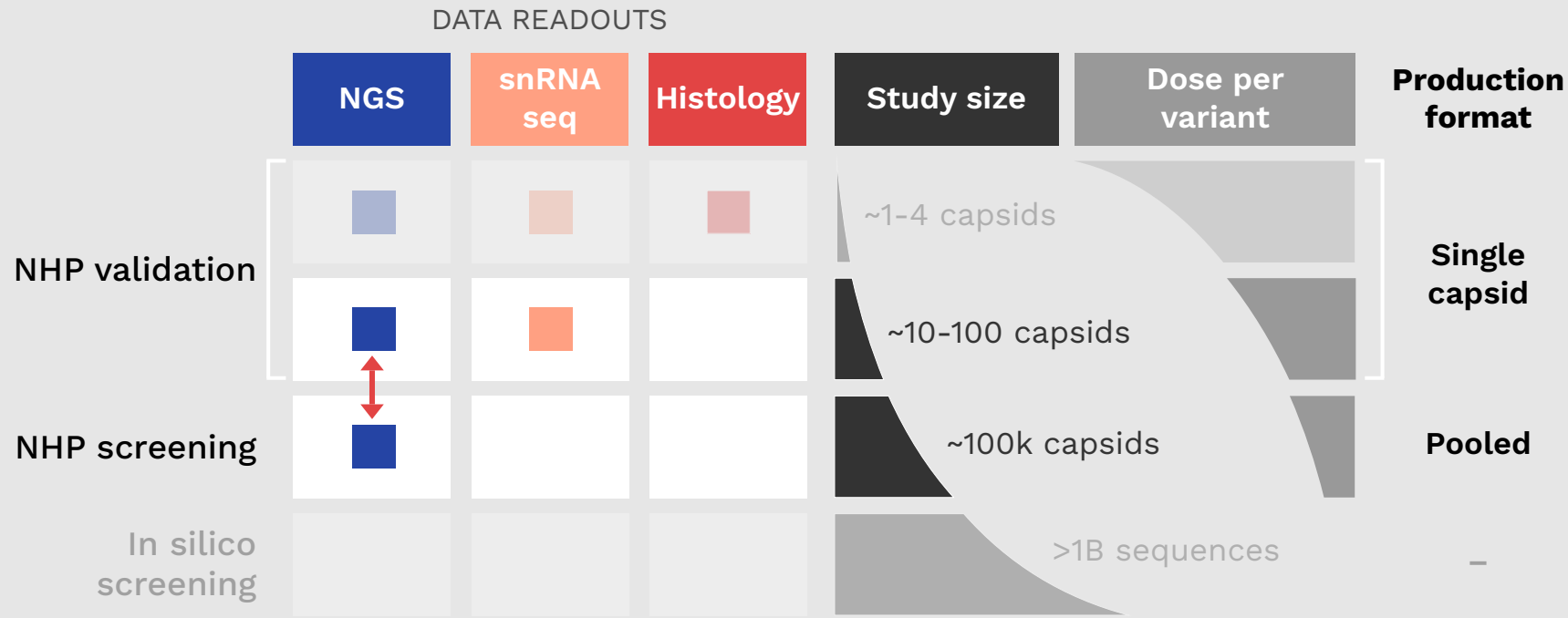


Transgene expression across organs

Our platform operates at four scales

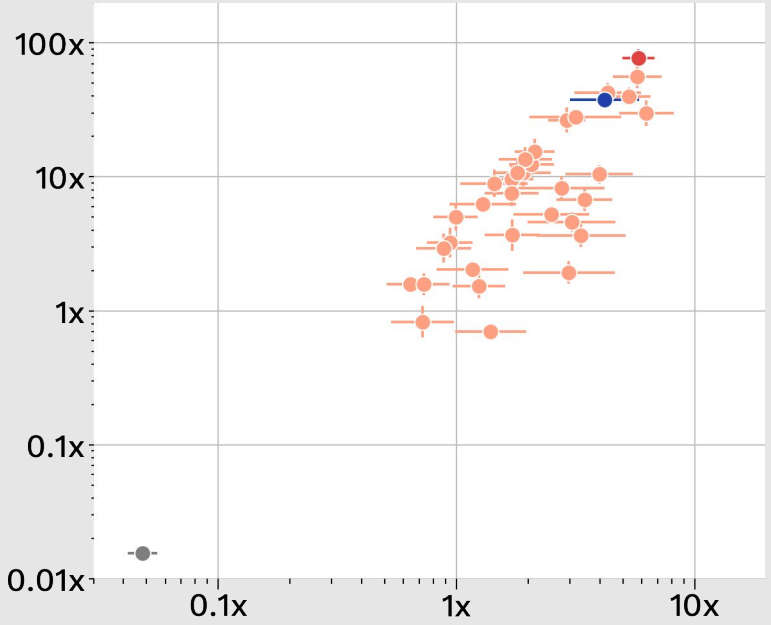


Our platform operates at four scales



Data agree across different scales

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



Dyno eCap 1

Dyno capsids

External engineered capsid

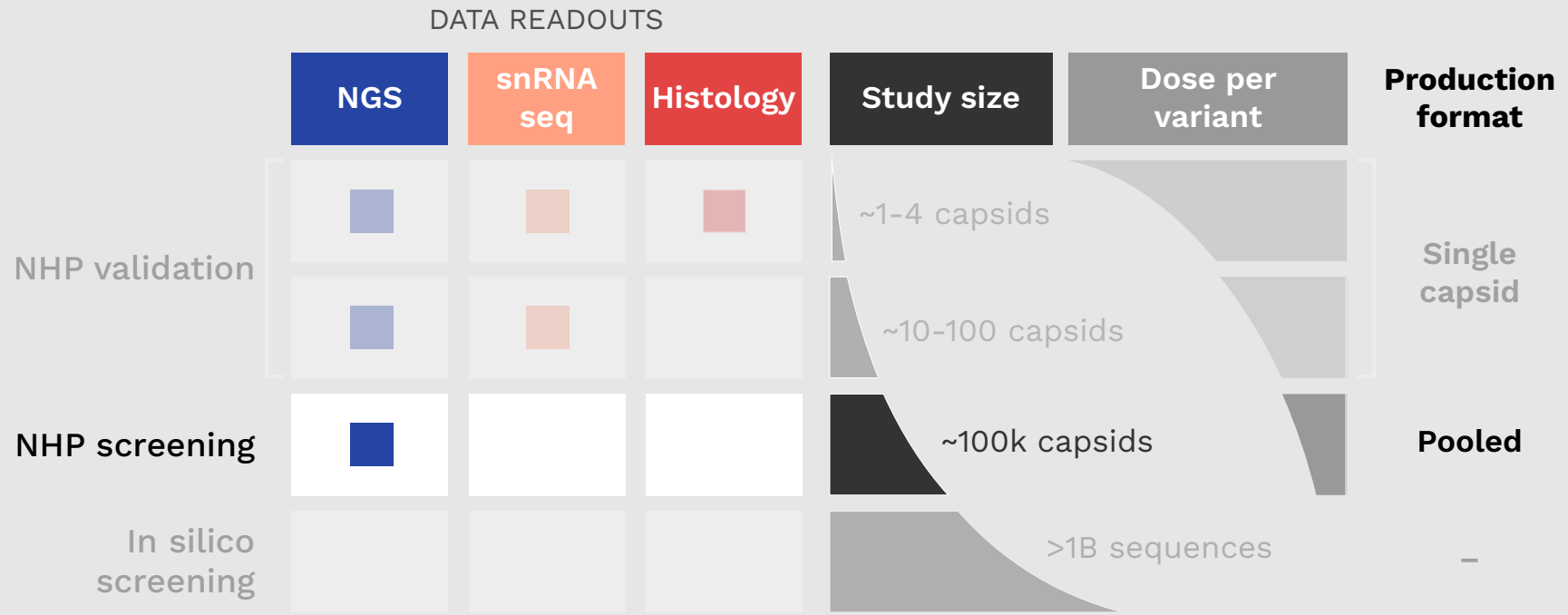
Negative control

Intravitreal delivery

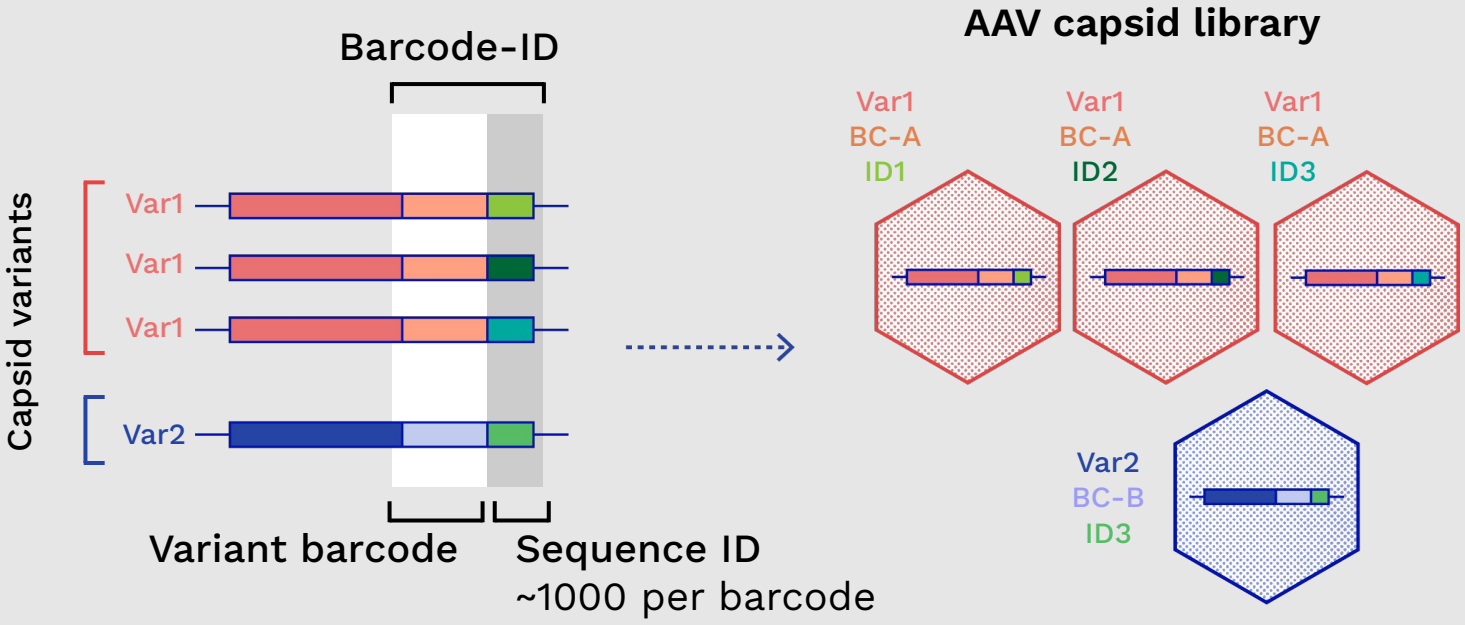
Measured with **~10-100** capsids multiplexed

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

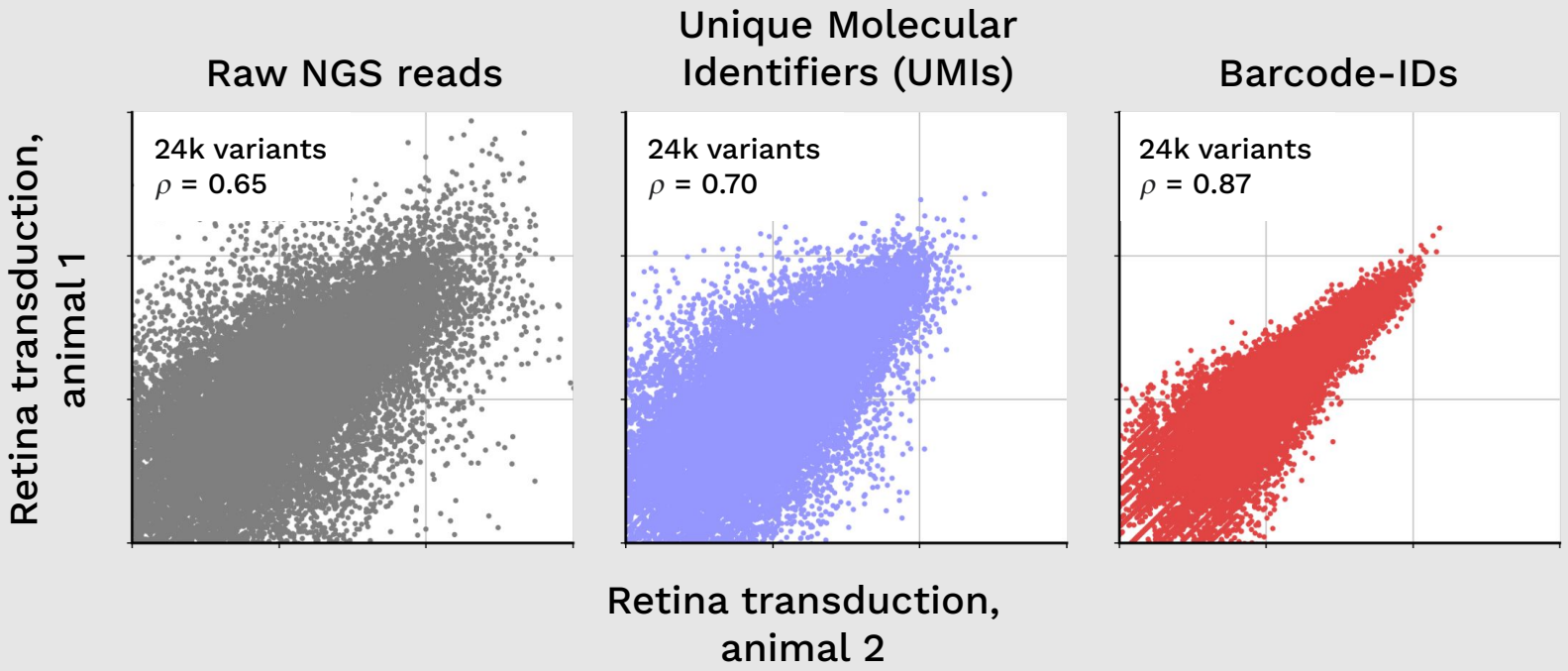
Our platform operates at four scales



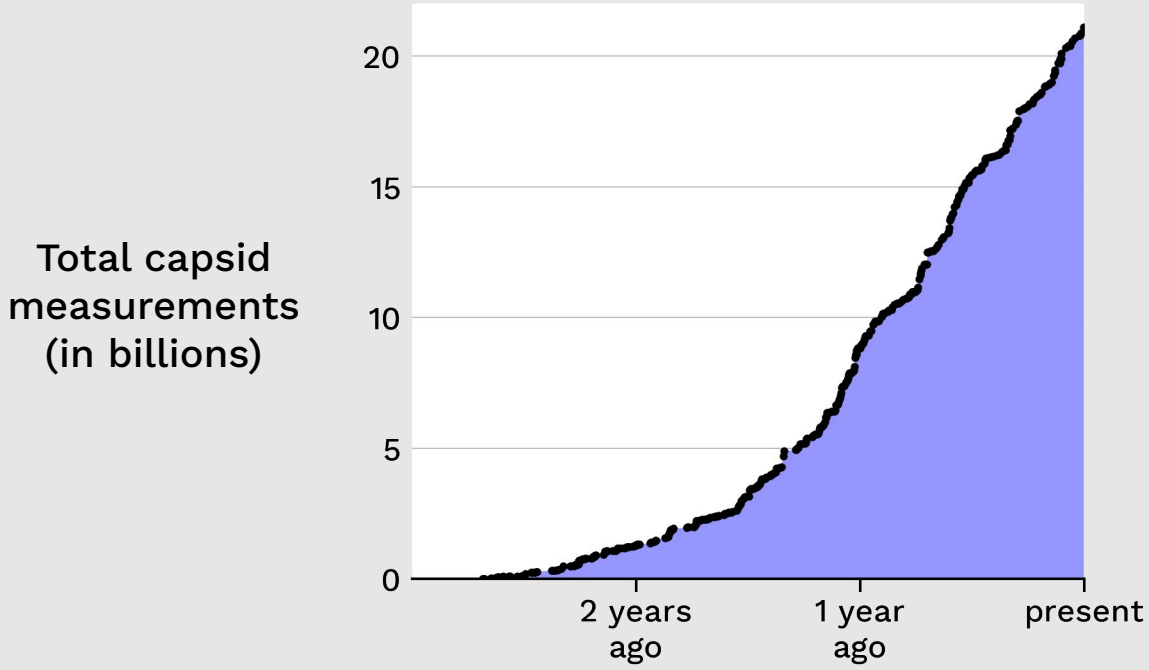
Molecular barcoding via barcode-IDs



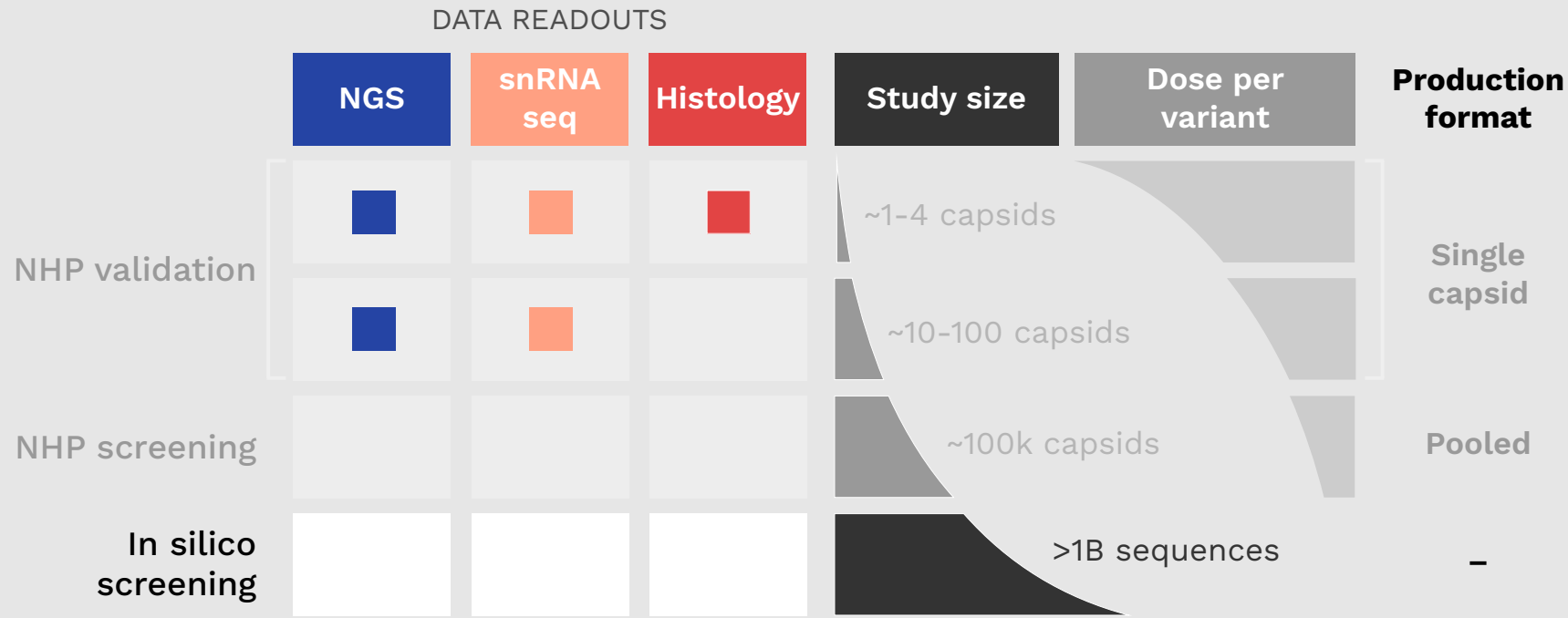
Quantification of transduction with barcode-ids increases data reproducibility



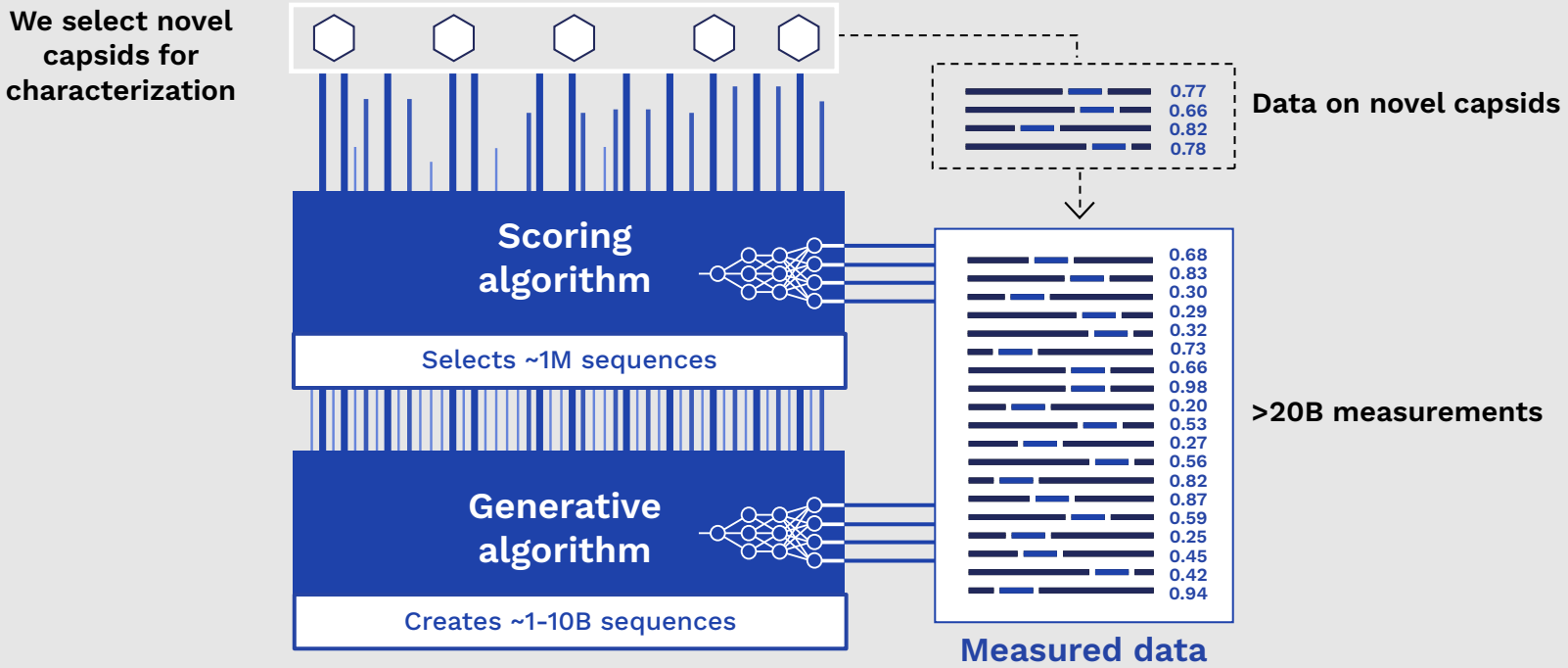
We make billions of measurements every month



