Engineered AAV Capsids Exhibit Improved Transduction of the Central Nervous System After CSF Administration in Adult Cynomolgus Macaques

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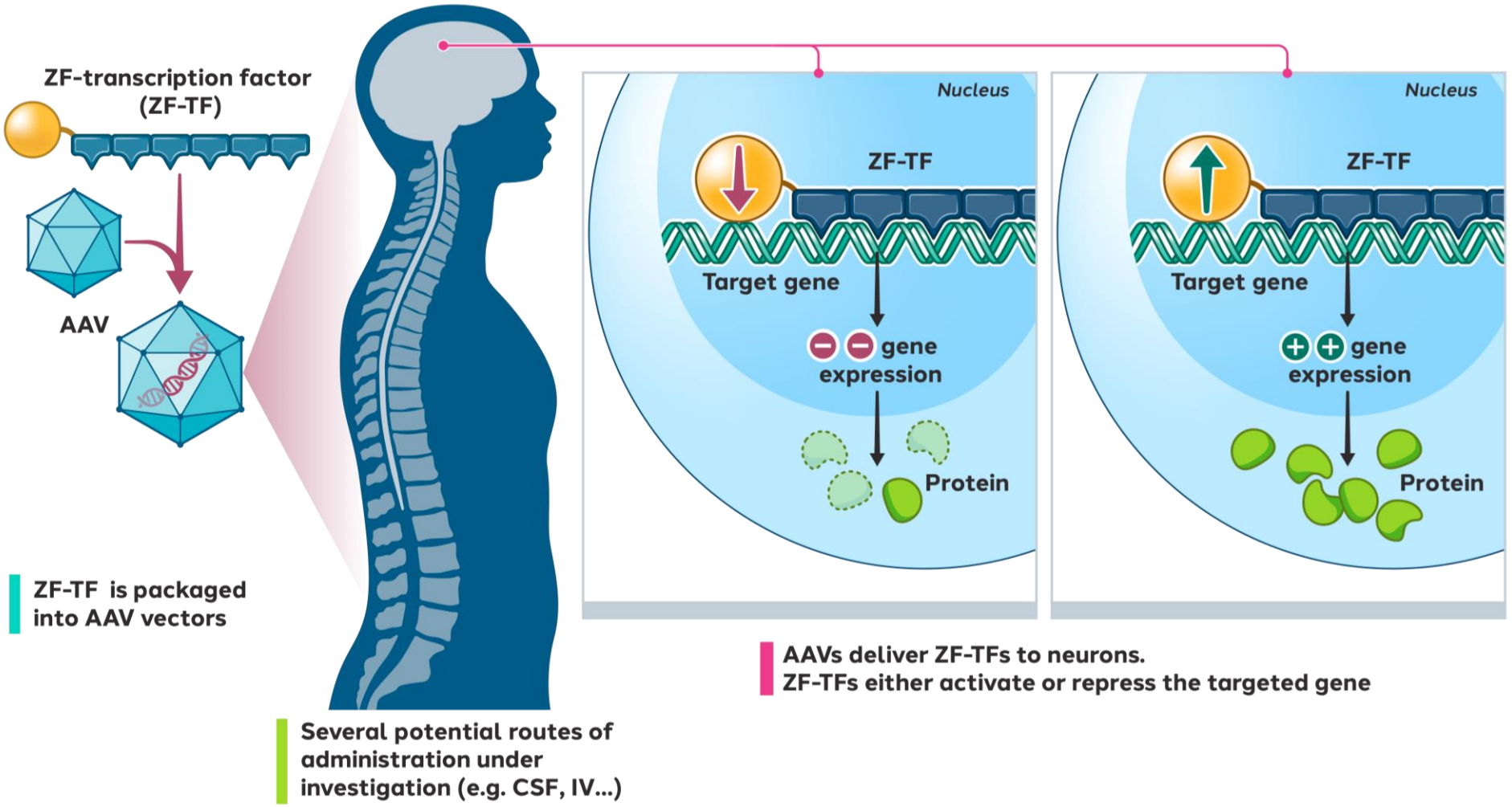
ASGCT 25th Annual Meeting – May 17, 2022