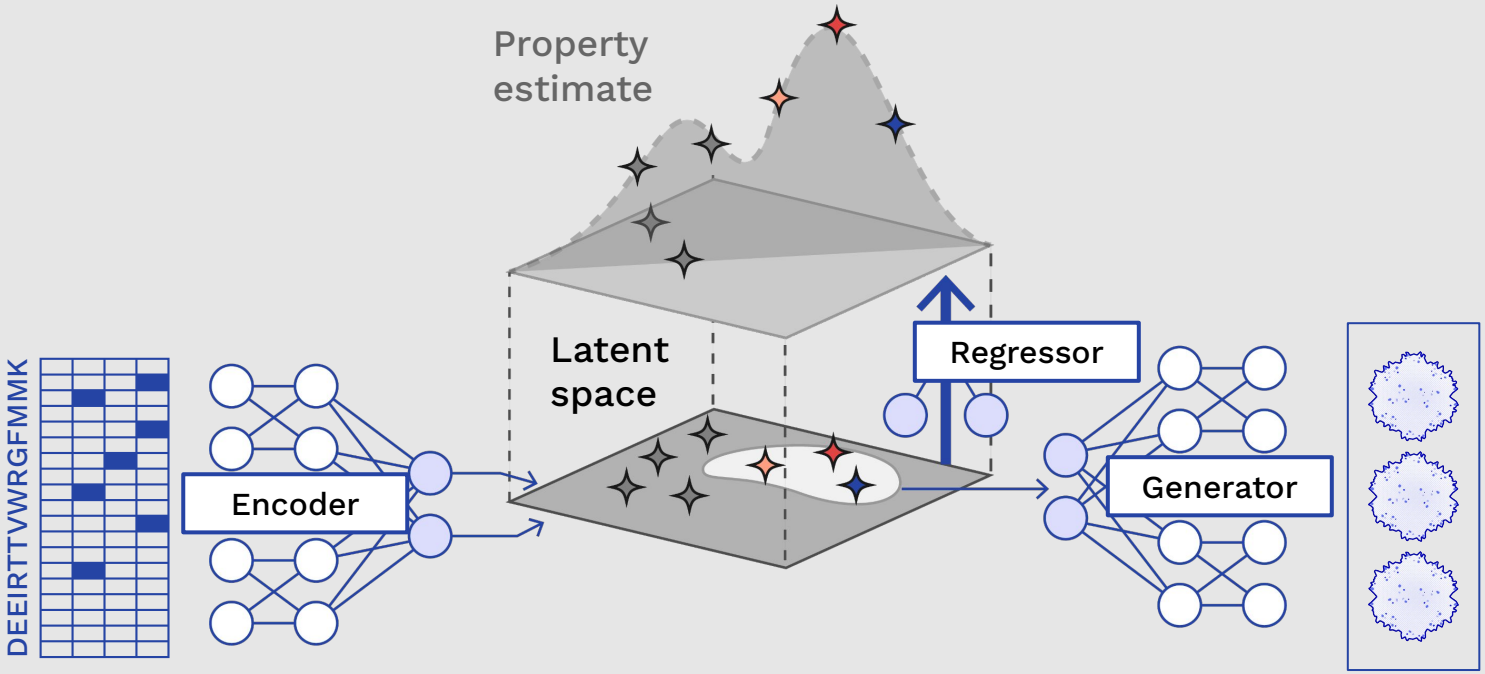
Our platform operates at four scales



We continuously improve our machine-learning models with each new measurement



We program AI to generate optimized capsid sequences



Sinai et al. arxiv 2017, Sinai et al. Biorxiv 2021, ESGCT23 Abstract P076

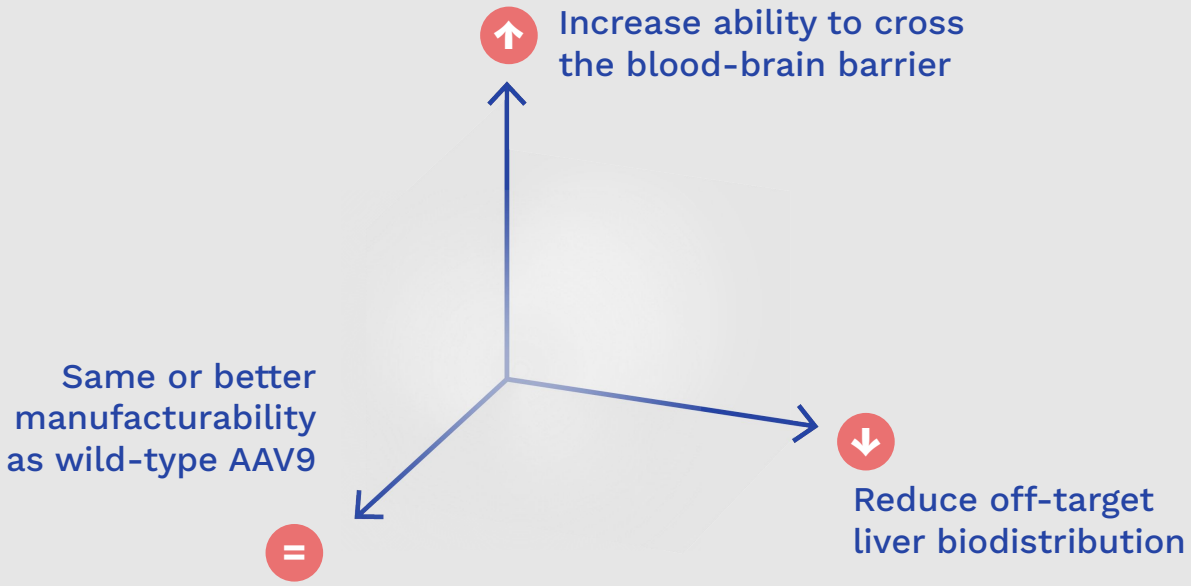
We program A … sid sequences

Abstract P076:

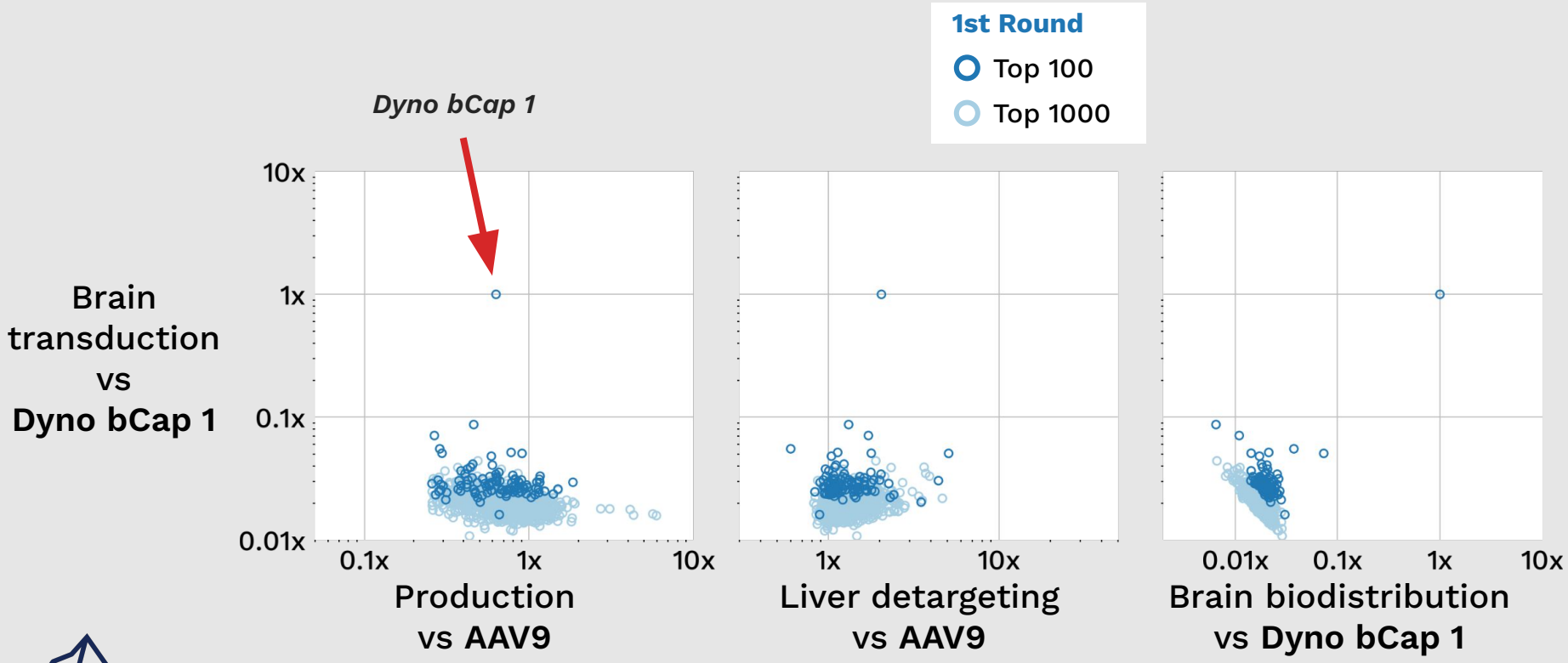
"VAEprop: A generative machine learning approach for designing high-performing AAV capsids for the non-human primate brain"

Sinai et al. arxiv 2017, Sinai et al. Biorxiv 2021, ESGCT23 Abstract P076

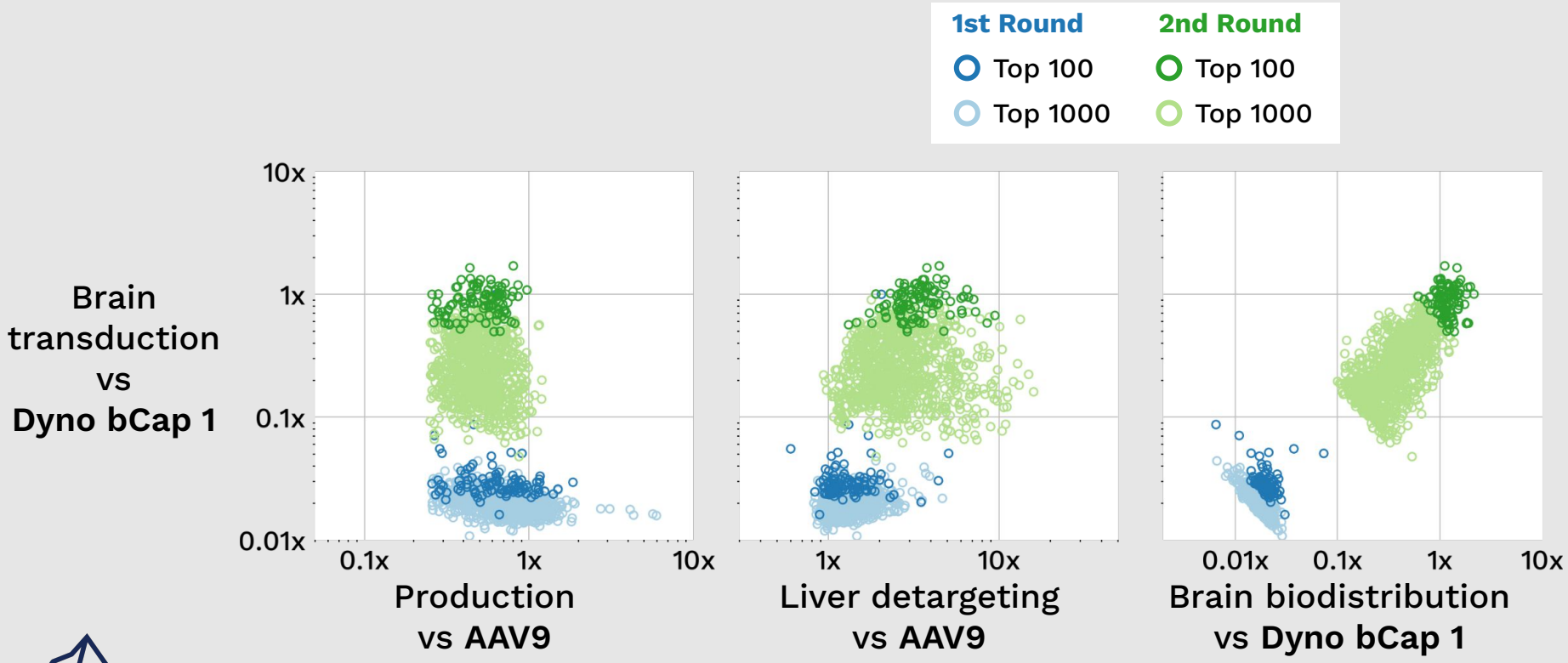
We optimize capsids across multiple properties



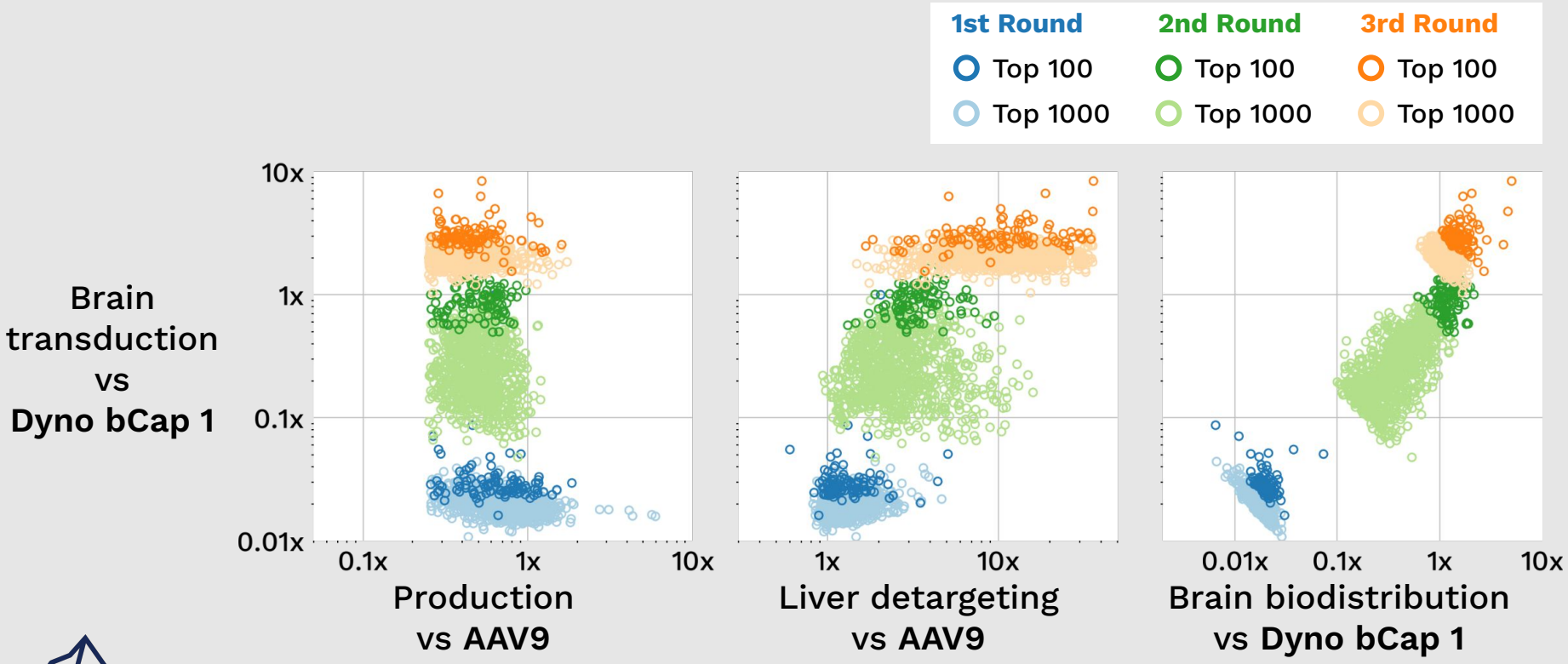
Example: Optimization for CNS gene delivery



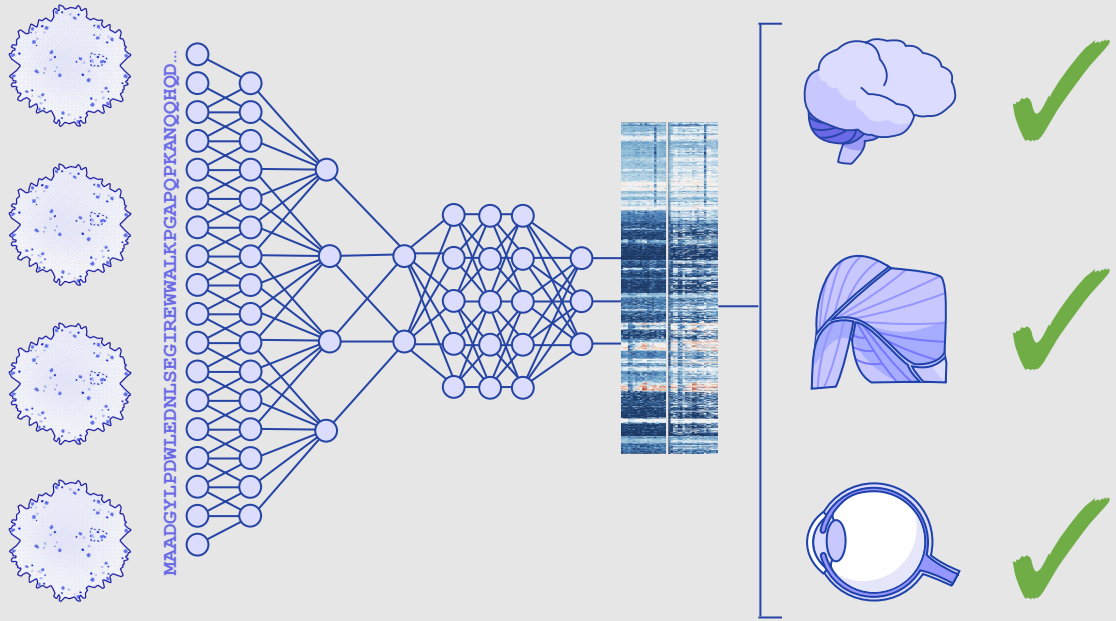
Example: Optimization for CNS gene delivery



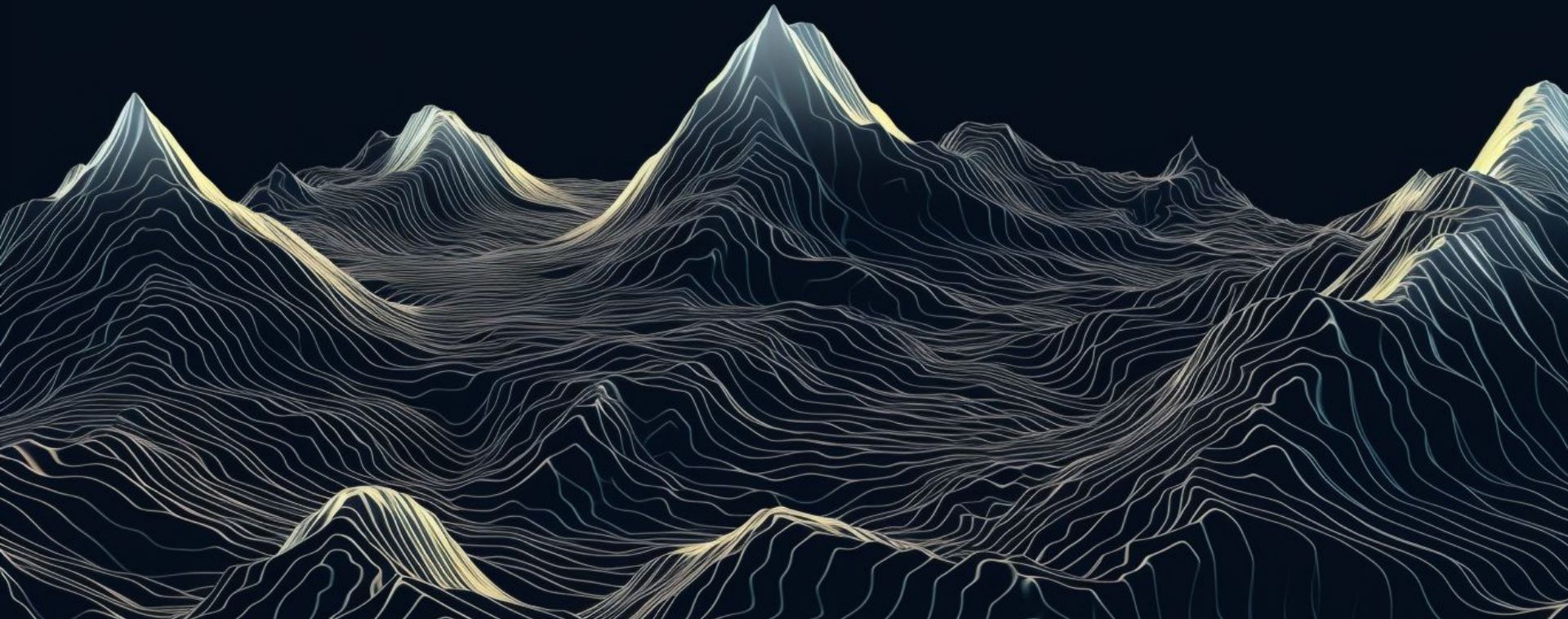
Example: Optimization for CNS gene delivery