Disclosure

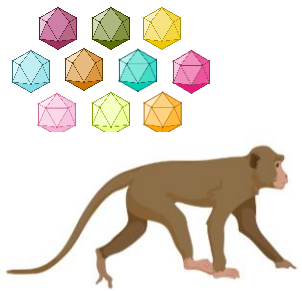
I am a full-time employee of Sangamo Therapeutics

Deploying AAV and zinc finger platforms for CNS therapeutics



SIFTER platform enables functional selection of AAV capsids that transduce target cells

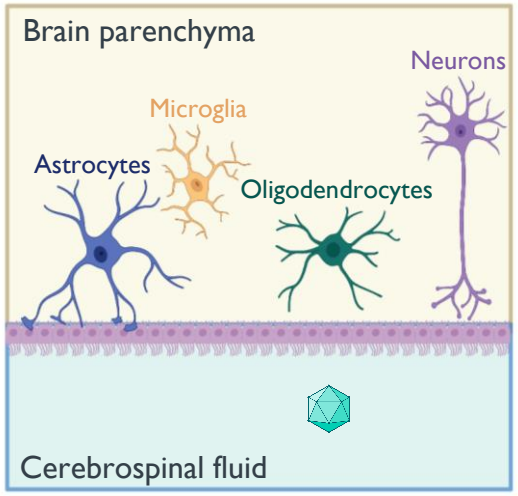
AAV library screen in non-human primates



Some AAV capsids reach brain tissue

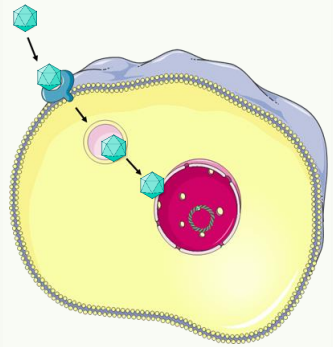
Traditional methods stop here

Overcome biological barriers



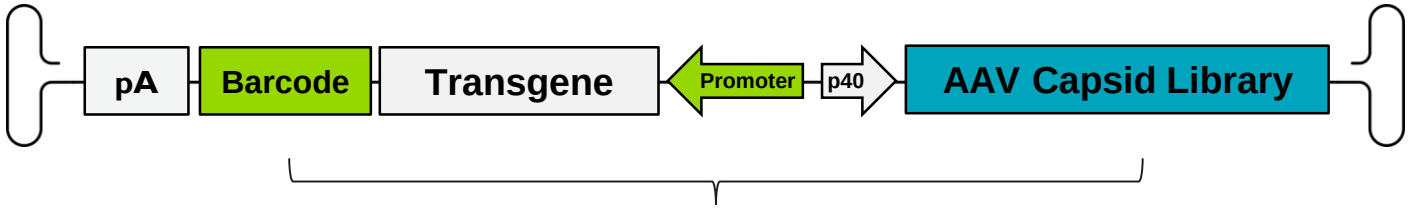
Transduce neurons

Express transgene in neurons



Delivery goal

SIFTER™ = Selecting In vivo For Transduction and Expression of RNA

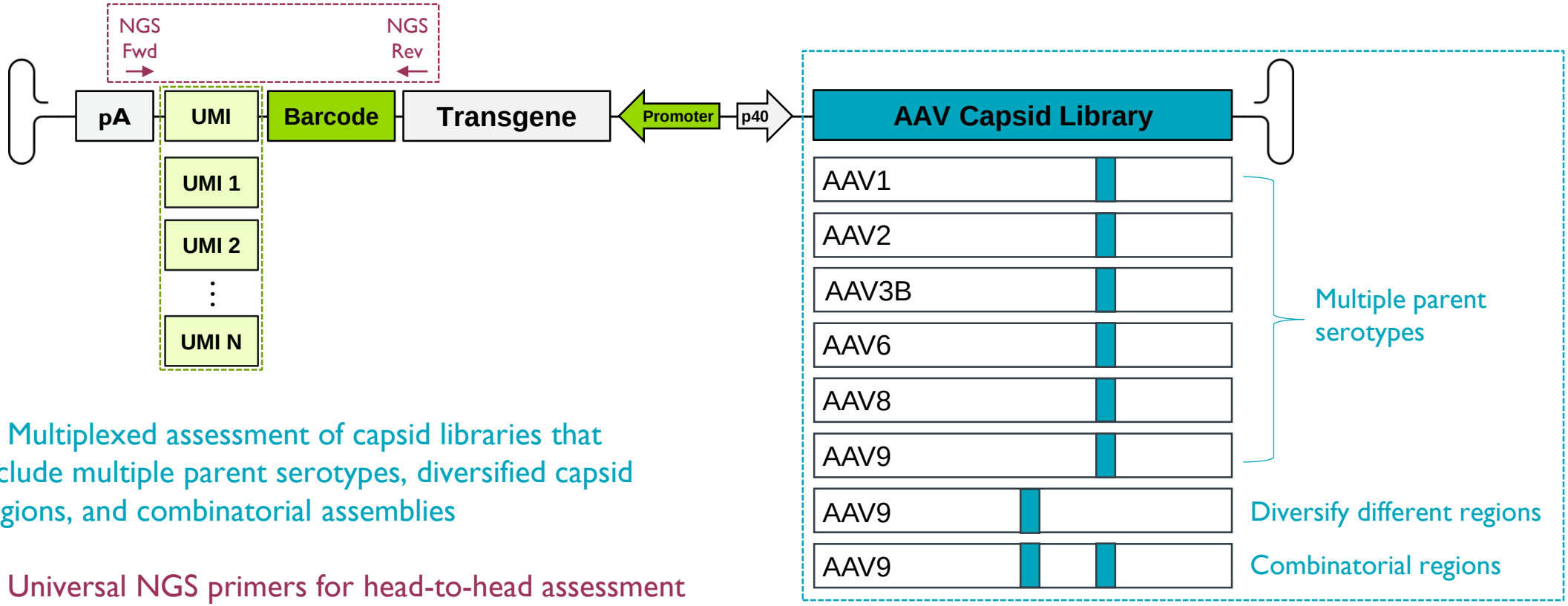


Barcode linked to capsid variant through a pre-established look-up table

Readouts

- DNA ✓
- mRNA ✓
- Cell type ✓

Differentiating features of SIFTER platform



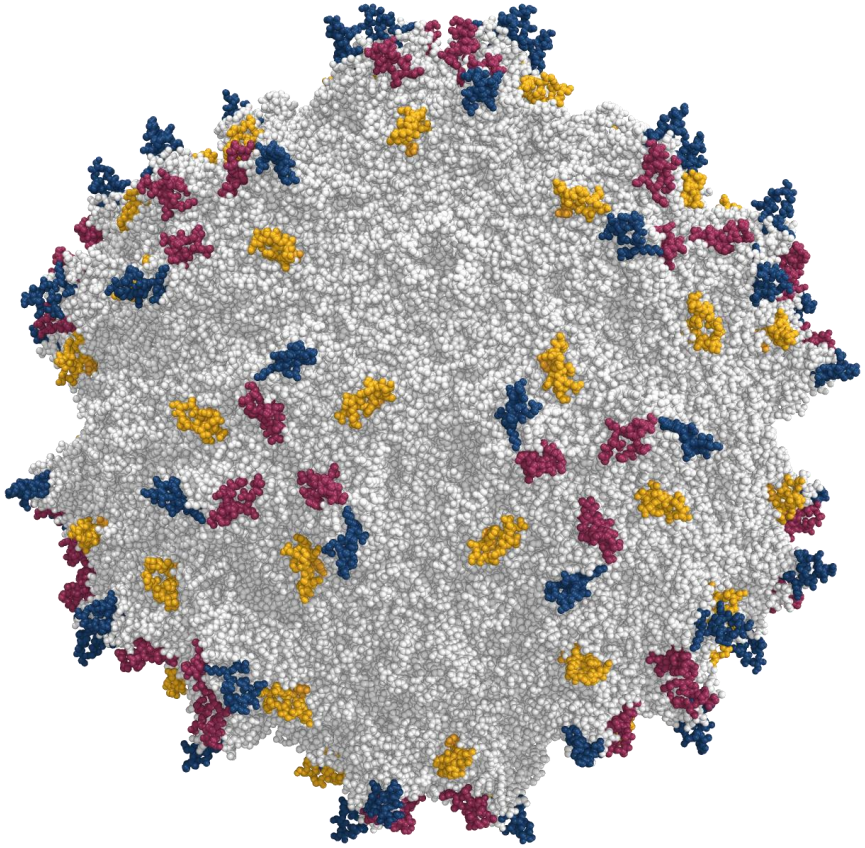
1) Multiplexed assessment of capsid libraries that include multiple parent serotypes, diversified capsid regions, and combinatorial assemblies