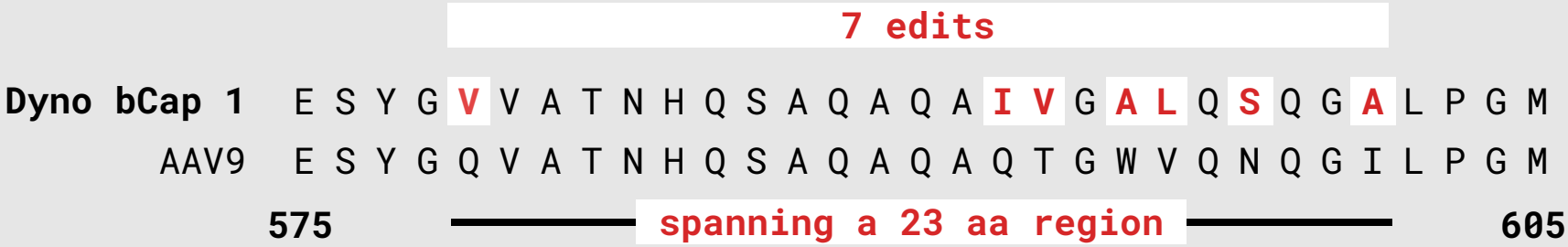
We created field-leading AAV capsids



Dyno Brain capsids



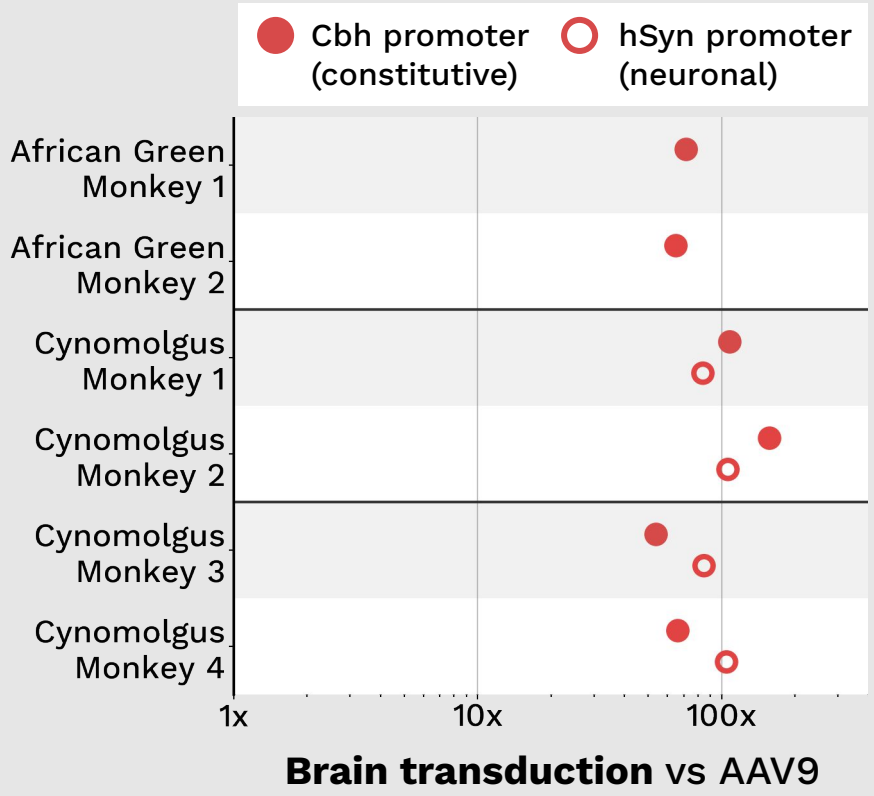
The power of Dyno’s sequence design platform



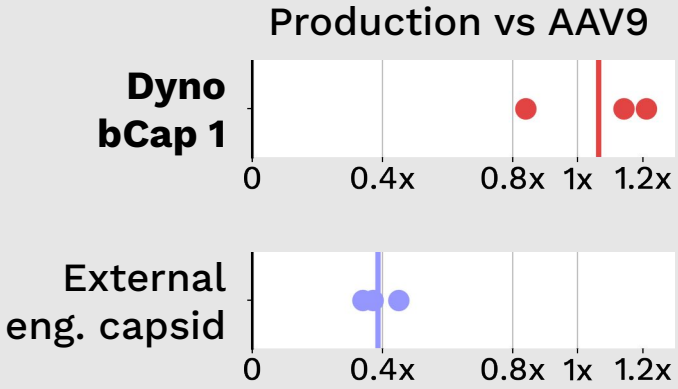
Dyno bCap 1: Cross-species 100x improved CNS transduction

>100,000 capids scale

***in vivo* NHP** measurement



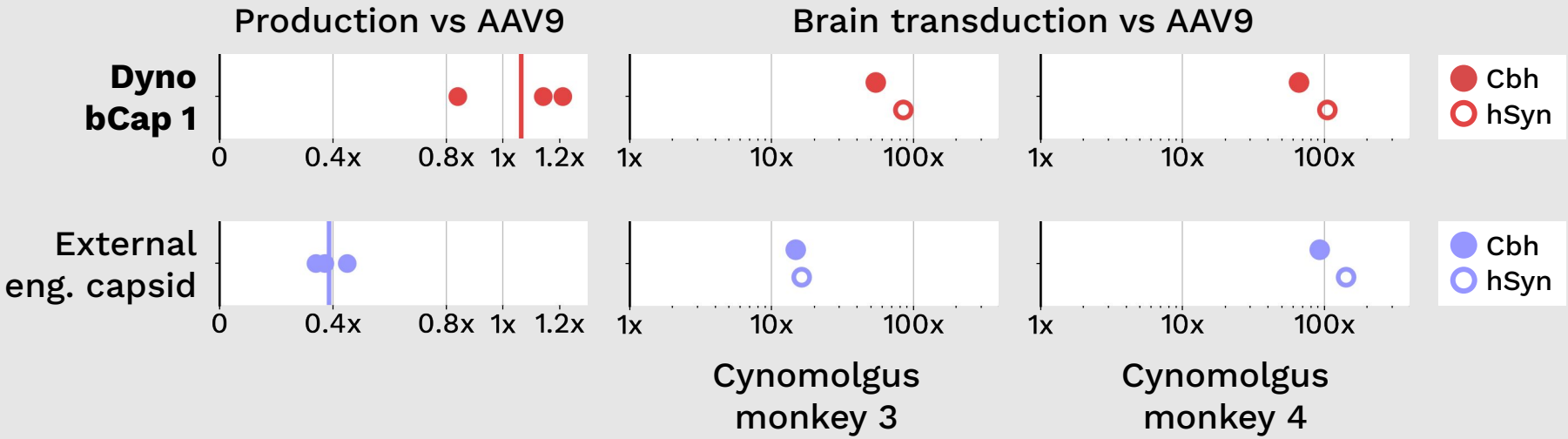
Dyno bCap 1 delivery has the greatest all-around potential for CNS gene therapy



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

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

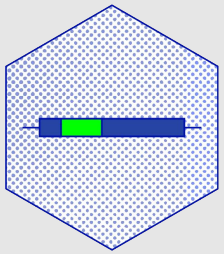
Dyno bCap 1 delivery has the greatest all-around potential for CNS gene therapy



Dyno bCap 1 shows better **consistency** across NHPs in multiplexed studies

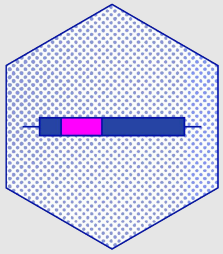
Validating Dyno bCap 1 delivery

50%



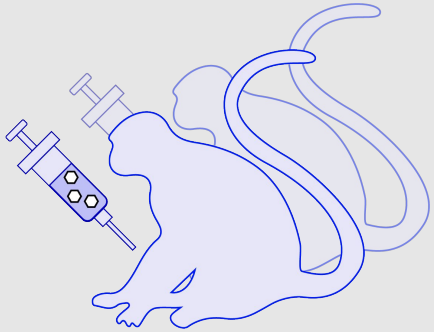
Dyno bCap 1 capsid with CBh-**GFP**

50%



AAV9 capsid with CBh-**mCherry**

2 Cyno NHPs

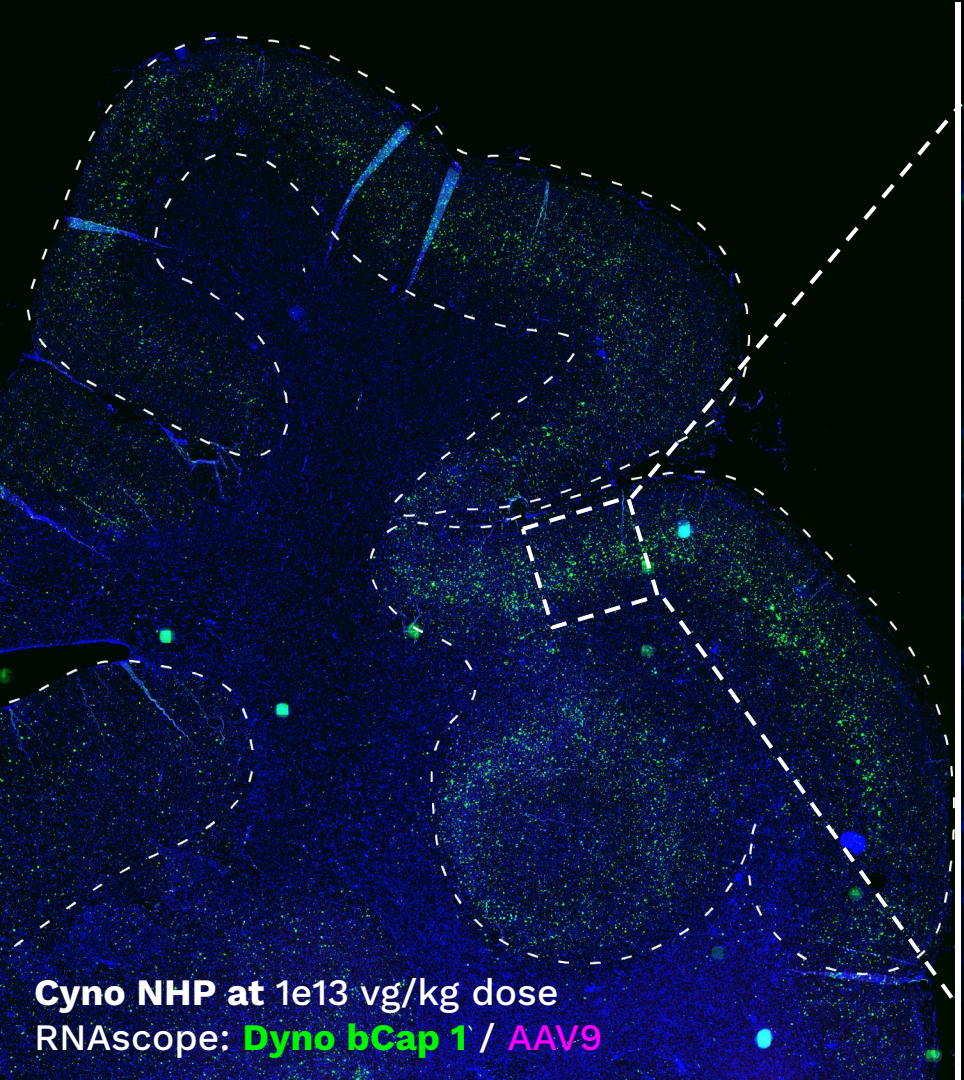


1e13 vg/kg/capsid

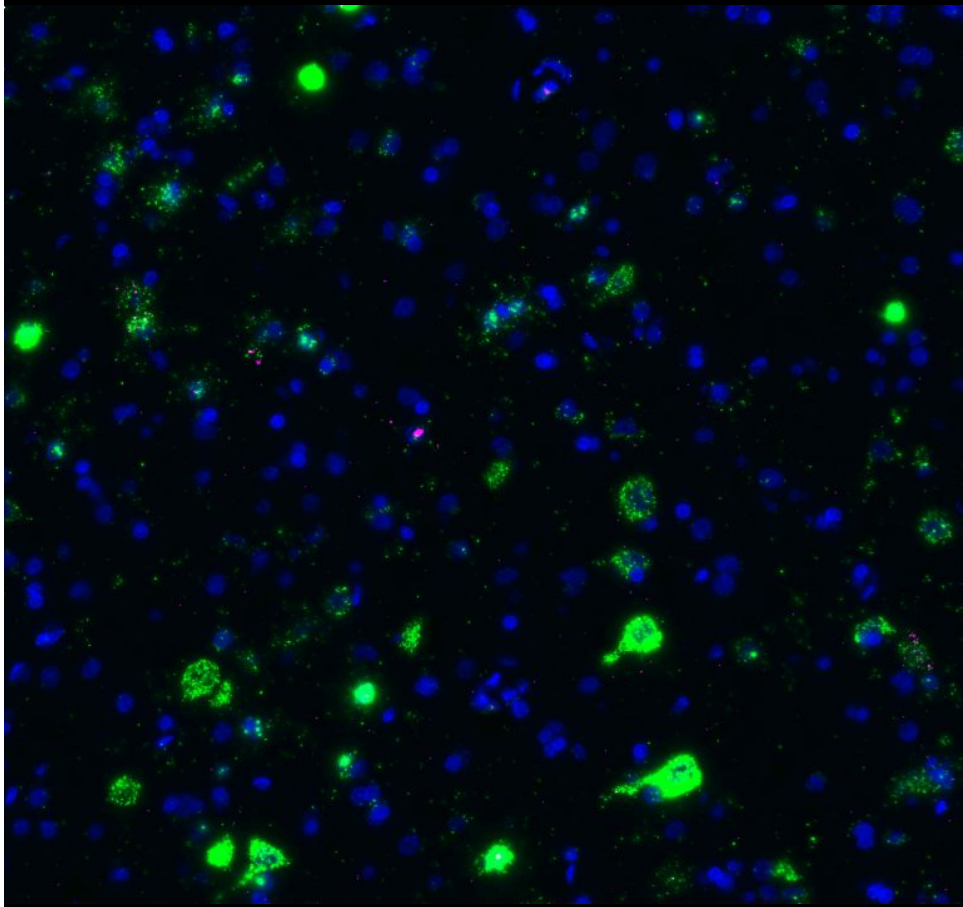
5e12 vg/kg/capsid

28 days in life

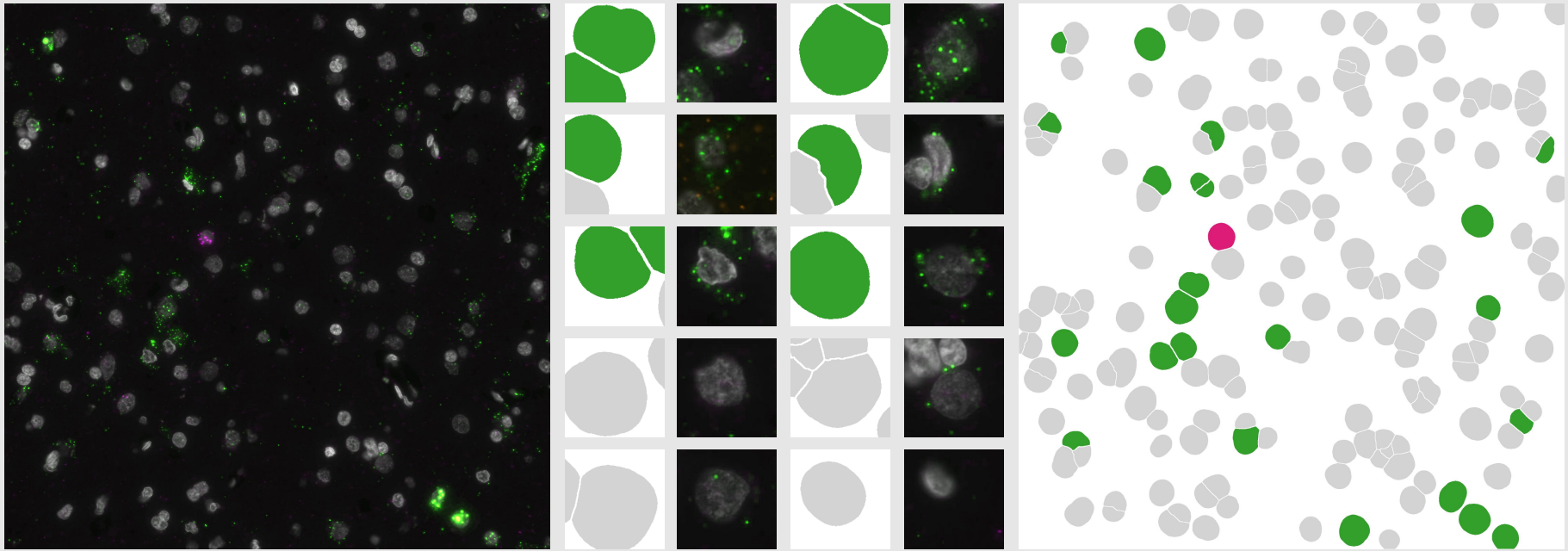
- **Transduction (NGS)**
- **Biodistribution (NGS)**
- **RNAscope histology**
- **IF and IHC histology**



Motor cortex: 11% of cells transduced



Quantification of % total cells transduced

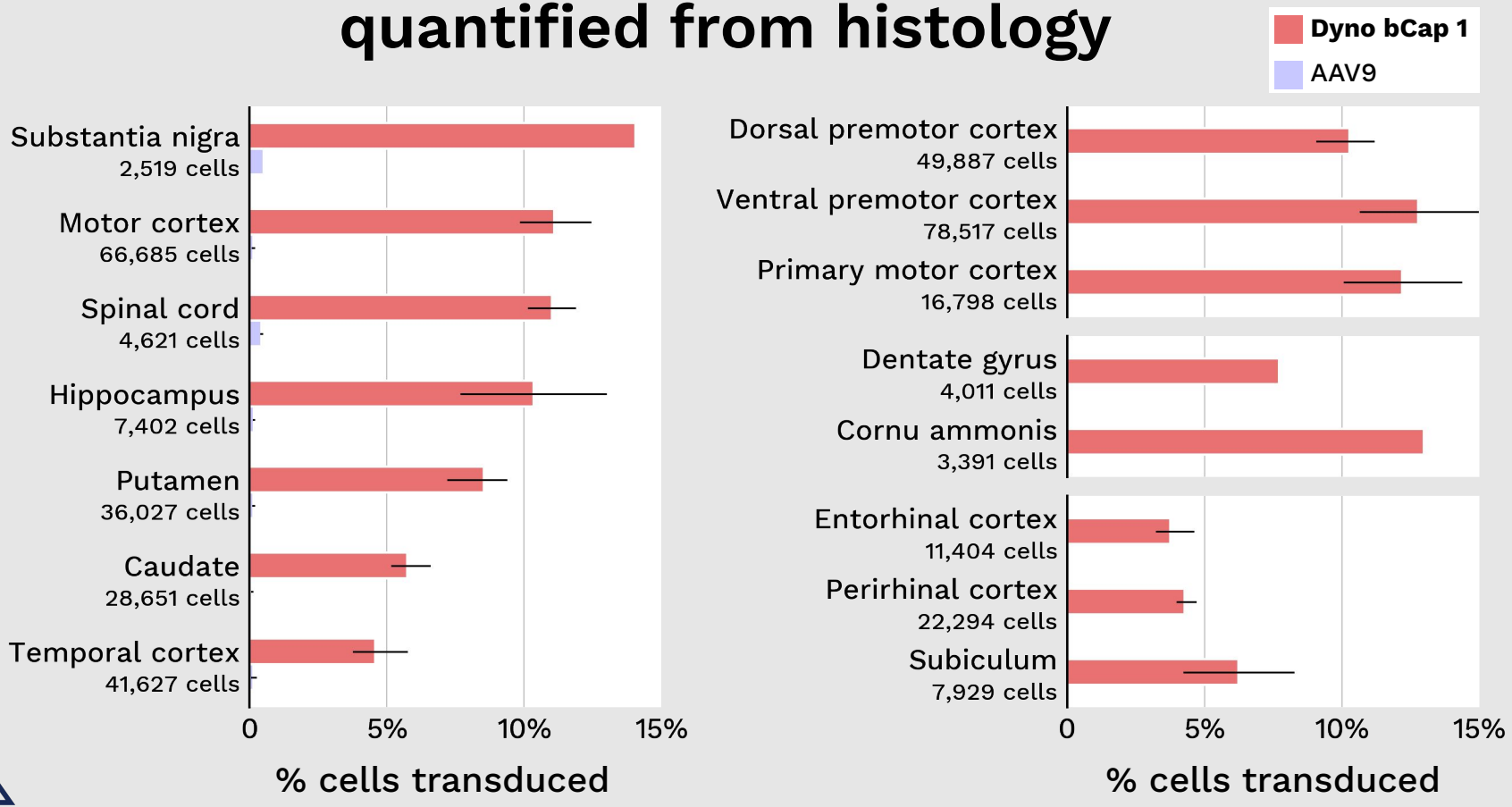


Dyno bCap 1 transduction

Cell without transduction

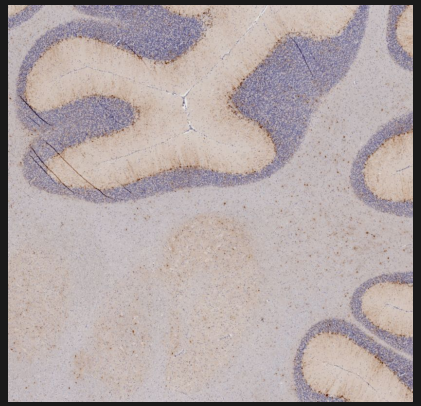
AAV9 transduction

Dyno bCap 1: Pan-brain transduction quantified from histology

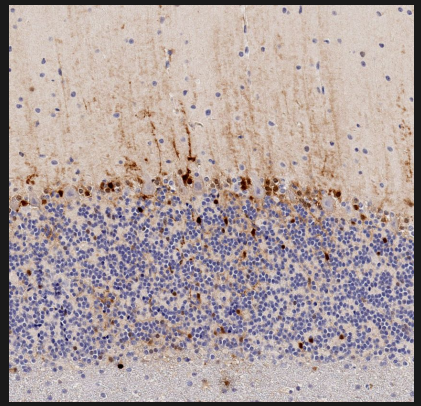


Dyno bCap 1: IHC confirms RNAscope quantification

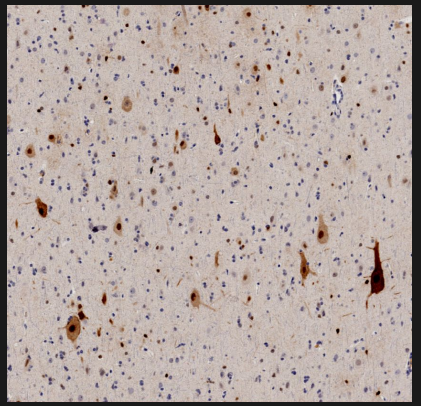
Dyno bCap 1
(nuclear)