2) Universal NGS primers for head-to-head assessment of different libraries in the same animal

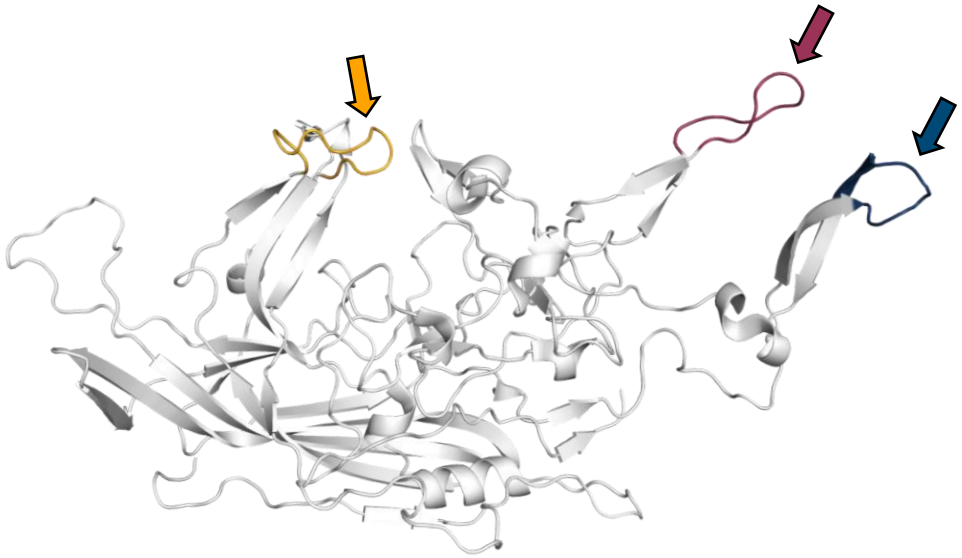
3) Each barcode tagged with additional unique molecular identifiers (UMIs) to improve signal to noise ratio in library screens

Engineered AAV capsid libraries explore a diverse sequence space

Peptide insertion regions in full icosahedral capsid



Peptide insertion regions in viral protein monomer



Parent serotypes: AAV1, 2, 3B, 6, 8, and 9

Peptide insertion sites: VR I, VR IV, VR VIII

Peptide insertion sizes: 7, 10, and 15 amino acids

Head-to-head multiplexed evaluation of different serotypes, insertion sites, and insertion sizes in a single screening campaign

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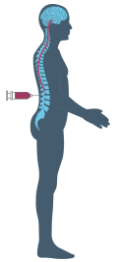
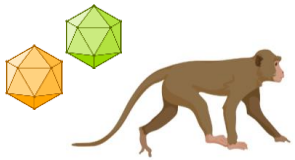
Application of SIFTER platform for identification of CNS-tropic capsids

CSF Delivery Route



+ Lower dose and cost of goods
+ Low levels of anti-AAV antibodies in the CSF
- Distribution to deep brain regions is challenging

Status of selection campaign



Lead capsid validation in non-human primates

(Today's focus)

Intravenous Delivery Route

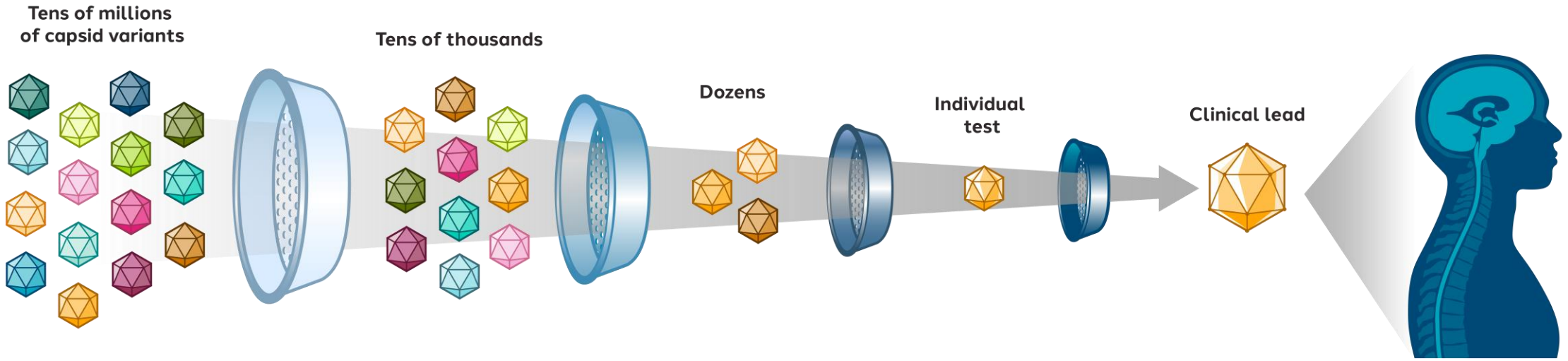
+ Potential to access all neurons throughout the brain
+ Least invasive delivery route
- Blood-brain barrier limits transduction
- Exposure to pre-existing anti-AAV antibodies

Status of selection campaign

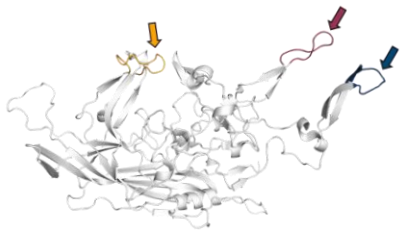
Library screening in non-human primates

Matt Tiffany - Abstract 899, Poster 1371
Wednesday May 18, 5:30-6:30

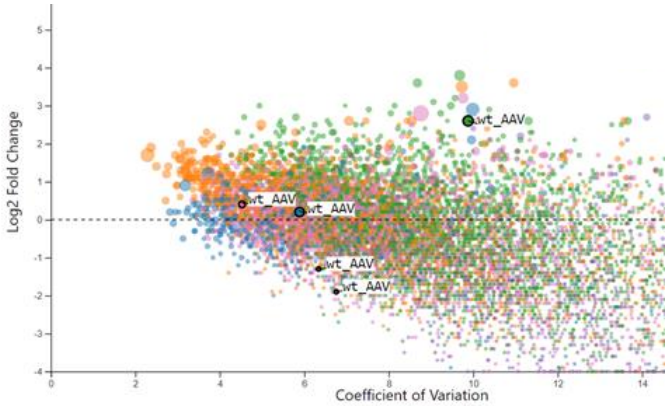
Library selections identify AAVs with improved CNS delivery after CSF administration



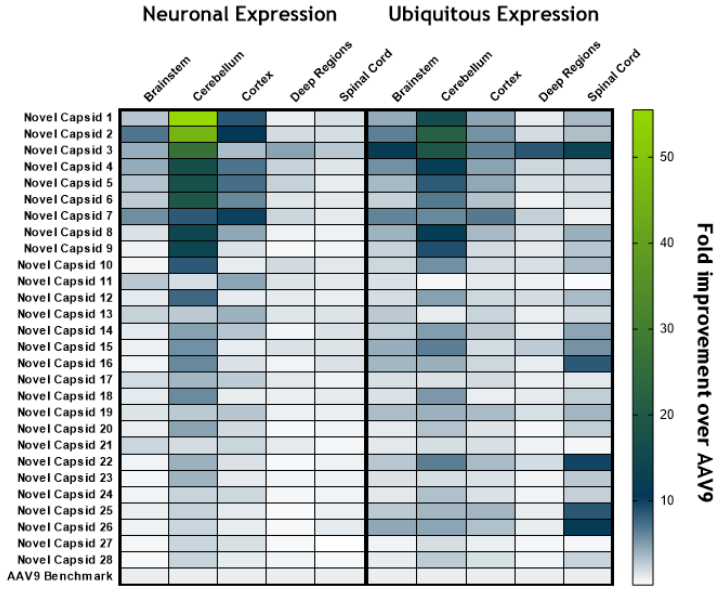
① **Generation of capsid library and screening in NHP**



② **Multiplexed ranking of AAV capsids after CSF delivery in NHP**



③ **Pooled evaluation of barcoded AAVs in NHP with dual neuronal and ubiquitous expression cassette**