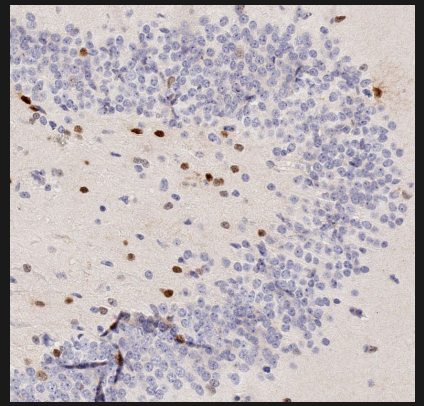
Cerebellum



Purkinje Layer

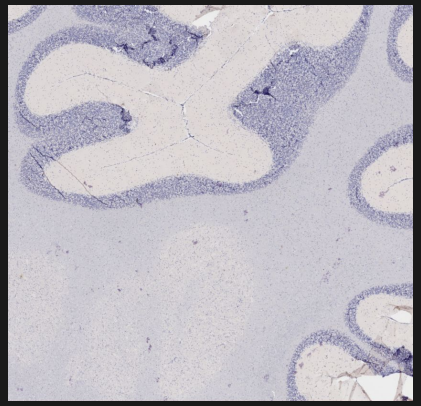


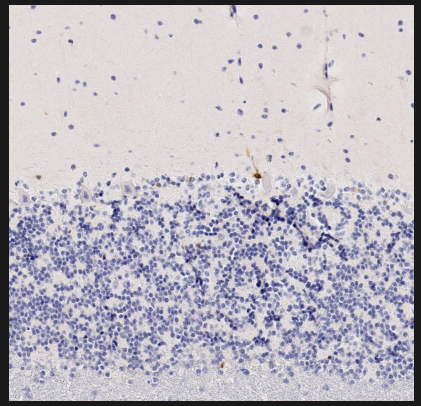
Motor Cortex

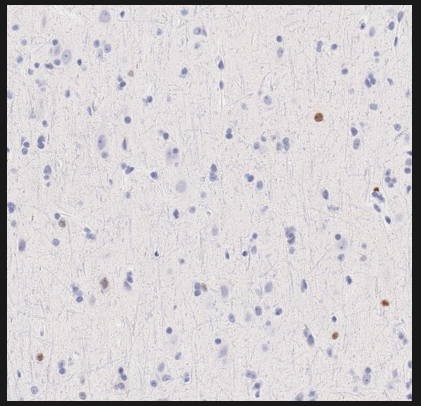


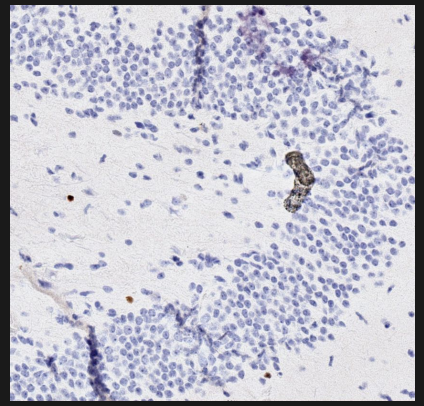
Dentate Gyrus

AAV9
(nuclear)

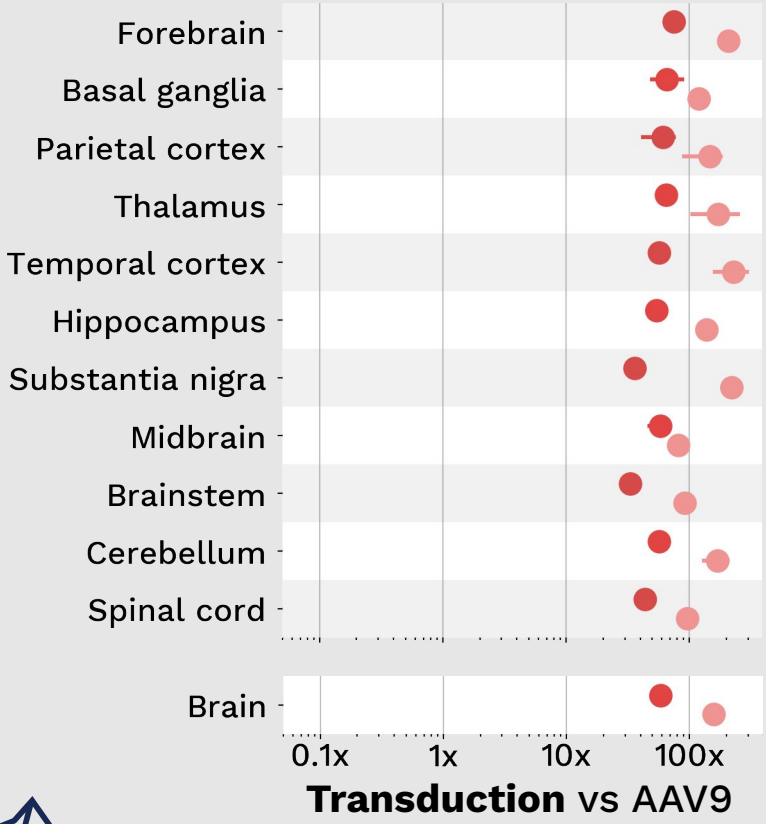


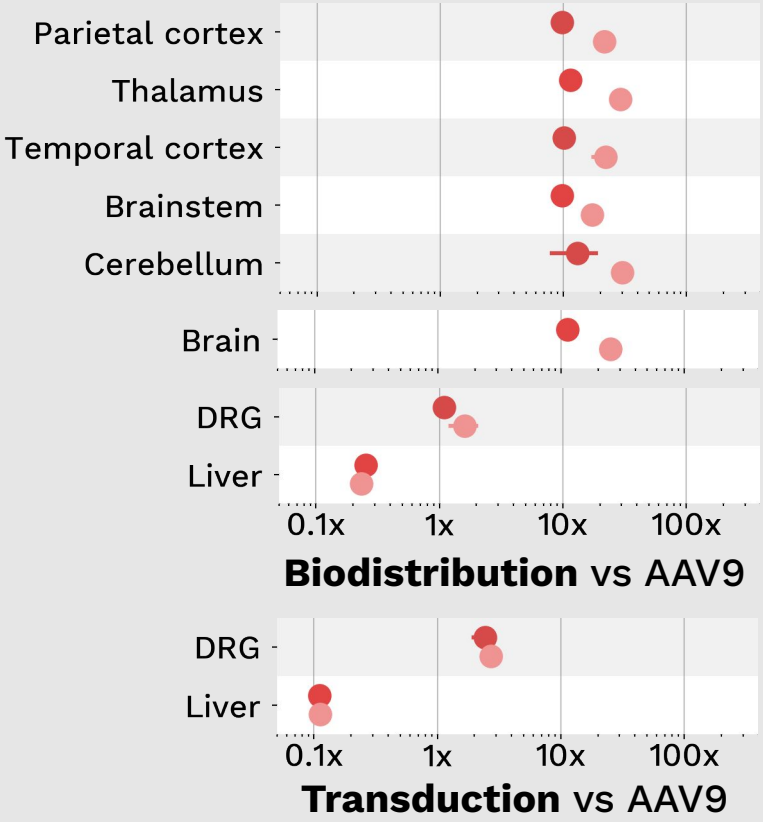


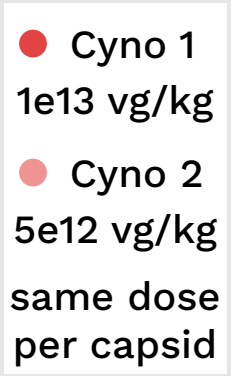




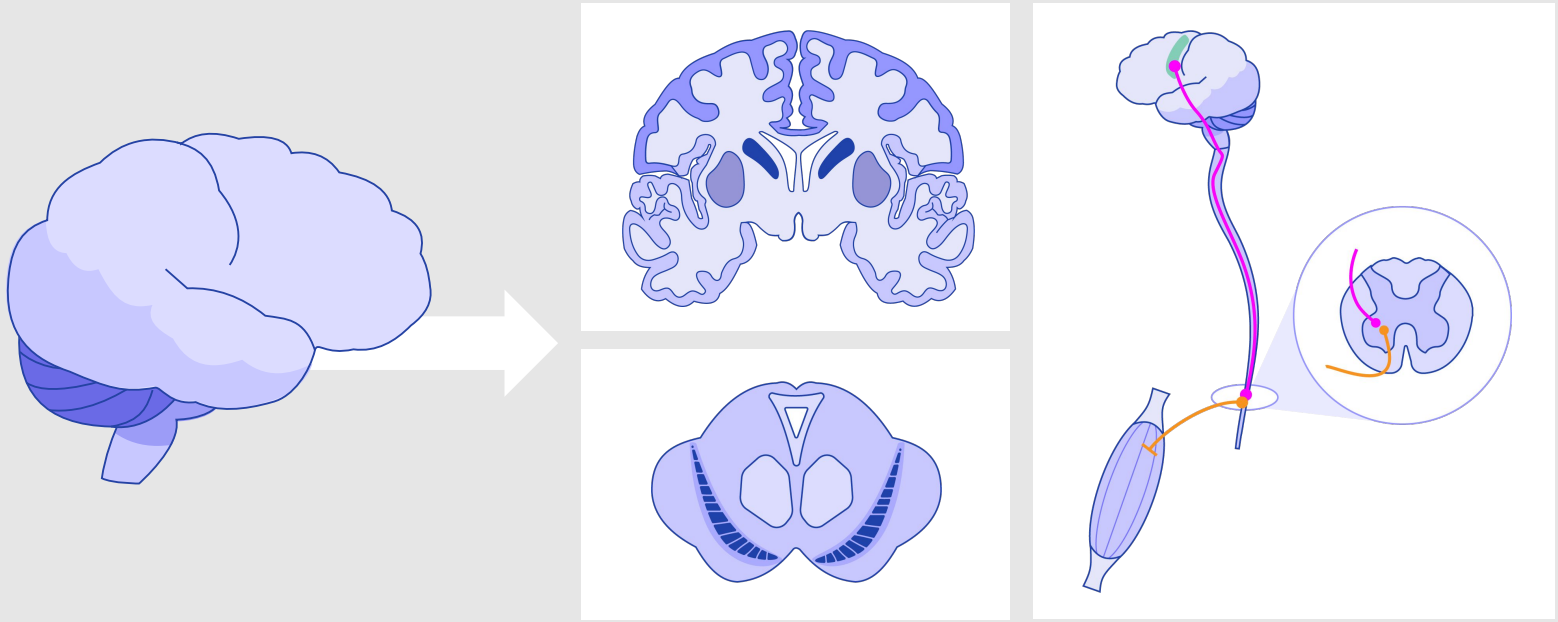
Dyno bCap 1: Pan-brain transduction quantified from NGS







Validating Dyno bCap 1 delivery for specific use cases with cell-specific histology

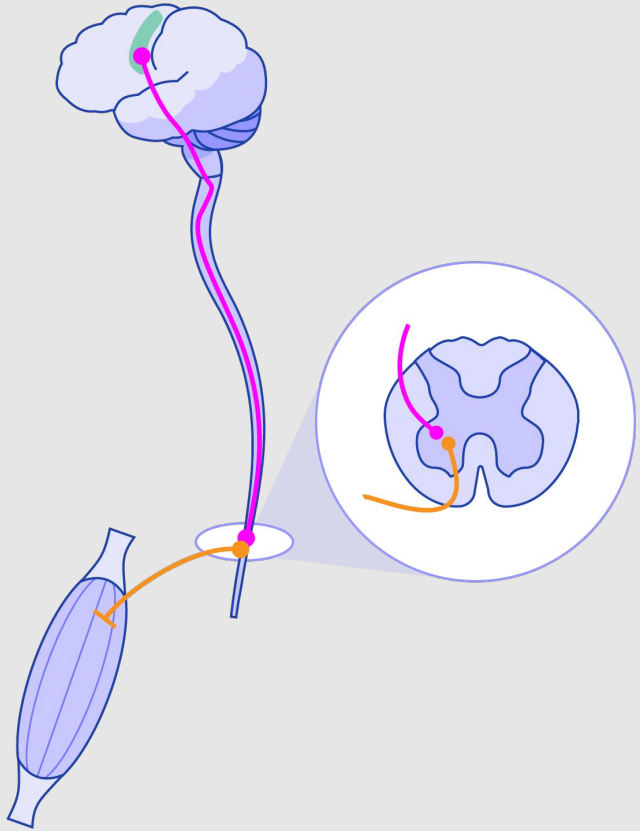


USE CASE 1

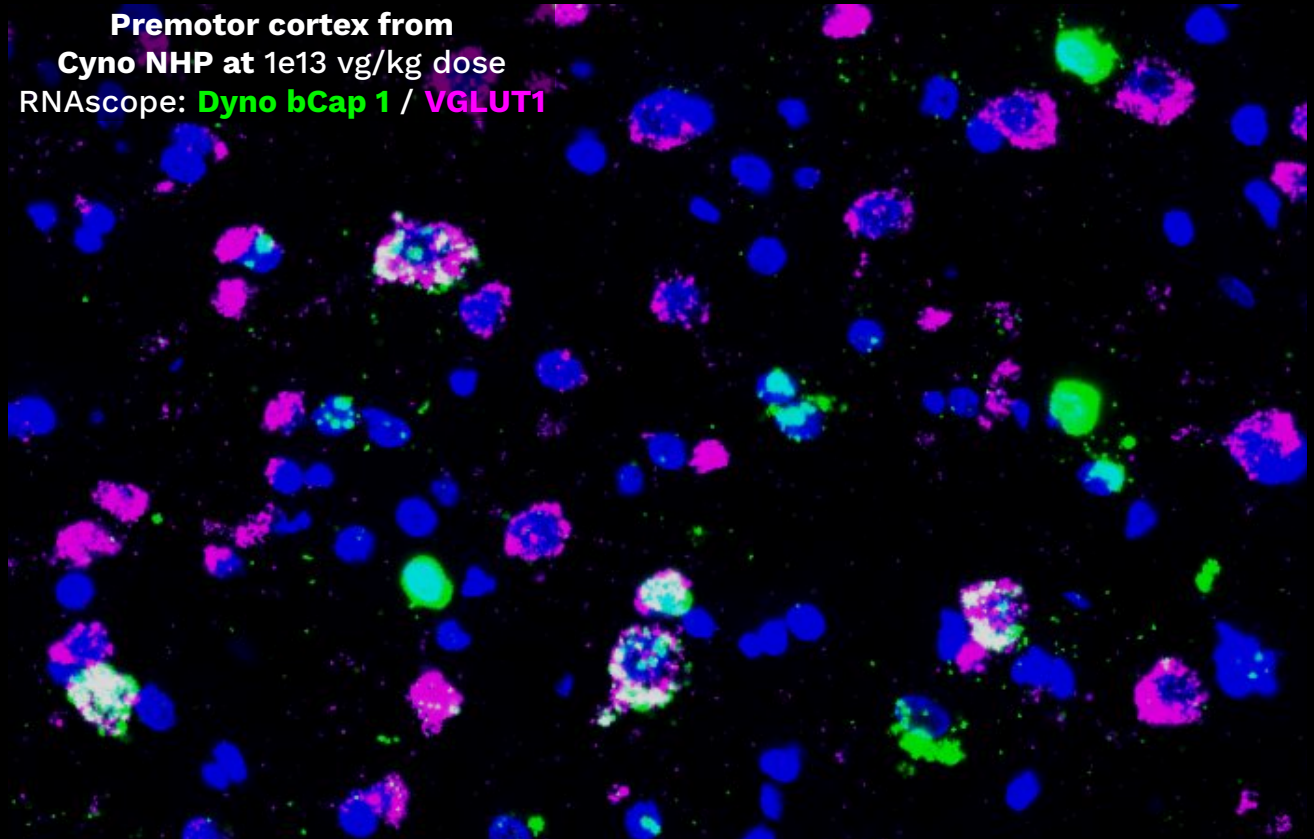
Amyotrophic Lateral Sclerosis (ALS)

Unmet patient need:
Severe and rapidly progressing motor neuron disorder

Delivery challenge:
Reaching enough **upper and lower motor neurons** so that a given treatment strategy can achieve therapeutic efficacy

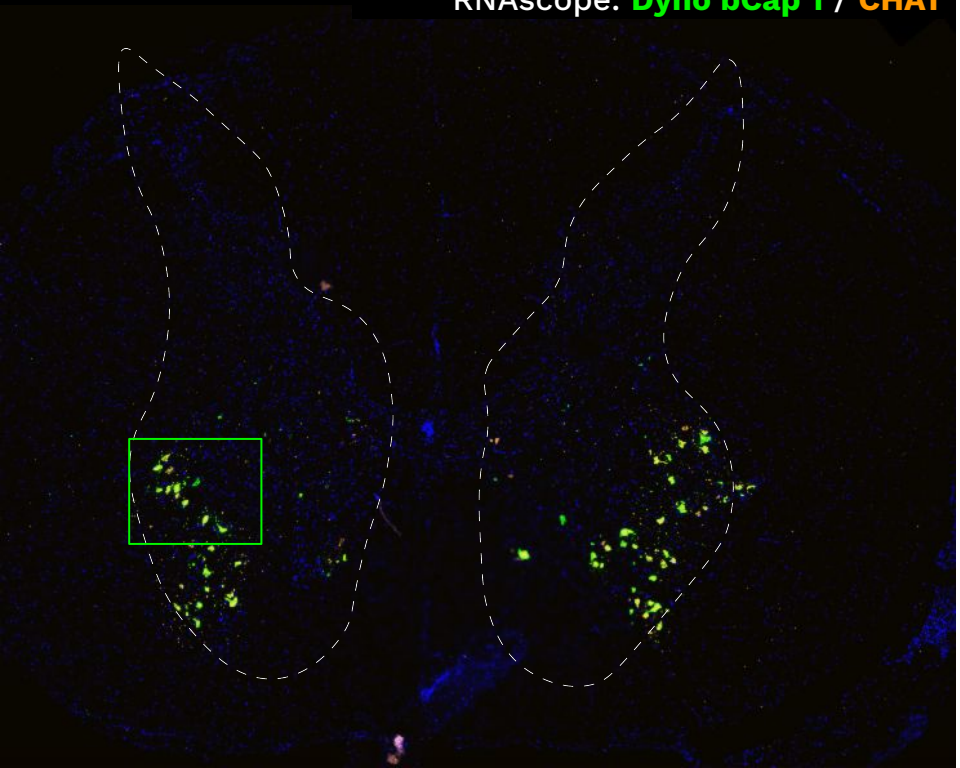


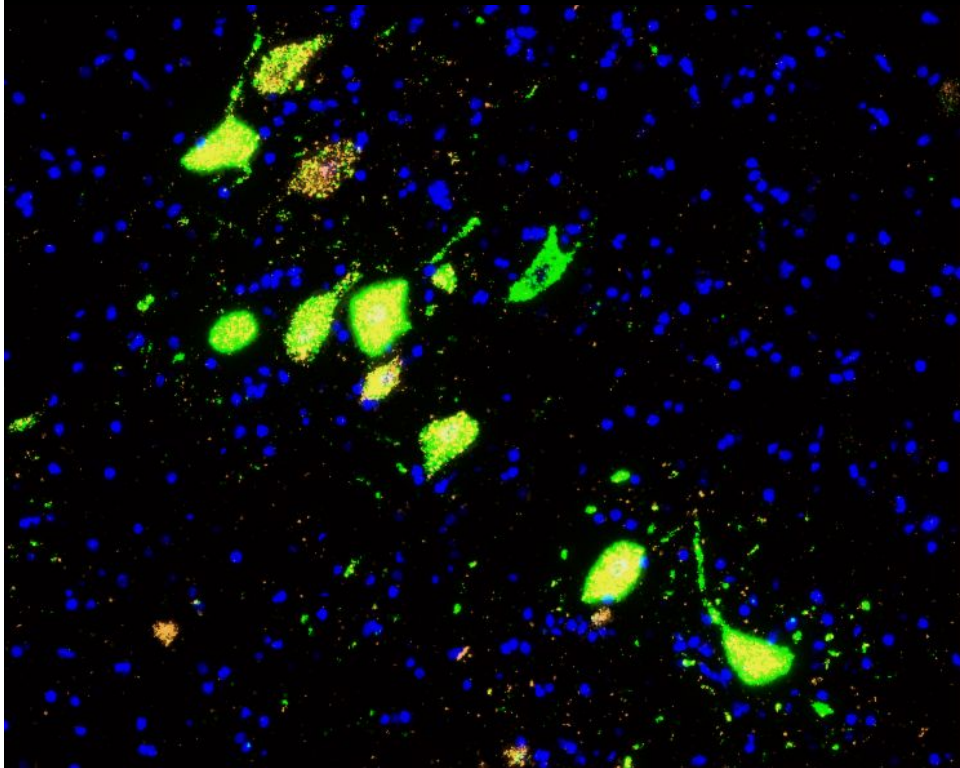
Dyno bCap 1 efficiently transduces upper motor neurons



Dyno bCap 1 efficiently transduces lower motor neurons

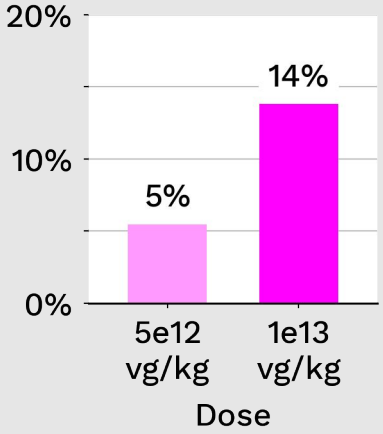
Cyno NHP at 1e13 vg/kg dose
RNAscope: **Dyno bCap 1** / **CHAT**





Dyno bCap 1 efficiently transduces upper and lower motor neurons

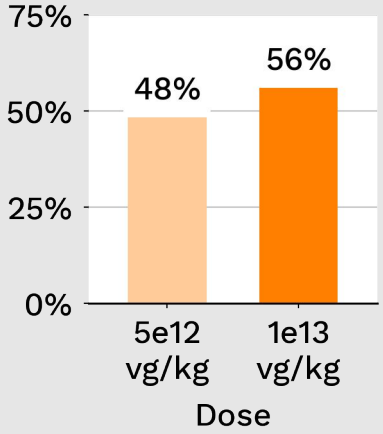
% transduced upper motor neurons (VGLUT1+)



24551 43560

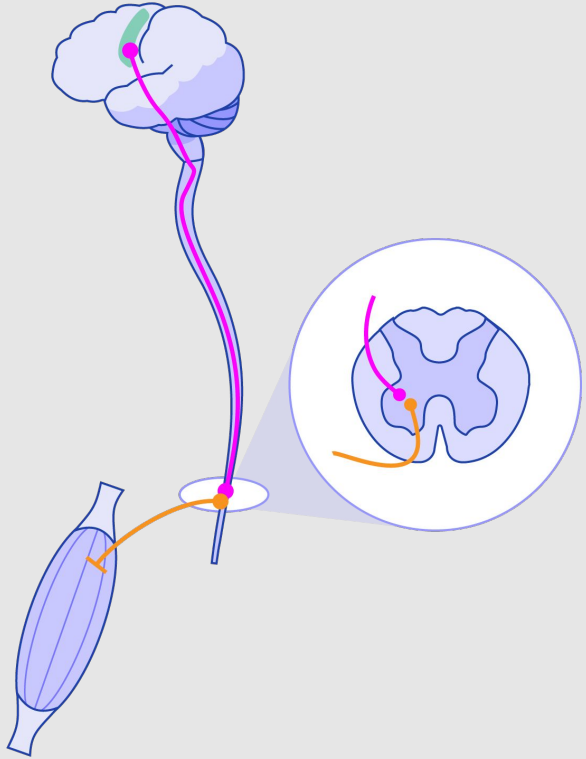
Total counted VGLUT1+ cells

% transduced lower motor neurons (CHAT+)



184 25

Total counted CHAT+ cells



USE CASE 2

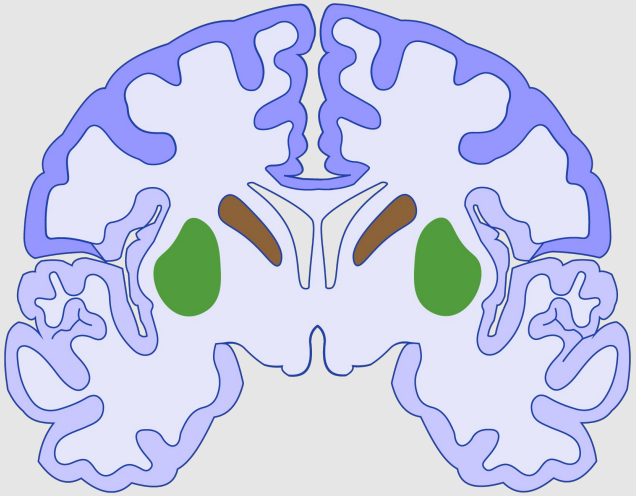
Huntington’s Disease

Unmet patient need:

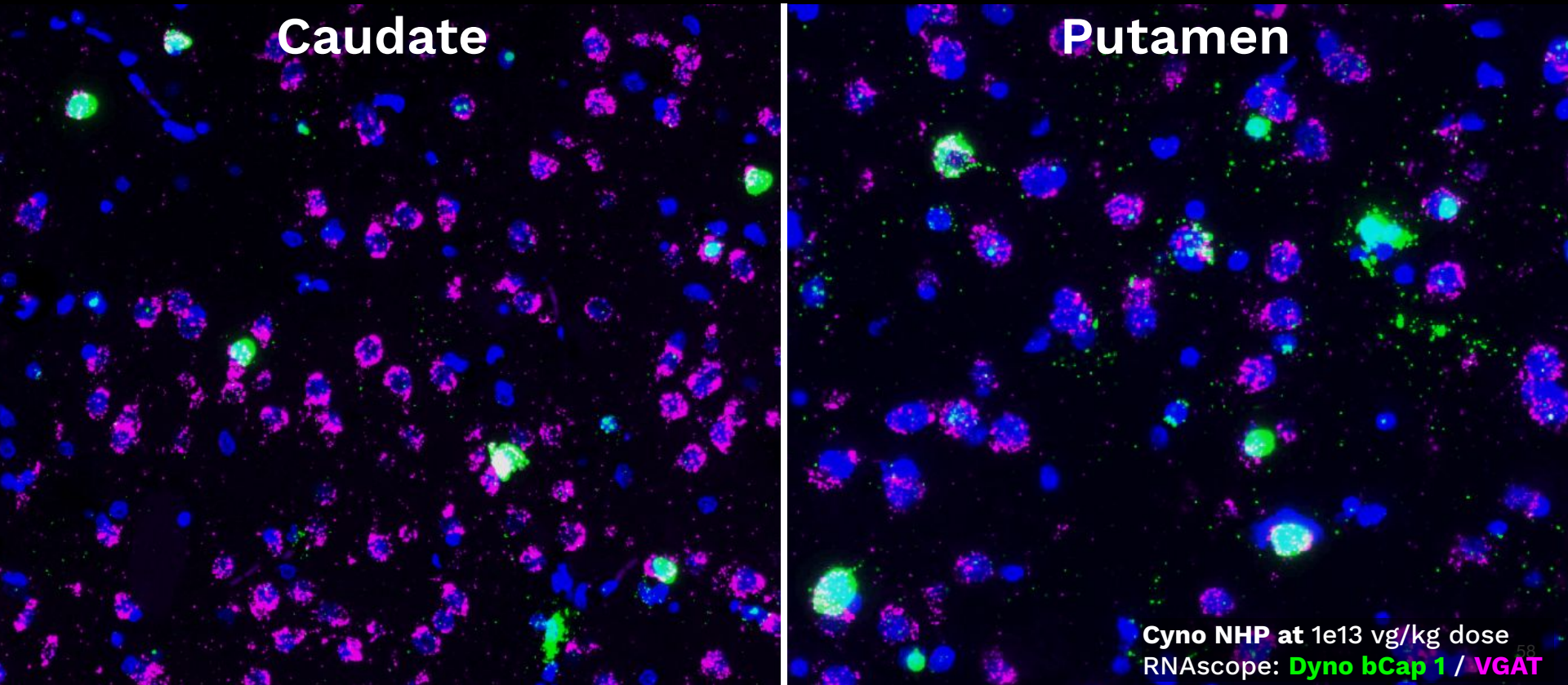
Severe inherited motor disorder causing loss of specific neuronal populations

Delivery challenge:

Pan-brain transduction, especially in **caudate**, **putamen** and motor cortex circuits where neuronal degeneration causes most symptoms



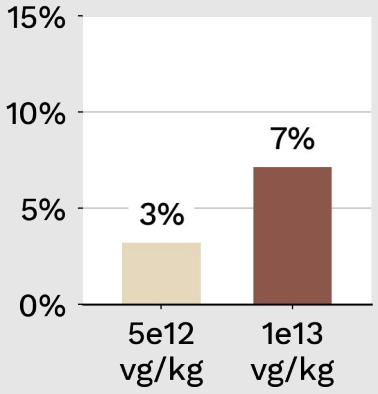
Dyno bCap 1 efficiently transduces medium spiny neurons in striatum



Dyno bCap 1 efficiently transduces medium spiny neurons in striatum

medium spiny neurons (VGAT+)

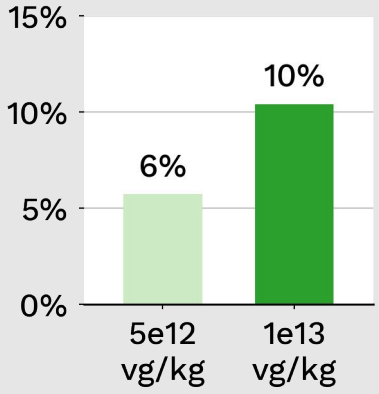
% transduced in caudate



16916 21371

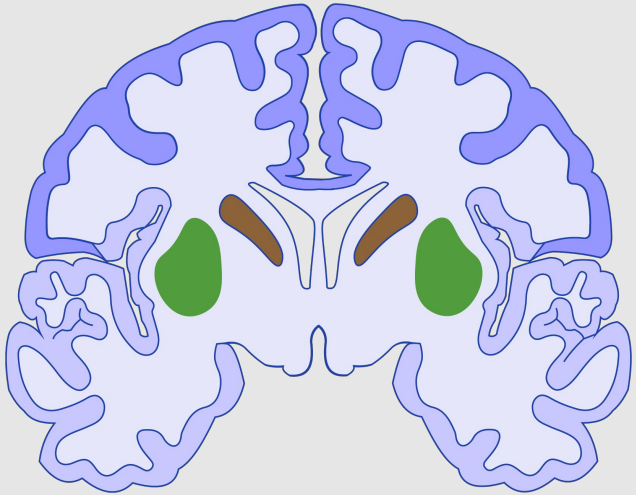
Total counted VGAT+ cells

% transduced in putamen



21957 30255

Total counted VGAT+ cells

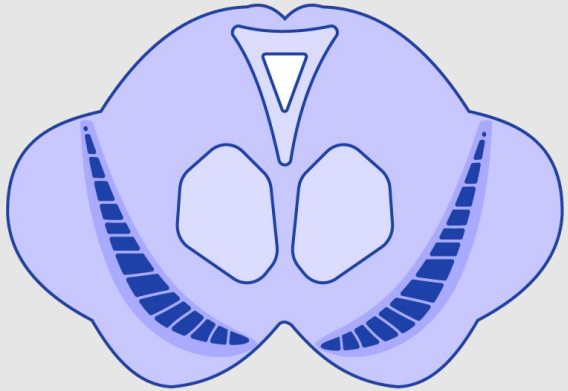


USE CASE 3

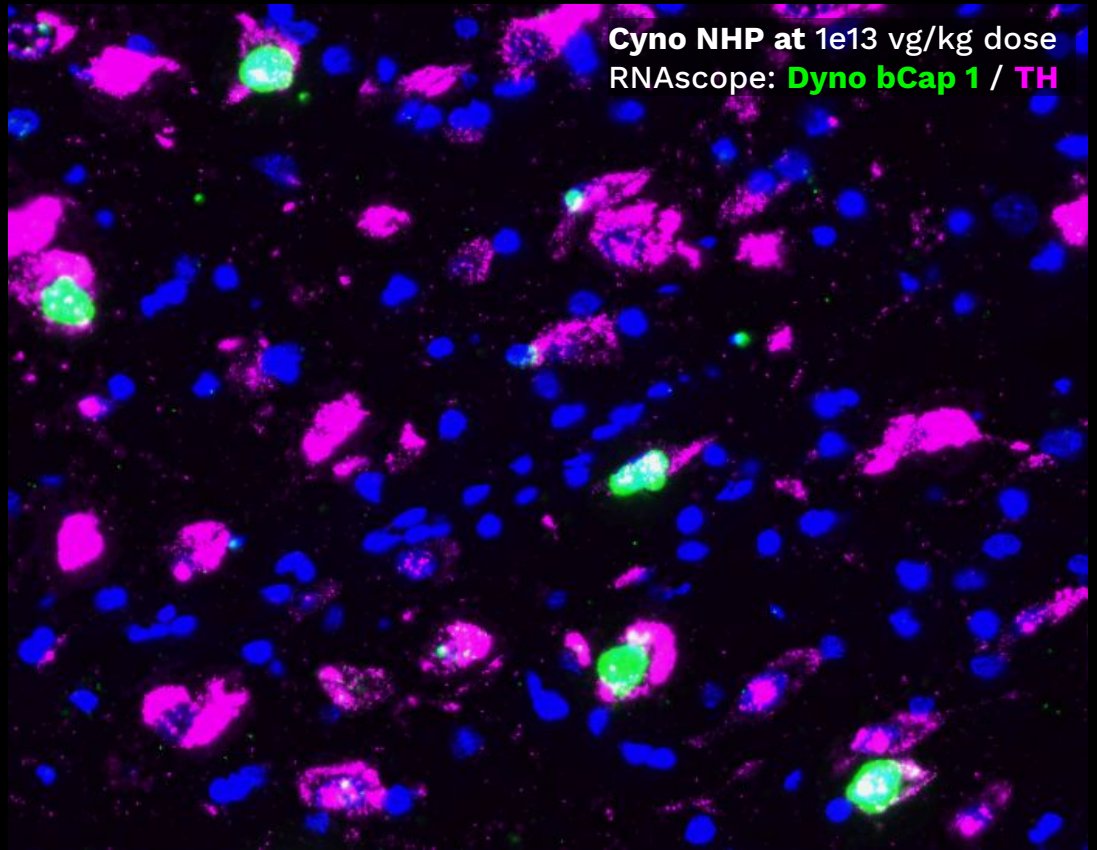
Parkinson’s disease

Unmet patient need:
Prevalent and progressive neurodegenerative disorder caused by loss of specific neuronal populations

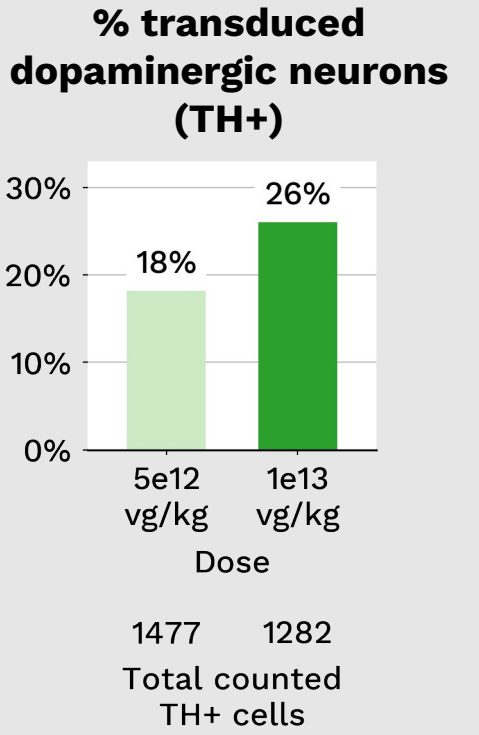
Delivery challenge:
Transducing **dopaminergic neurons** in **substantia nigra**

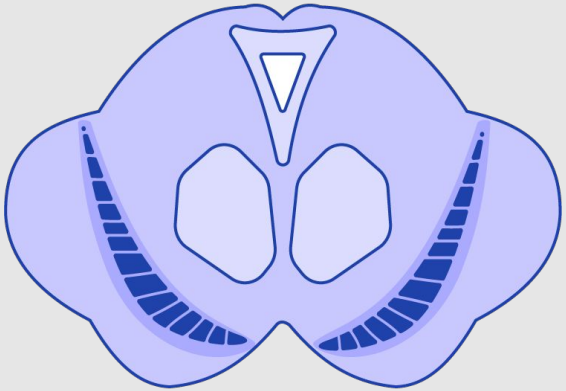


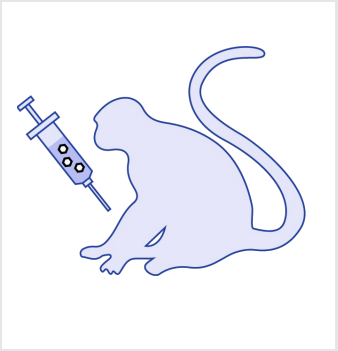
Dyno bCap 1 efficiently transduces dopaminergic neurons in the substantia nigra

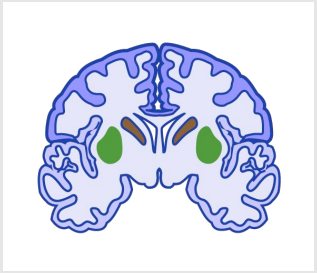


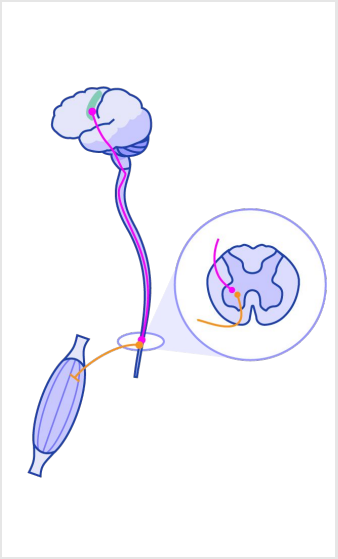
Dyno bCap 1 efficiently transduces dopaminergic neurons in the substantia nigra

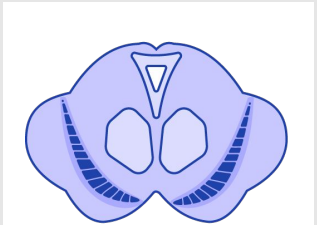












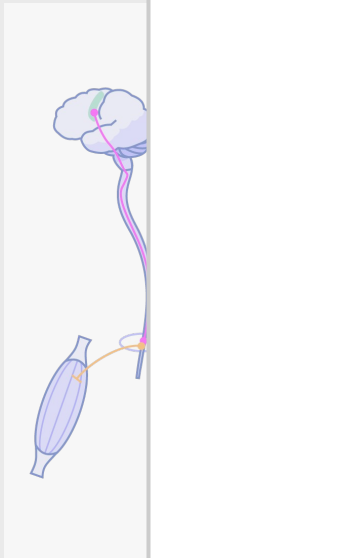
Dyno bCap 1 delivery

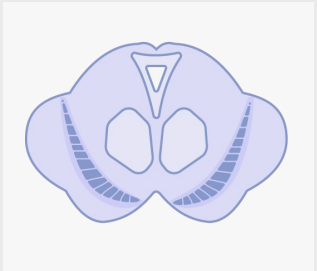
Delivers **pan-brain** and across the **CNS,** crossing the **blood-brain-barrier** after IV administration

1x production vs AAV9
10x liver detargeting vs AAV9
100x brain transduction vs AAV9

Transduces neurons and other therapeutically relevant cell-types

Transduction patterns relevant for **ALS**, **Huntington's** & **Parkinson's Disease**, ...





Dyno bCap 1 delivery

d across the **CNS,**

-brain-barrier

nistration

vs AAV9

ting vs AAV9

ction vs AAV9

ons and other

vant cell-types

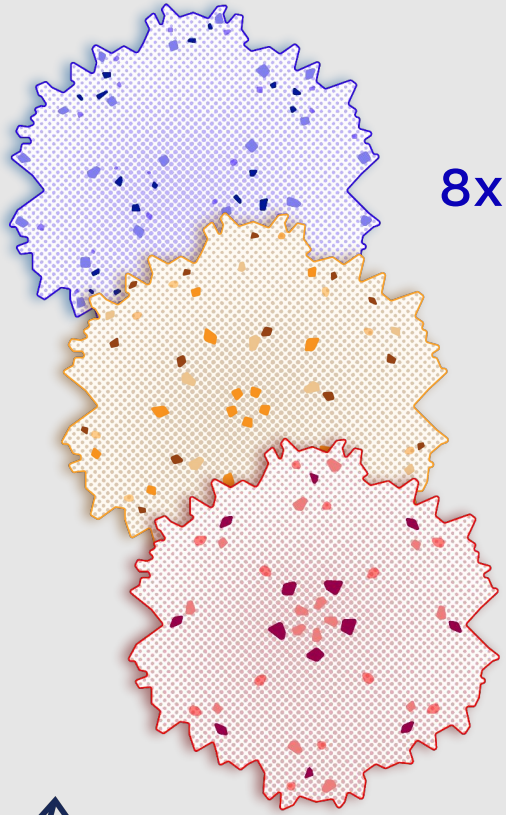
relevant for **ALS**,

Huntington's & **Parkinson's Disease**, ...