④ **Confirm individual capsid performance in NHP**

Sangamo Therapeutics AAV Capsid (STAC)
AAV9 benchmark
STAC-102
STAC-103

Evaluation of lead capsids in adult cynomolgus macaques

Study design

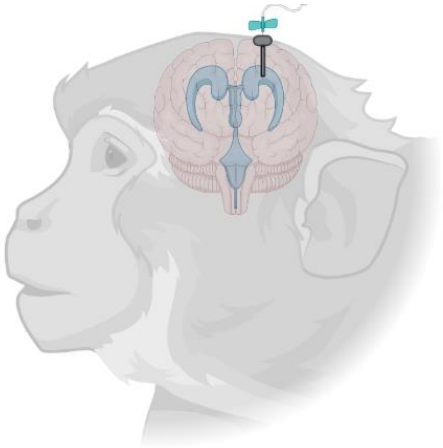
N	Capsid	Dose
3	AAV9 benchmark	3.9E13 total vg
3	STAC-102	3.9E13 total vg
3	STAC-103	3.1E13 total vg

Single stranded AAV vector

Neuron-restricted promoter

Zinc finger repressor

Target gene is predominately expressed in neurons



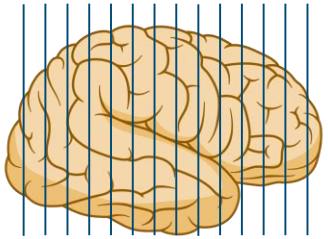
Adult female cynomolgus macaques

Unilateral intracerebroventricular (ICV) delivery

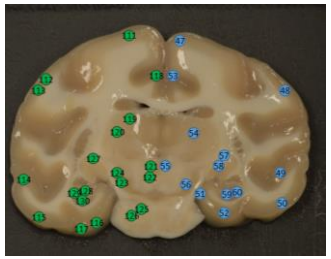
8-week study*

Transduction endpoints

Coronal brain slices



Brain punches



Formalin preservation

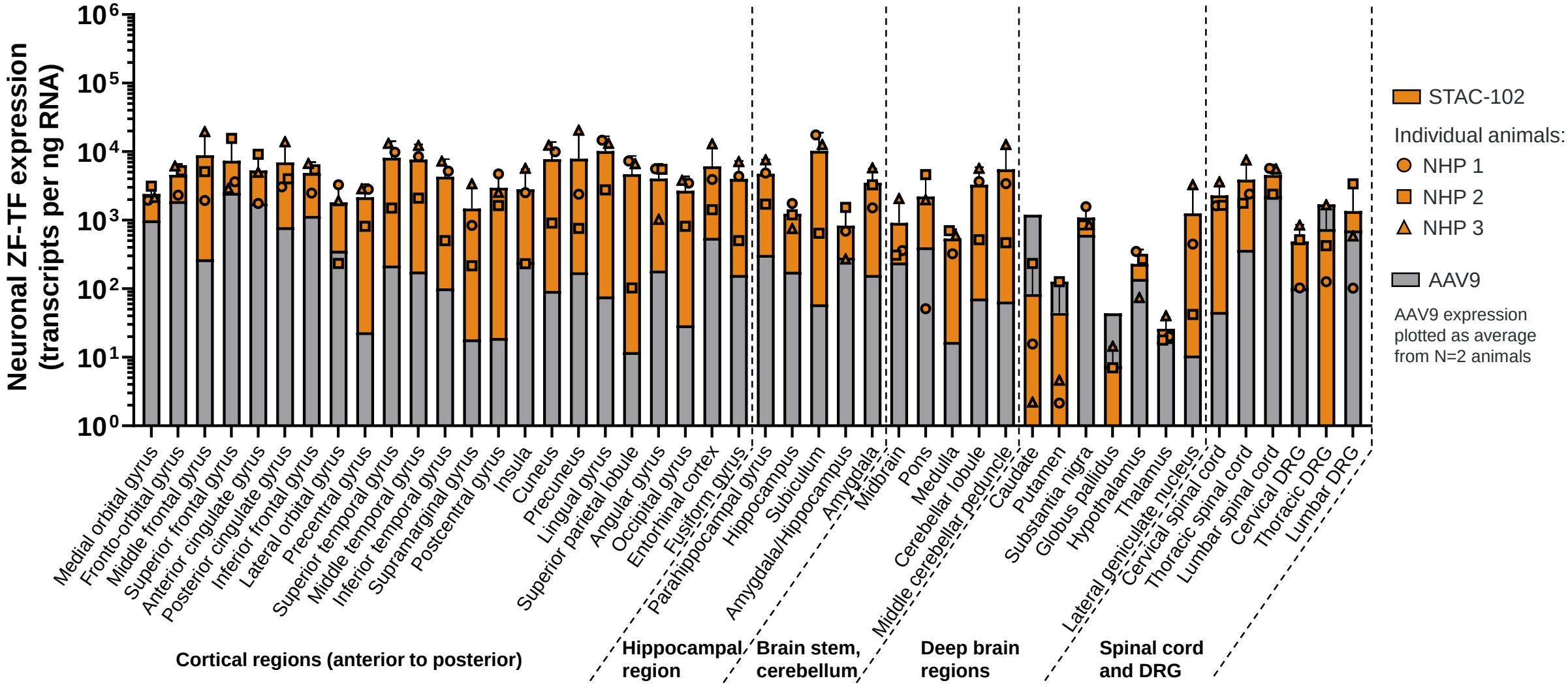
Vector genome biodistribution

Transgene expression in neurons

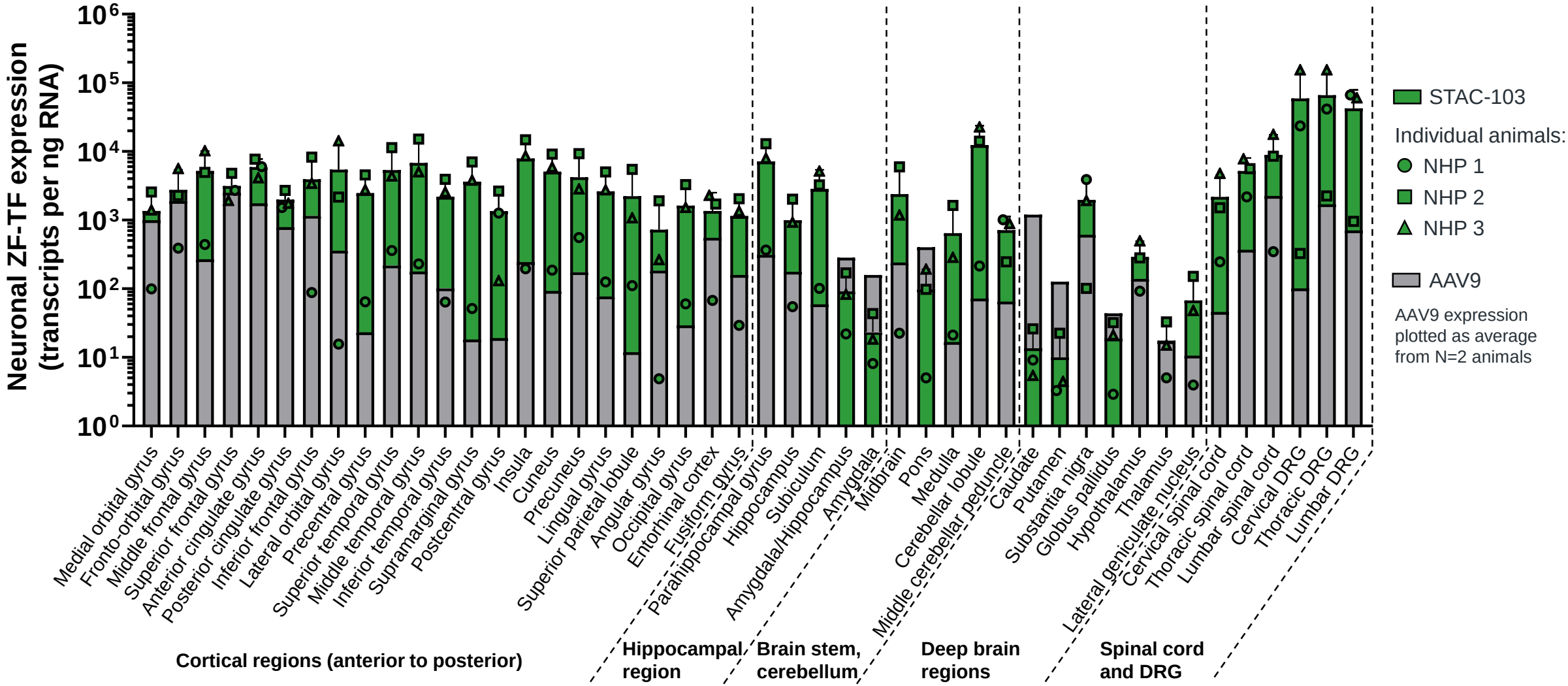
Single cell analysis of zinc finger expression and target repression