Abstract P012:

"Dyno bCap 1 delivery: Cell-type resolved characterization of CNS transduction by intravenously administered AAV capsid in non-human primates"

Emerging CNS capsids, choose from:

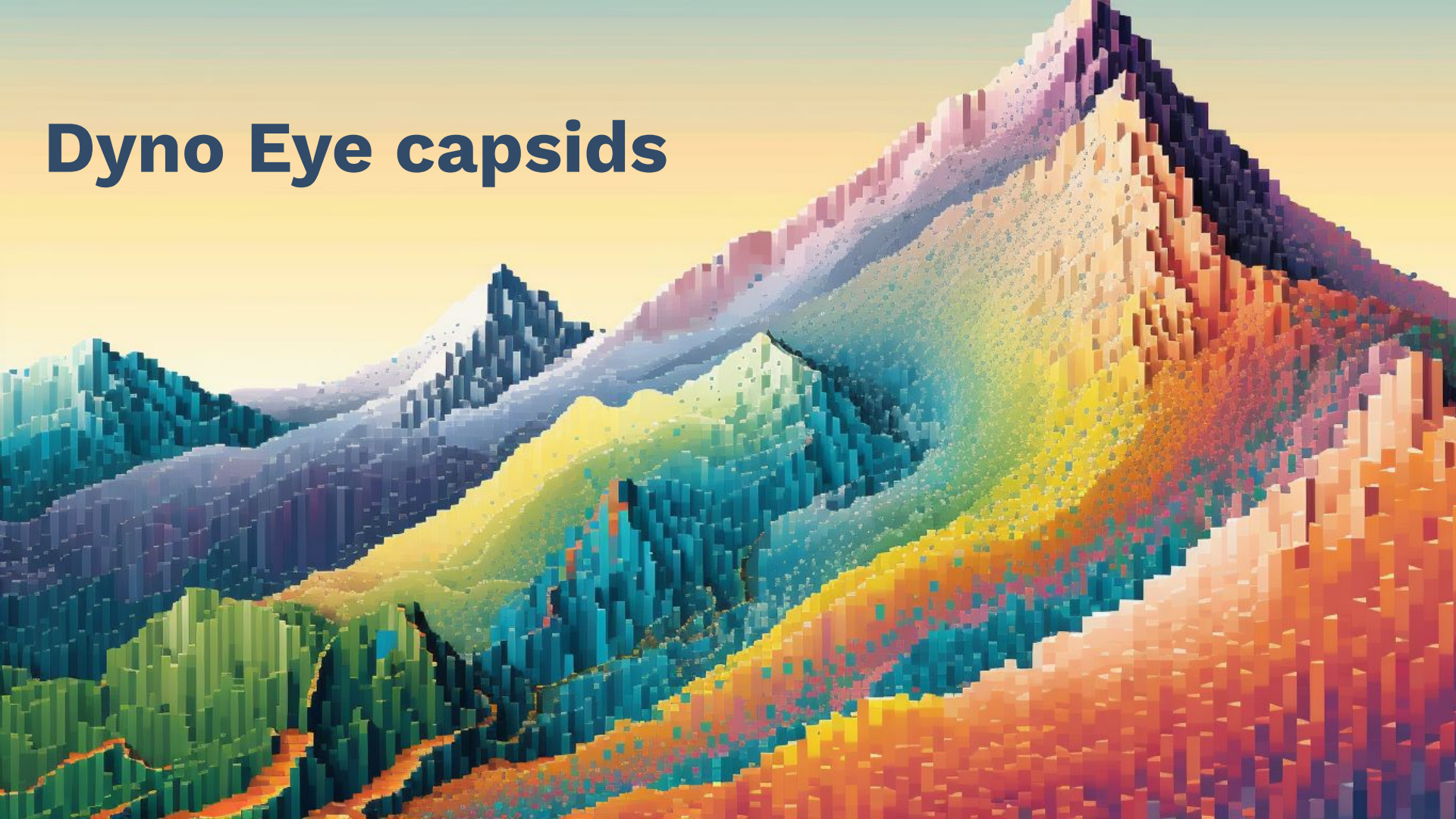


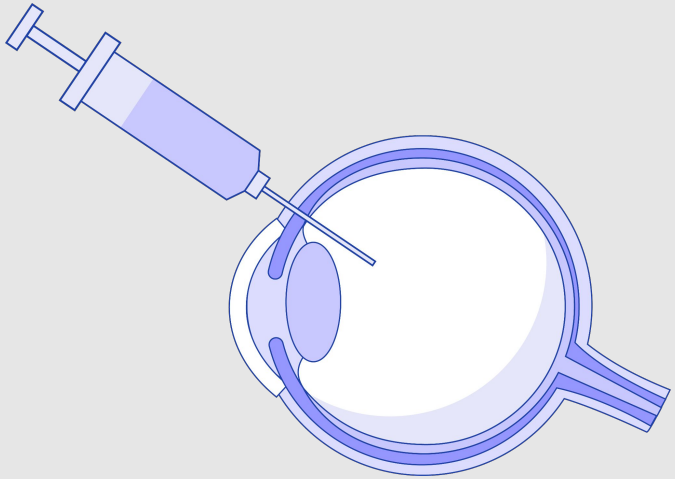
8x more **CNS transduction** vs Dyno bCap 1

3x further **liver detargeting** vs Dyno bCap 1

>8x greater **muscle transduction** vs AAV9

Dyno Eye capsids

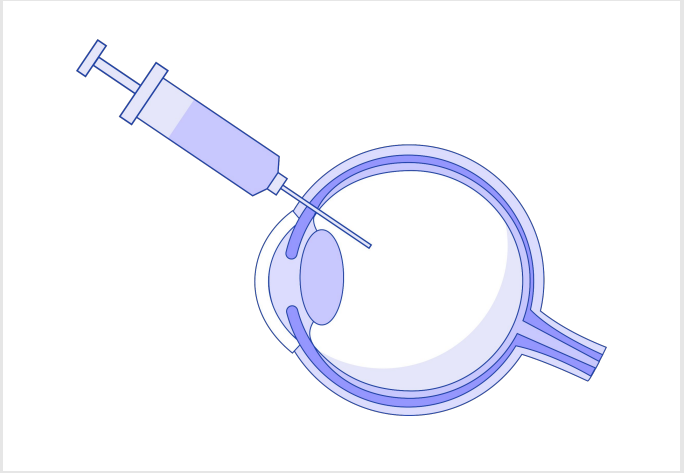


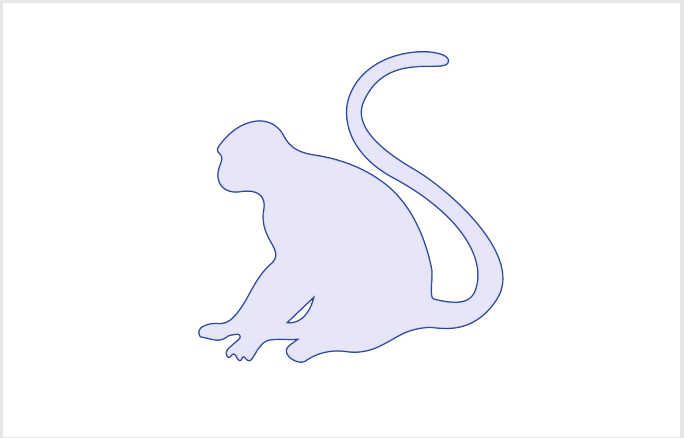


Eye delivery via IVT injection

Safe, non-surgical method for ocular gene therapy delivery

Minimal transduction using AAV2 intravitreal delivery





Dyno eCap 1 delivery

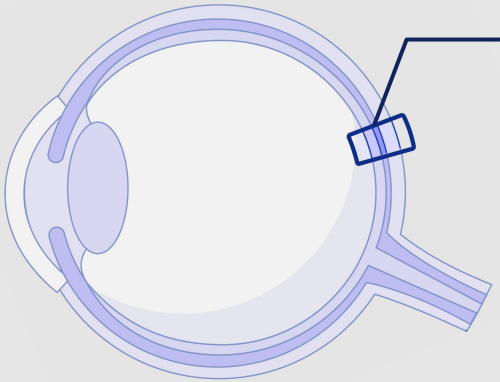
Designed for **IVT** eye delivery

1x production vs AAV2

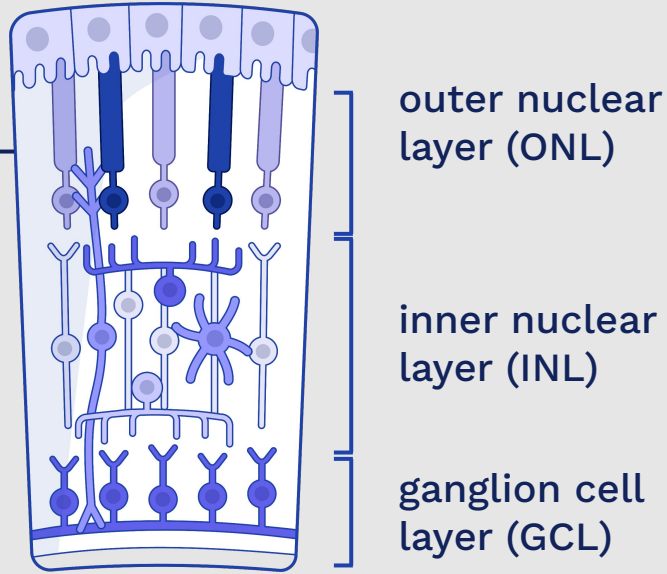
80x retina transduction vs AAV2
in Cyno monkeys

Dyno eCap 1 validation

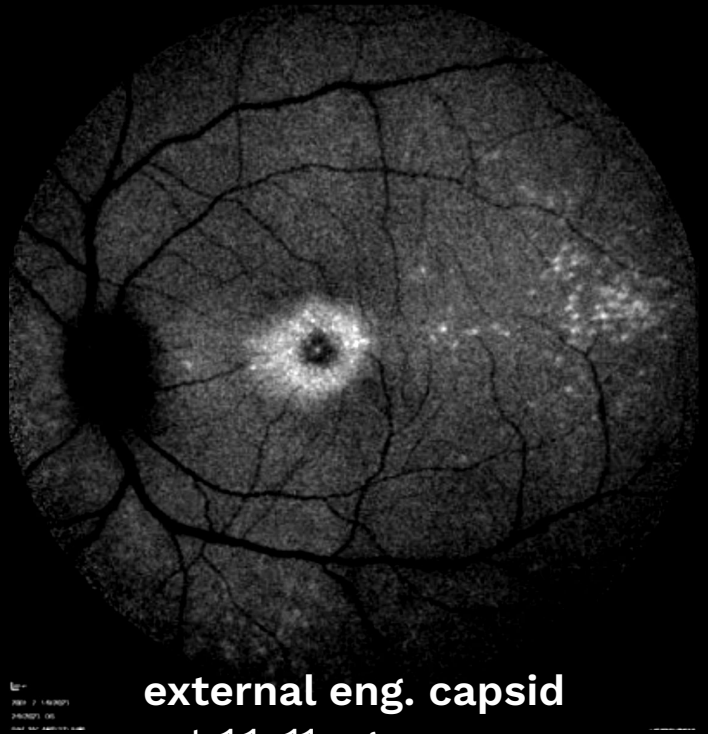
Where along the retina?



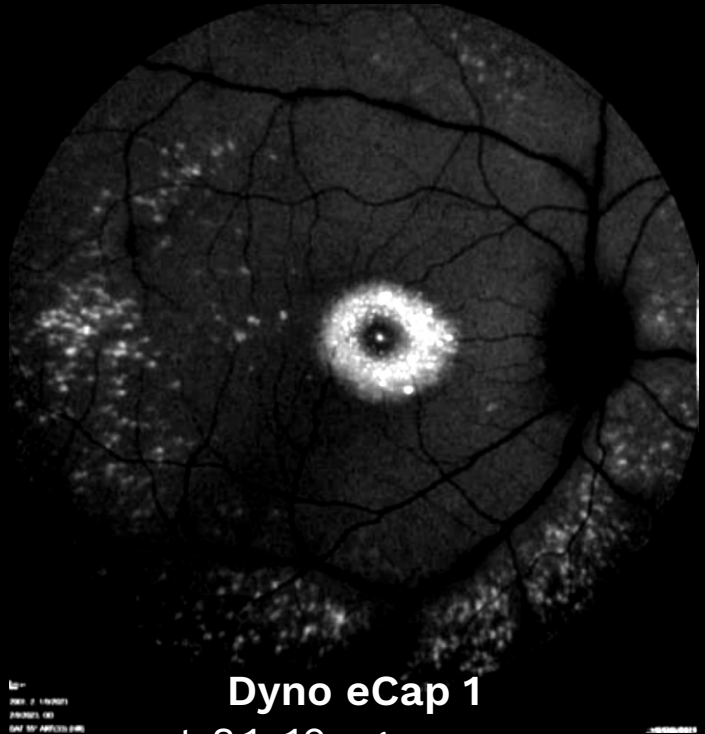
Which retinal layers?



Fluorescent fundus imaging confirms widespread delivery using single capsid delivery



external eng. capsid
at 1.1e11 vg per eye

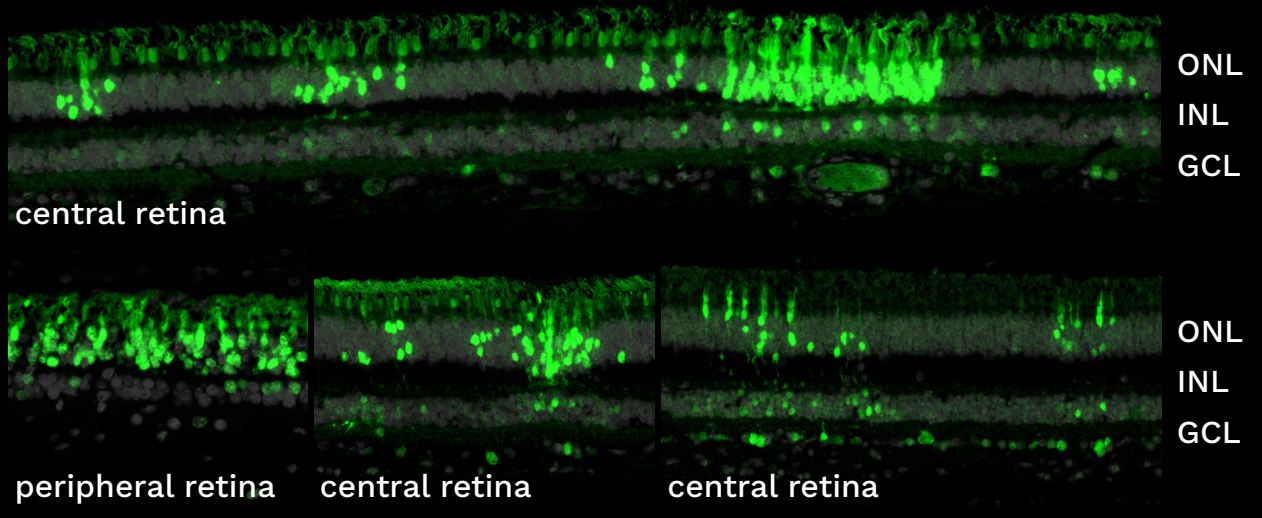


Dyno eCap 1
at 8.1e10 vg per eye

validation

Dyno eCap 1

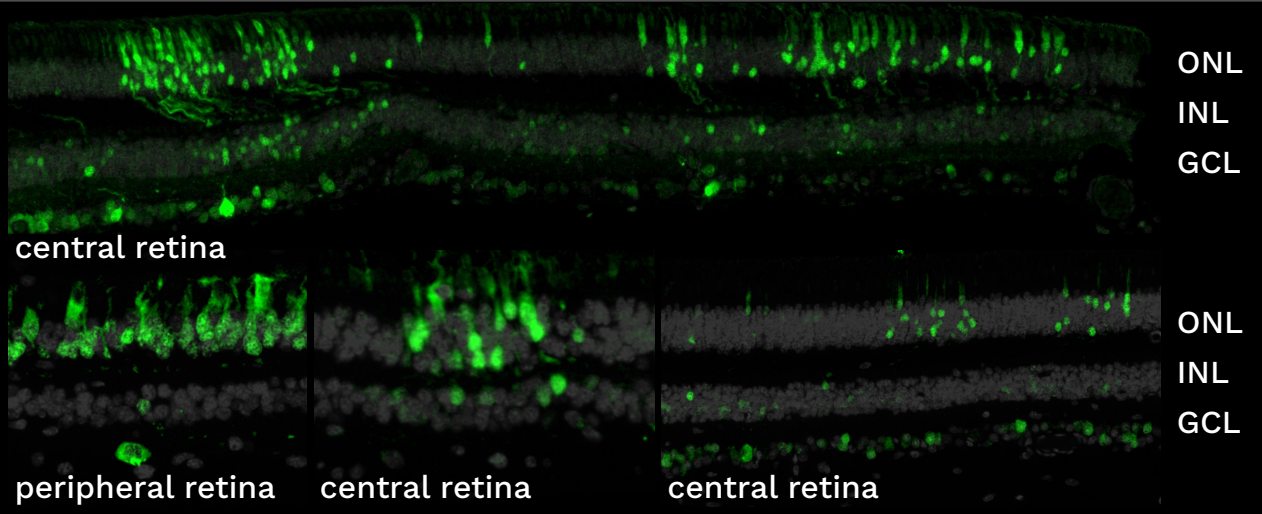
at 2.3e11 vg per eye



comparison

external engineered capsid

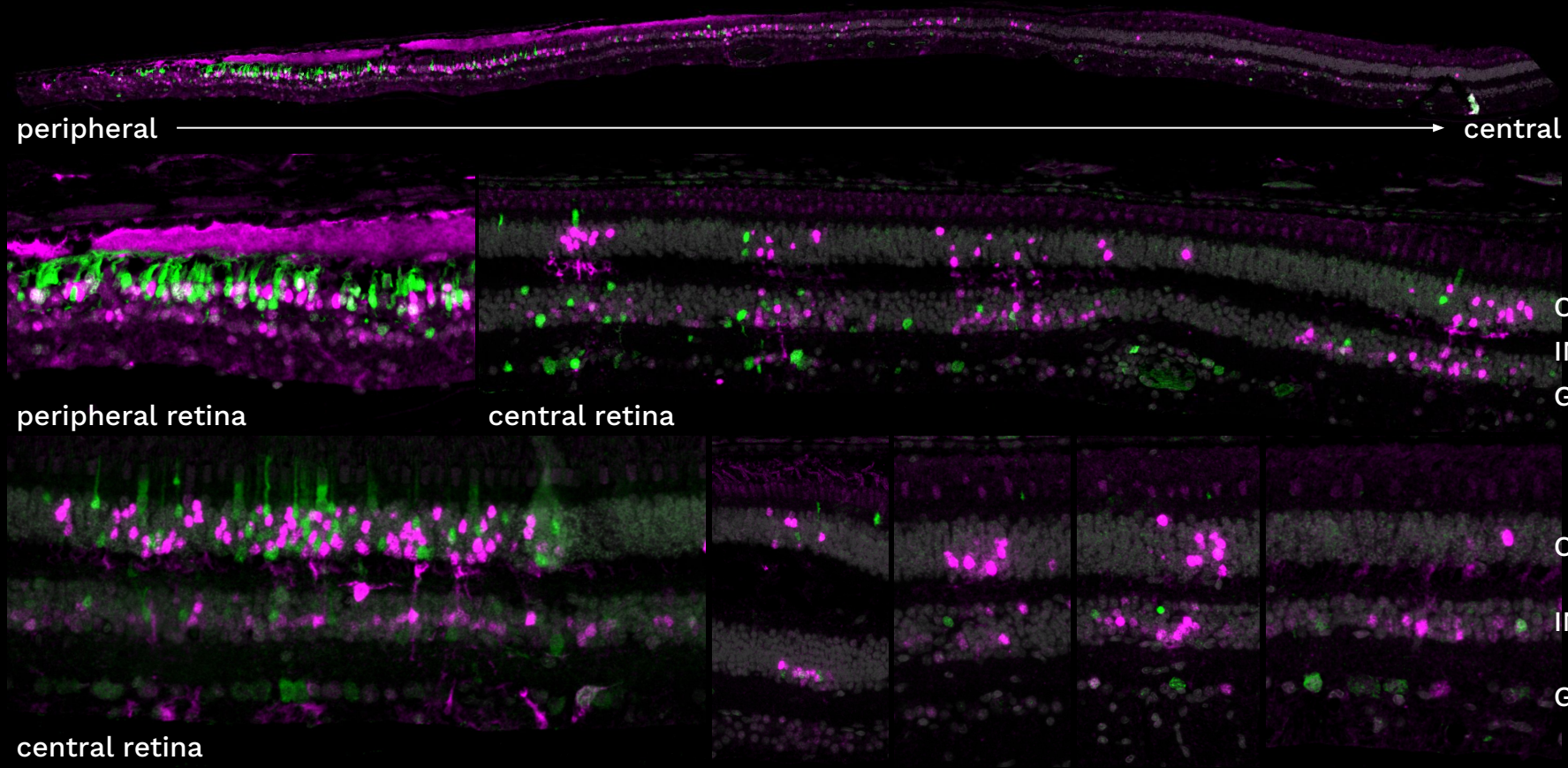
at 2.1e11 vg per eye



Head-to-head comparison

2 capsids co-injected

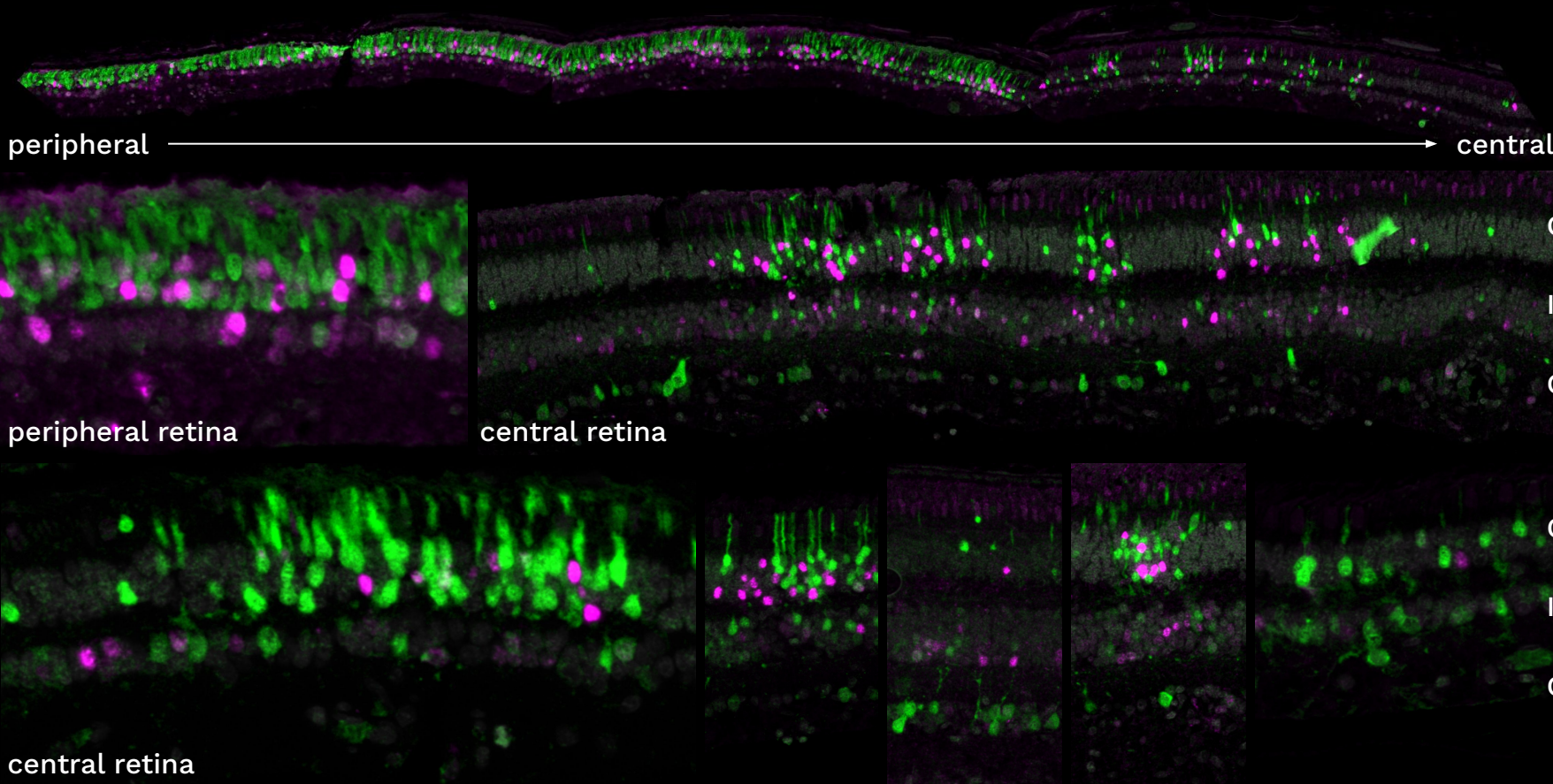
Dyno eCap 1 capsid (8.1e10 vg) +
External engineered capsid (9.3e10 vg)



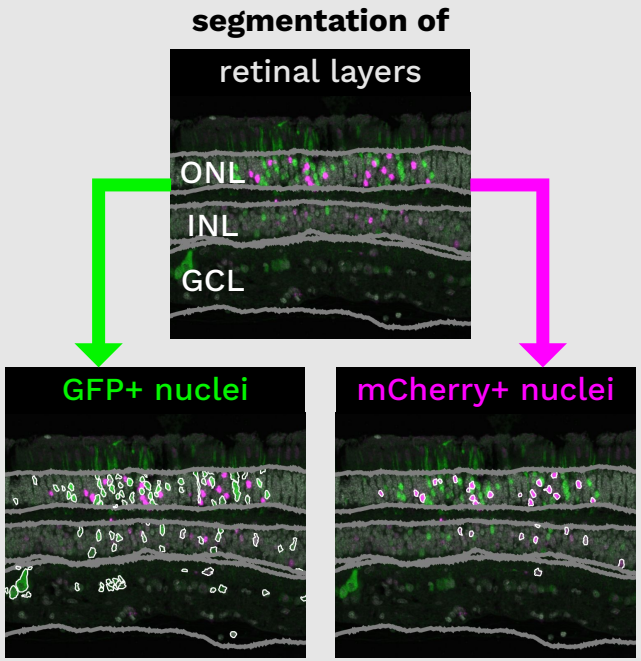
Head-to-head comparison

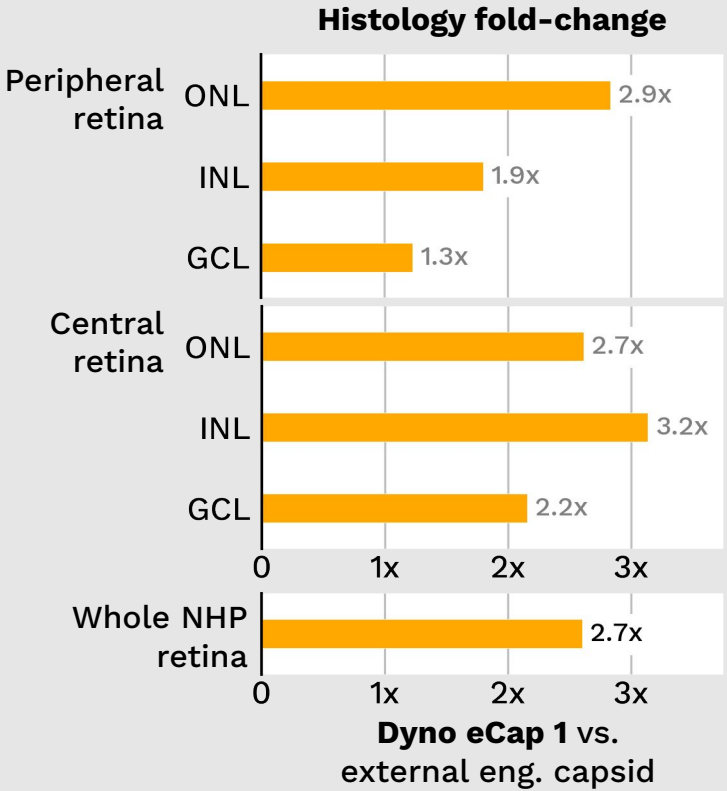
2 capsids co-injected, reporter swap

Dyno eCap 1 capsid (7.8e10 vg) +
External engineered capsid (1.1e11 vg)



Dyno eCap 1 transduces 2-3x more cells

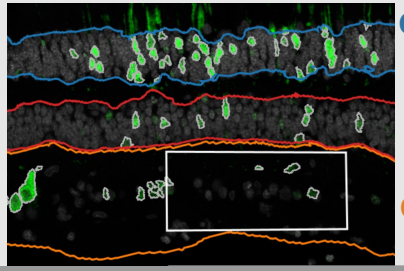




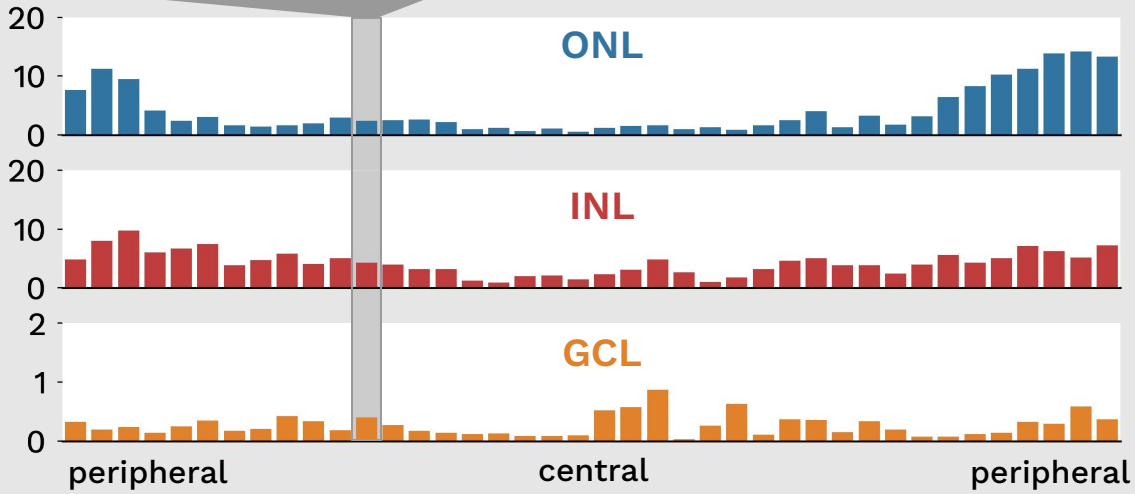
Dyno eCap 1 transduces cells all across the neural retina

5 µm² thick sections

10,000 µm² = white rectangle

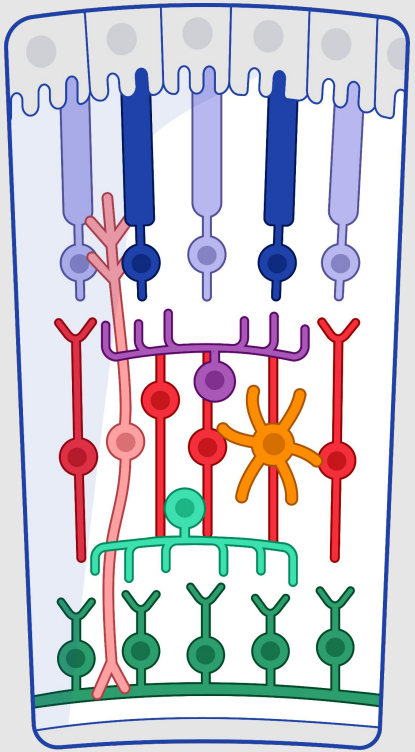


Number of transduced cells, **per 10,000 µm²** area of retinal layer, **per 5e10 vg** of Dyno eCap 1 injected



equally sized bins along retina

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

Cell-type resolved histology

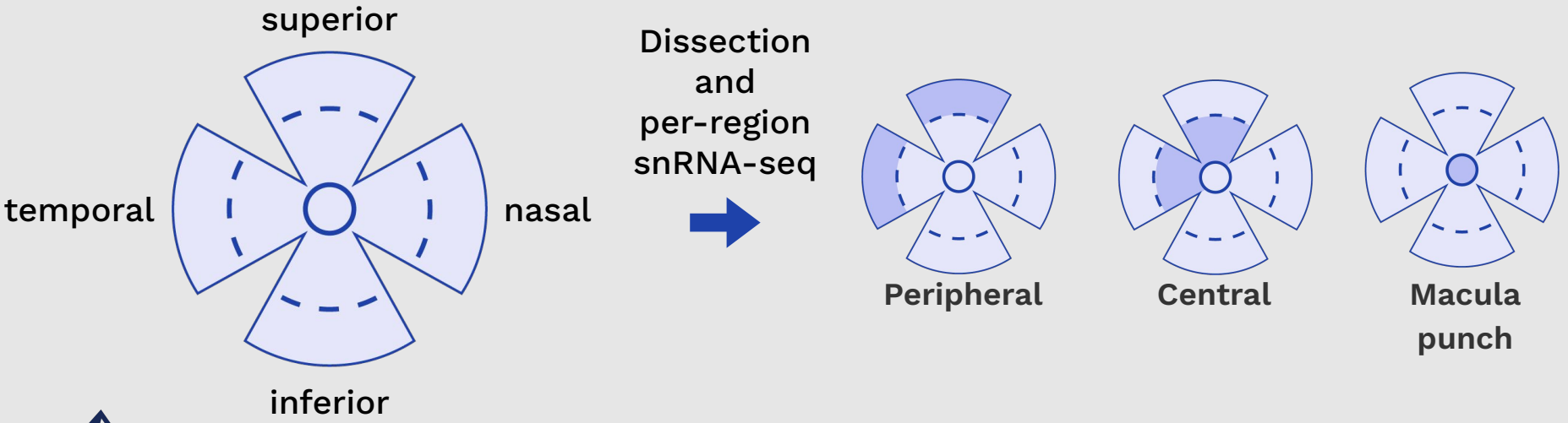
snRNA-seq of NHP retina

80 capsid pool

intravitreal co-injection

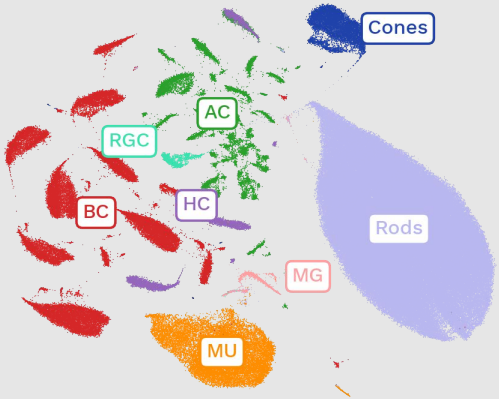
in Cyno NHPs for 28 day in-life period

2.7e11 vg total dose per eye

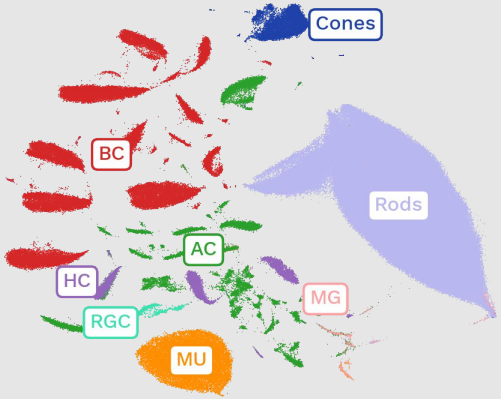


snRNA-seq of NHP retina

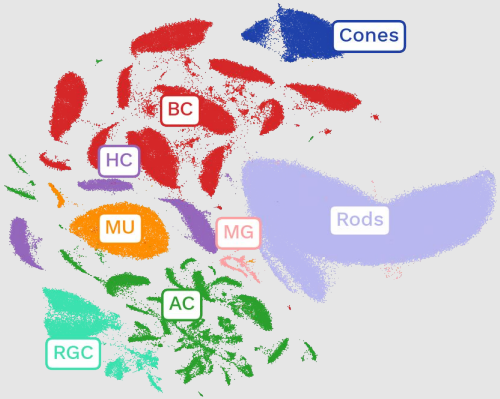
Peripheral retina (268,000 cells)



Central retina (321,000 cells)



Macula punch (307,000 cells)

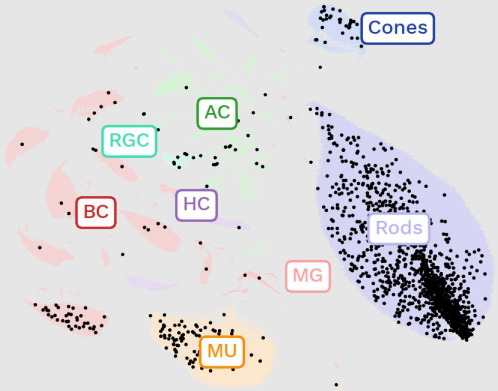


Cell types identified

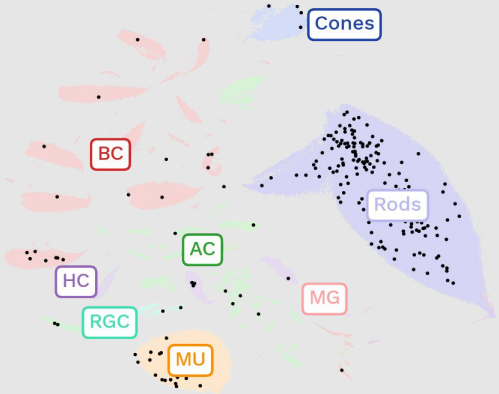
Rods	MU : Müller glia
Cones	MG : Microglia
HC : Horizontal cells	AC : Amacrine cells
BC : Bipolar cells	RGC: Retinal ganglion cells

Cell-type resolved measurement of Dyno eCap 1 transduction

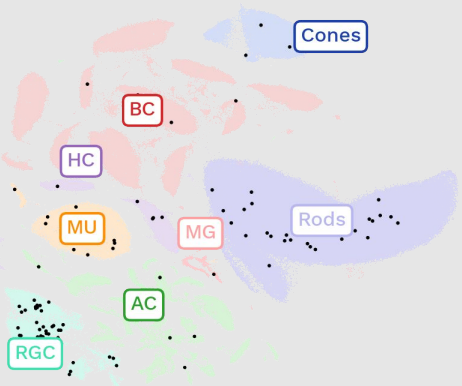
Peripheral retina (268,000 cells)



Central retina (321,000 cells)



Macula punch (307,000 cells)

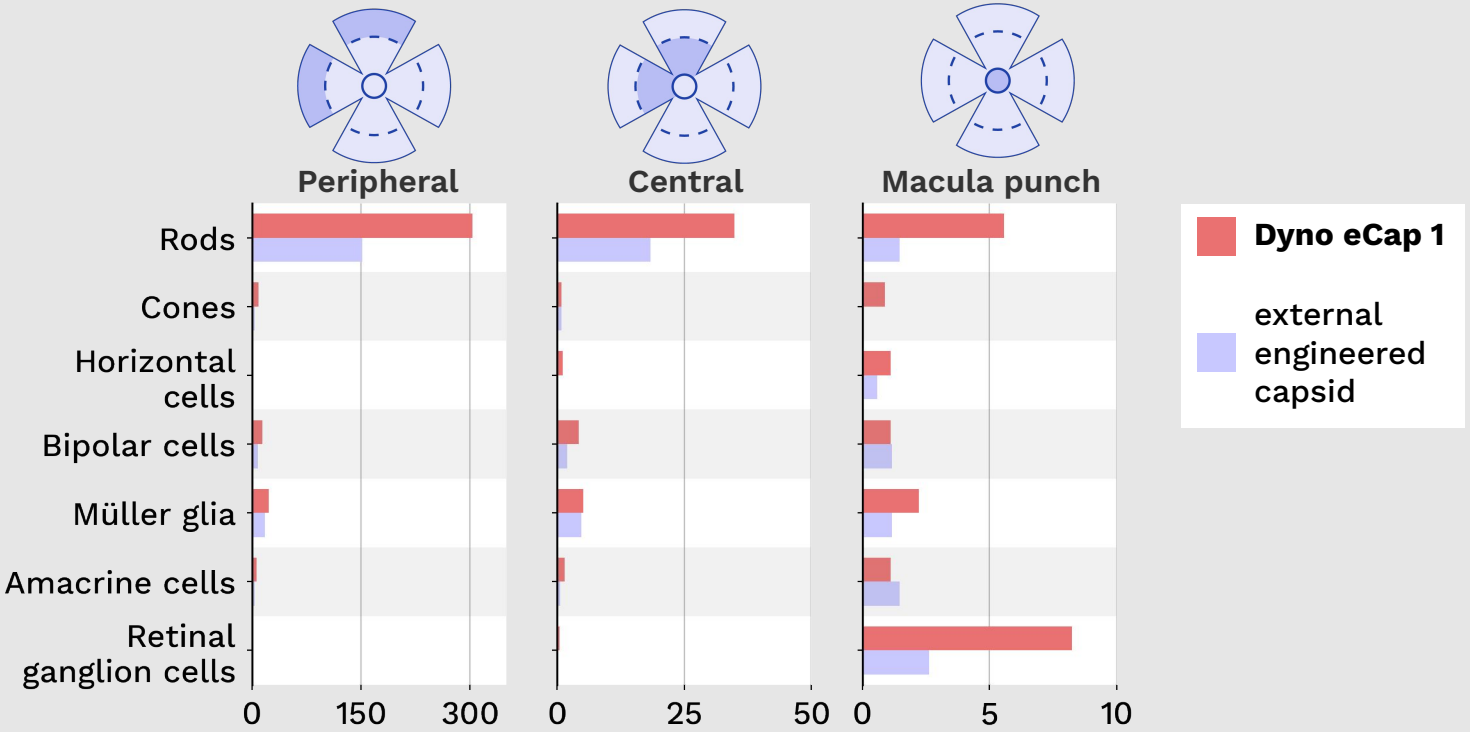


Cell types identified

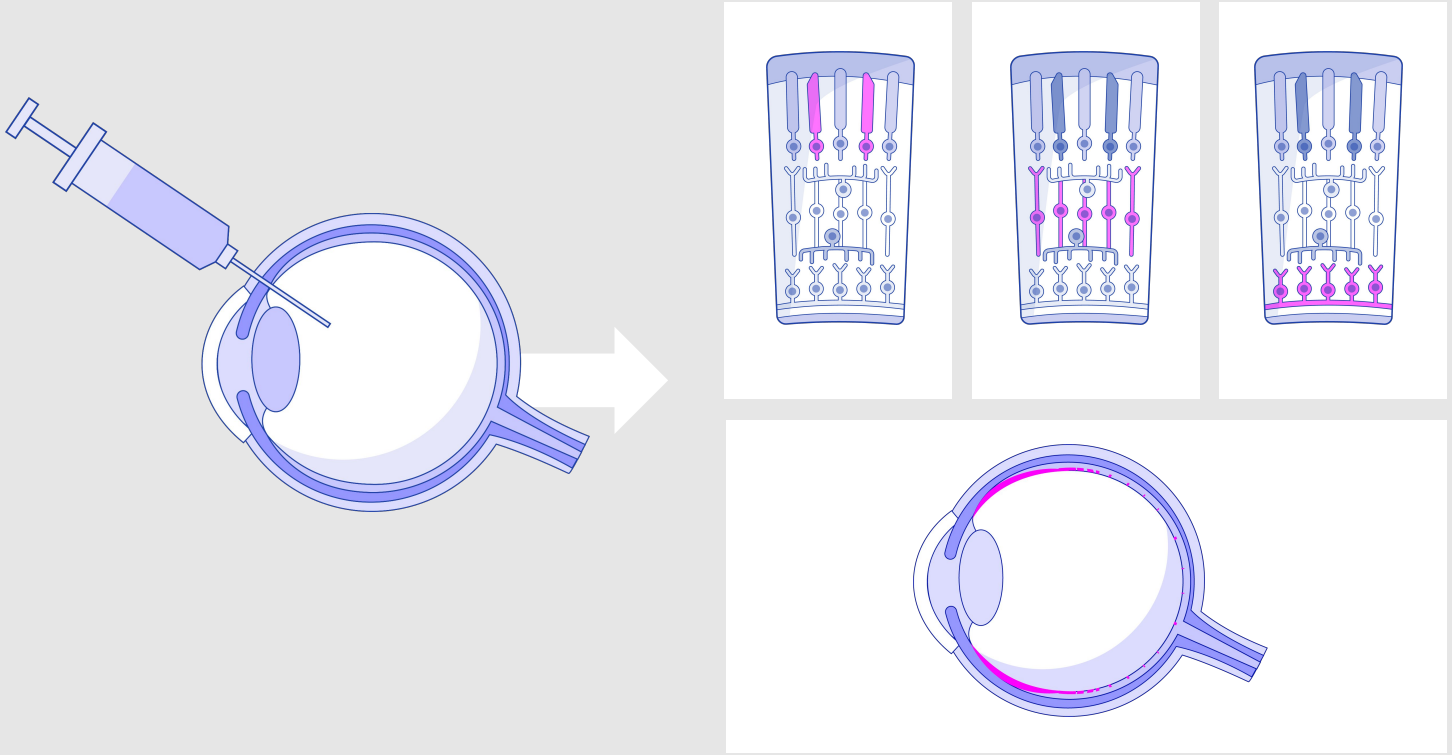
Rods
Cones
HC : Horizontal cells
BC : Bipolar cells
MU : Müller glia
MG : Microglia
AC : Amacrine cells
RGC: Retinal ganglion cells

Black dots denote cells transduced by Dyno eCap 1

Dyno eCap 1 delivery outperforms external capsids across cell types and regions



Validating Dyno eCap 1 delivery for specific use cases via histology

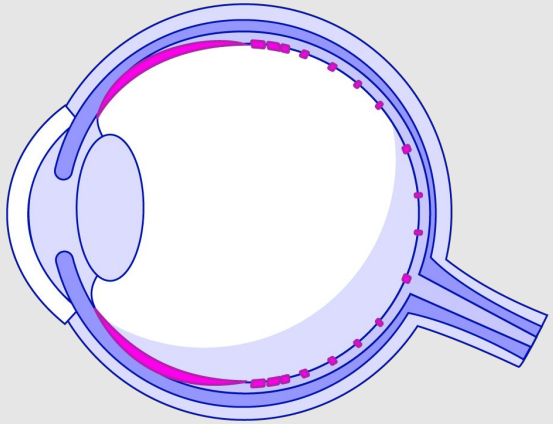


USE CASE 1

Secreted therapeutic biofactory

Patient unmet need:

Age-related Macular Degeneration (AMD)
Dry AMD with Geographic atrophy (GA)
Diabetic Macular Edema (DME)

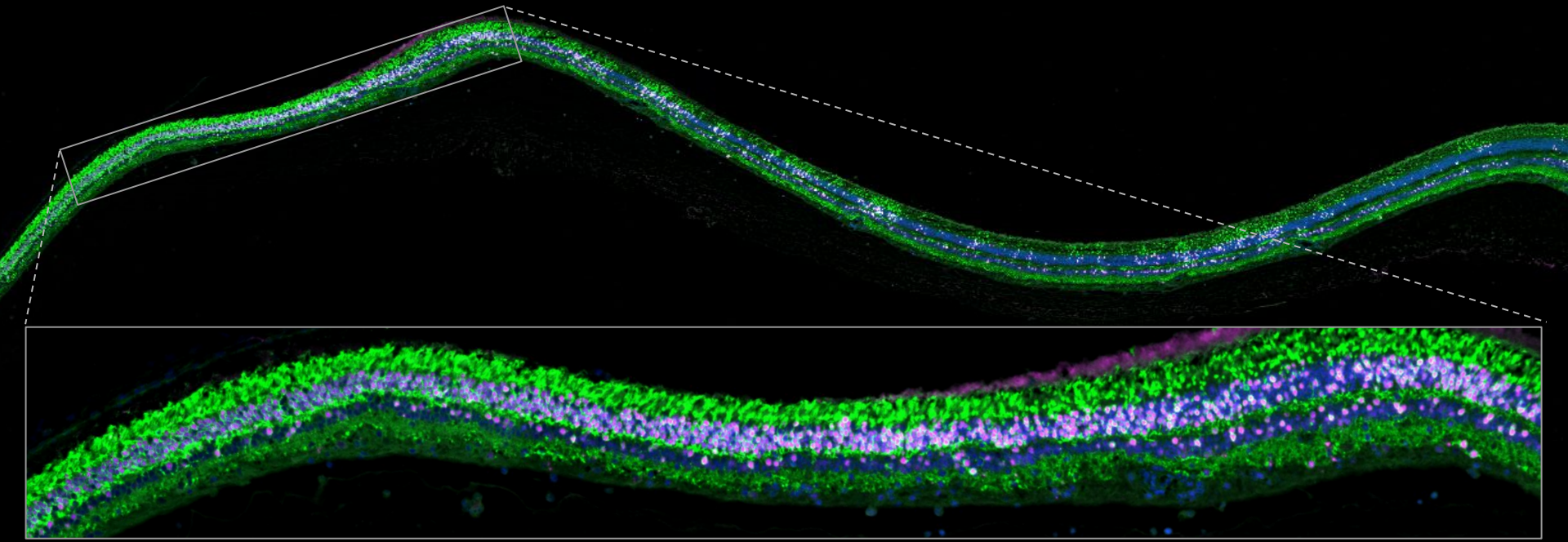


Delivery challenge:

Safe and easy-to-administer delivery across retina, reaching **enough cells** for secreted proteins to achieve a therapeutic dose

Secreted therapeutic biofactory

Dyno eCap 1 efficiently transduces more retinal cells after low dose IVT injection than external capsids



USE CASE 2

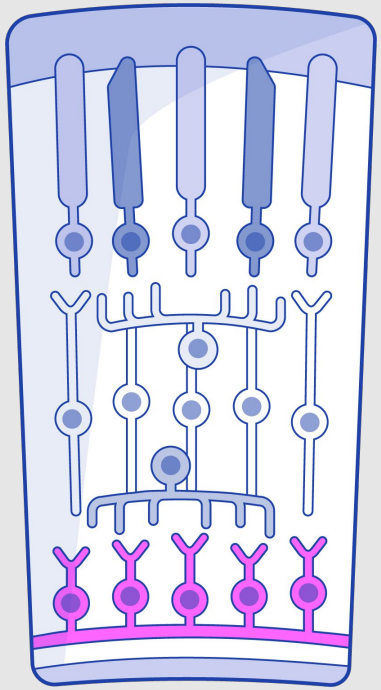
Glaucoma

Patient unmet need:

Degeneration of retinal ganglion cells (RGCs) in macula leading to central vision loss

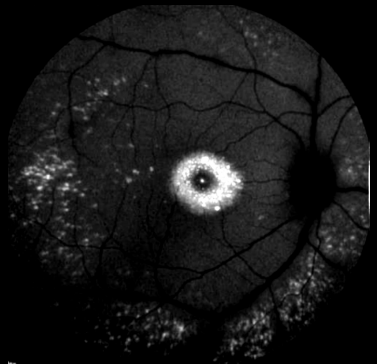
Delivery challenge:

Safe and easy-to-administer delivery to RGCs responsible for central vision in the **macula**

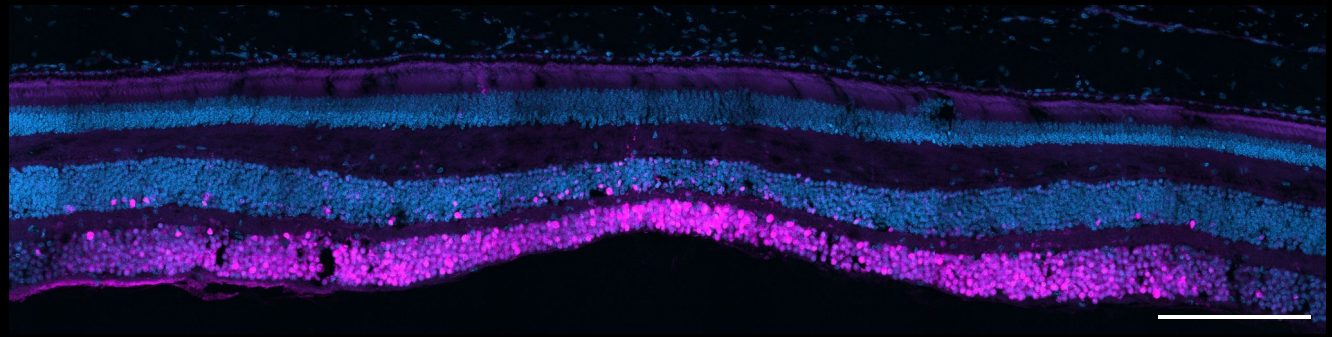


Glaucoma

Dyno eCap 1 achieves highly efficient transduction of RGCs in macular region, enabling therapeutic interventions that support survival of macular RGCs



cSLO imaging
(8.1e10 vg IVT)



Cyno macula with Dyno eCap 1 (1.4e11 vg IVT)

200 µm

USE CASE 3

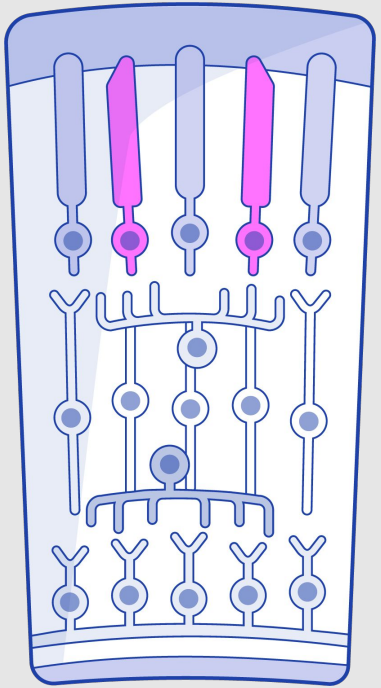
Inherited retinal diseases

Patient unmet need:

Retinitis pigmentosa is the leading cause of progressive vision loss early in life

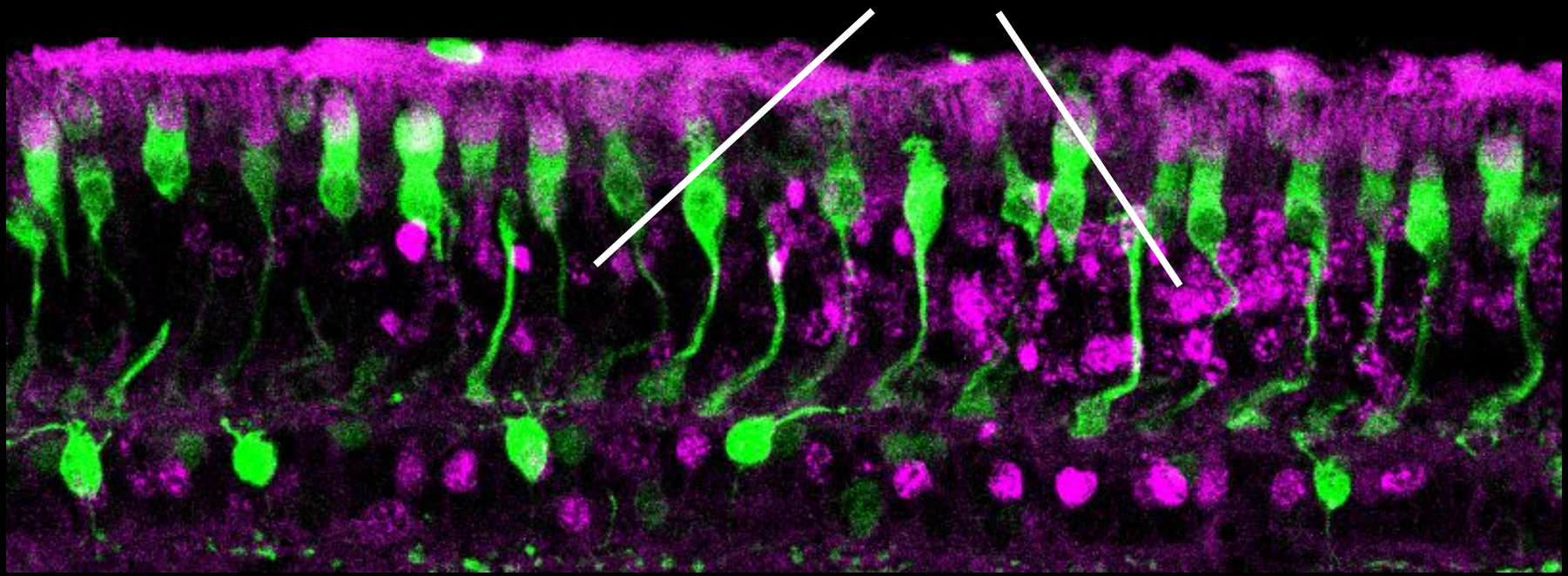
Delivery challenge:

Reach enough photoreceptors to prevent their degeneration and modify disease progression



Inherited retinal diseases

Dyno eCap 1 primarily transduces rod photoreceptors



Calbindin (cones) / **Dyno eCap 1**

USE CASE 4

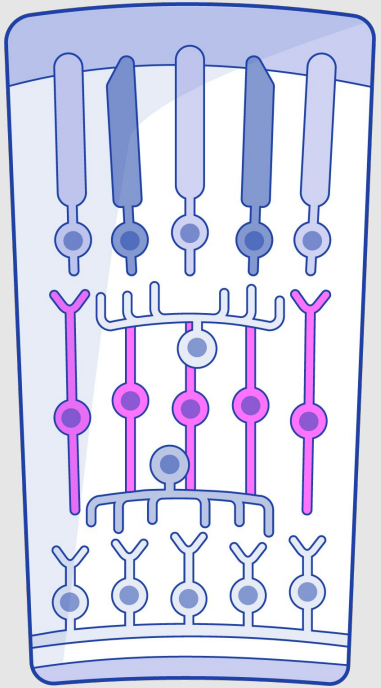
Optogenetic therapy

Patient unmet need:

Total vision loss due to advanced retinal disease progression

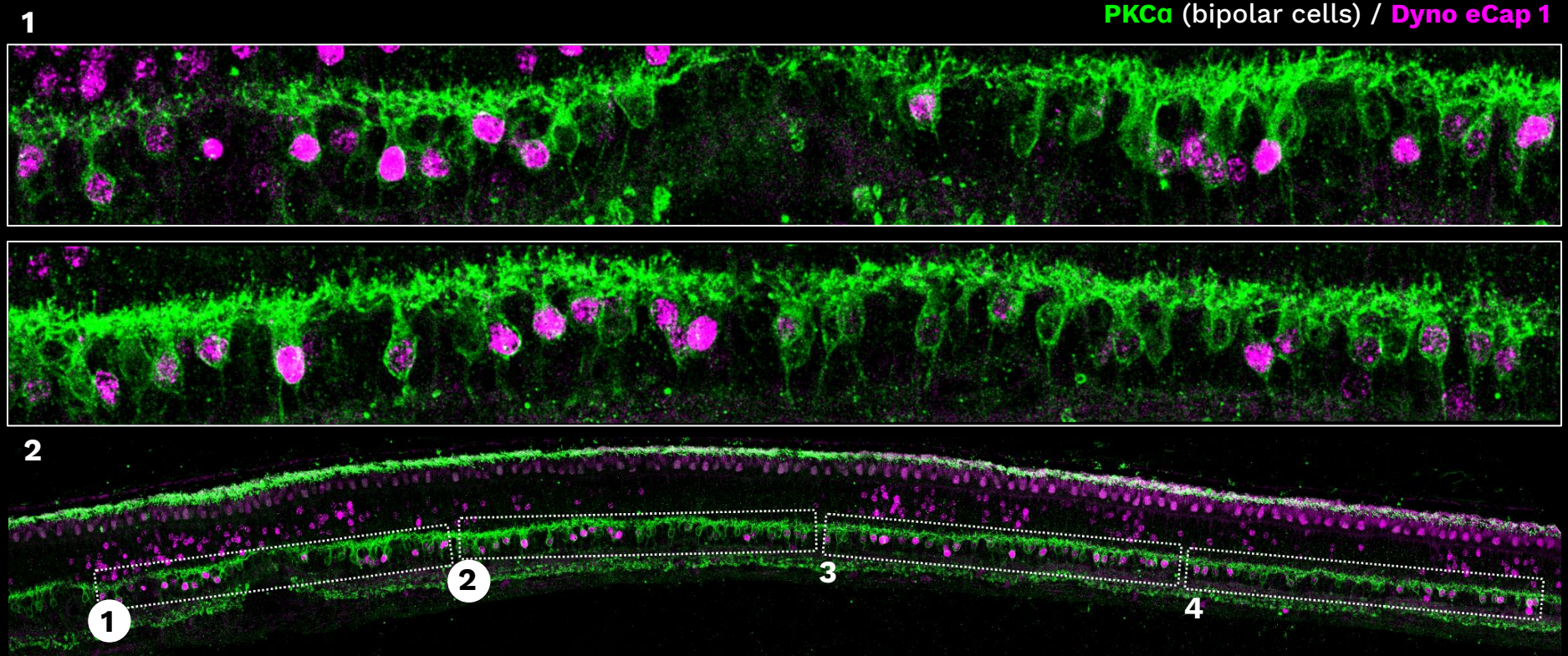
Delivery challenge:

Reaching enough bipolar cells, to enable an optogenetic intervention to have an impact



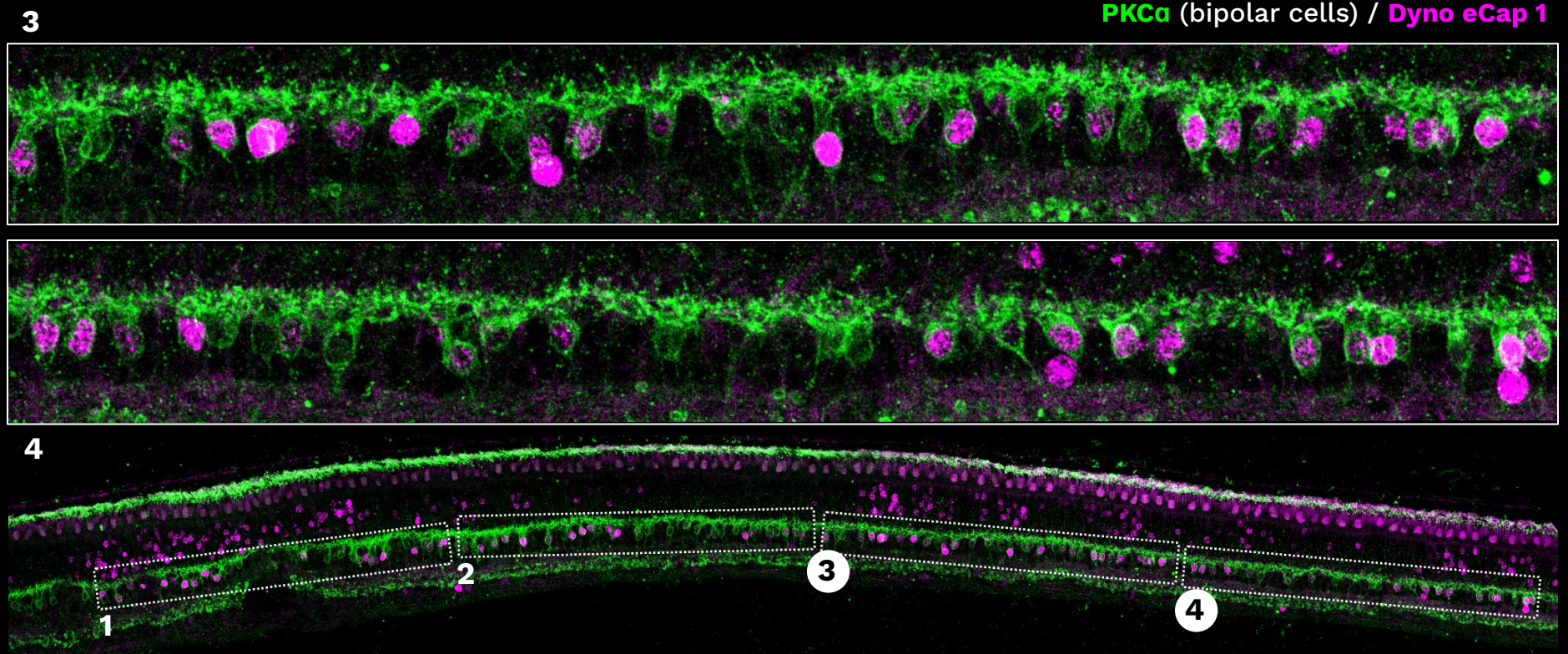
Optogenetic therapy

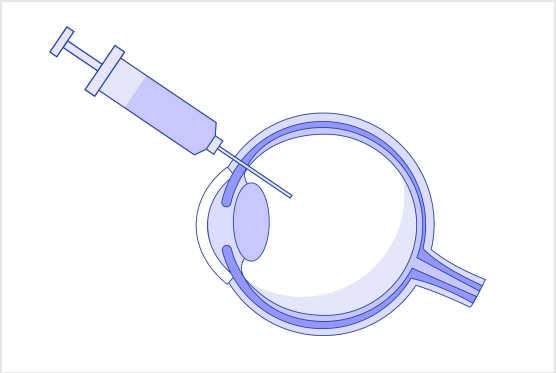
Highly efficient transduction of bipolar cells with Dyno eCap 1

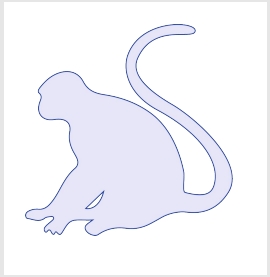


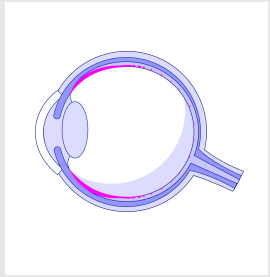
Optogenetic therapy

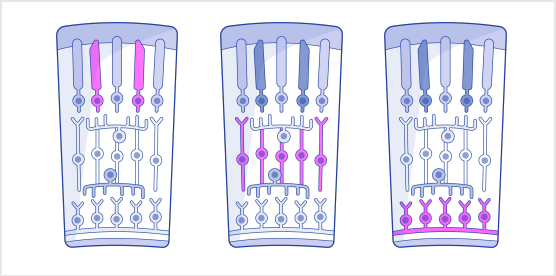
Highly efficient transduction of bipolar cells with Dyno eCap 1











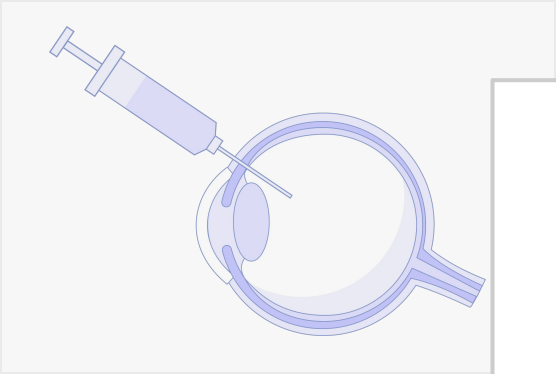
Dyno eCap 1 delivery

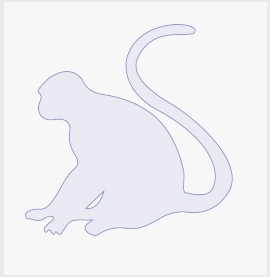
Designed for **IVT eye delivery**

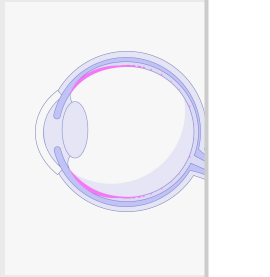
1x production vs AAV2
80x retina transduction vs AAV2

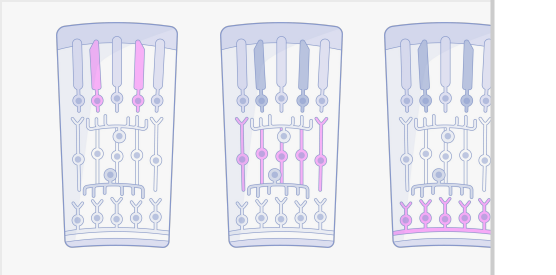
Transduces key **NHP retina** cell types including **retinal ganglion cells, bipolar cells & rod photoreceptors**

Ready for use in **biofactory, neuroprotection, optogenetic & photoreceptor targeted ocular gene therapies**









Dyno eCap 1 delivery

e delivery

AAV2

on vs AAV2

ina cell types

glion cells,

toreceptors

neuroprotection,

eptor targeted

ocular gene therapies

Abstract P038:

"Dyno eCap 1 Capsid: Cell-type resolved validation of an AAV Capsid optimized for intravitreal delivery to the non-human primate retina"

Reaching greater heights together

Dyno is **partnership-centric:** We succeed only when our partners win by helping patients

We are working to solve in vivo delivery for you

- We designed capsids with **field-leading potential** and validated their properties in **Cynomolgus monkeys:**
  - **Dyno bCap 1** for brain
  - **Dyno eCap 1** for eye
- All of **these capsids** and **additional emerging capsids** are available for licensing (bd@dynotx.com)

Dyno eCap 1 Vector

License ready

Extensively validated in Cyno monkeys

Enables best-in-class delivery for
a broad set of ocular indications

High-potential to help gene therapy patients