*No test-article related clinical signs, changes in body weight, food consumption, hematology parameters, or physical and neurological parameters

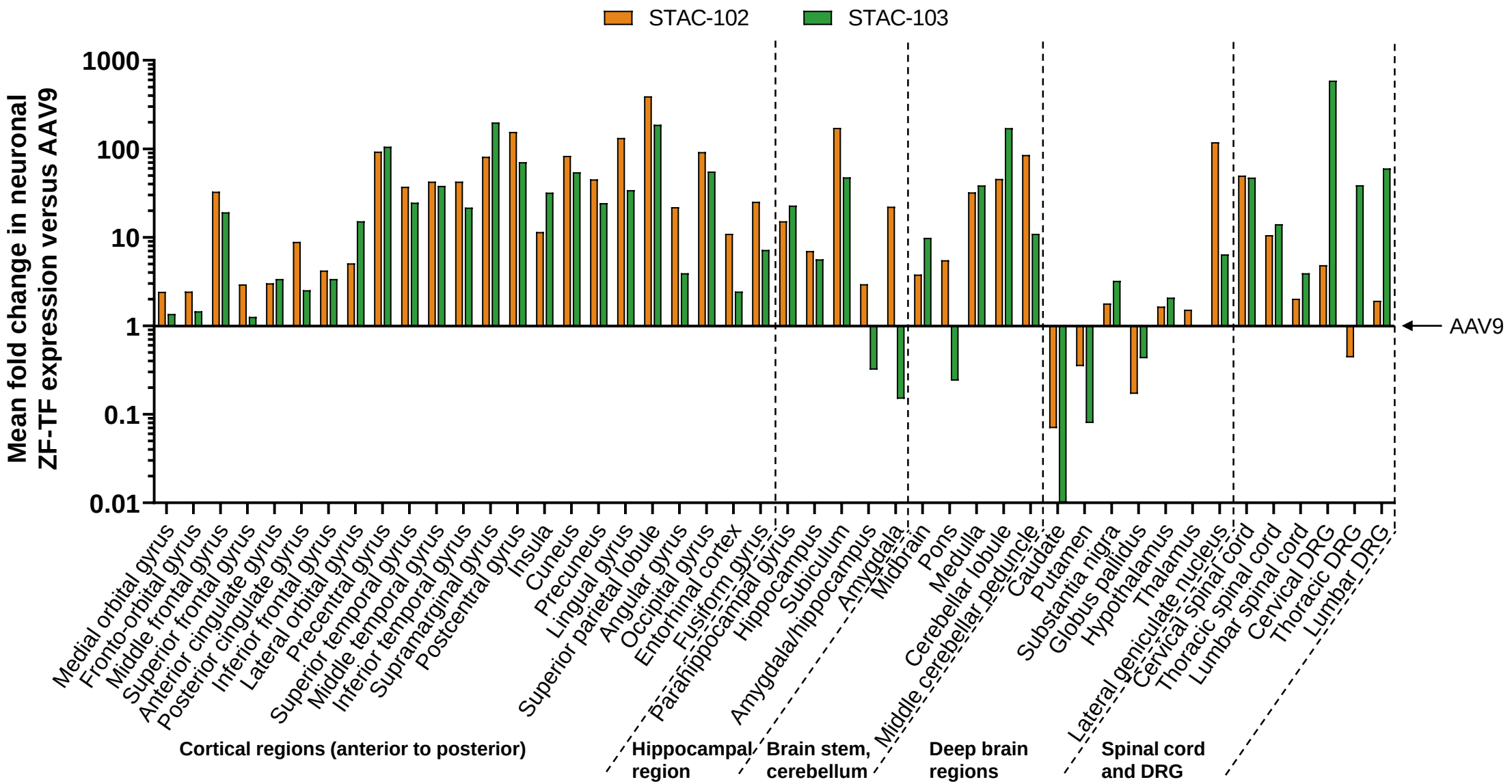
STAC-102 exhibits improved ZF-TF expression compared to AAV9



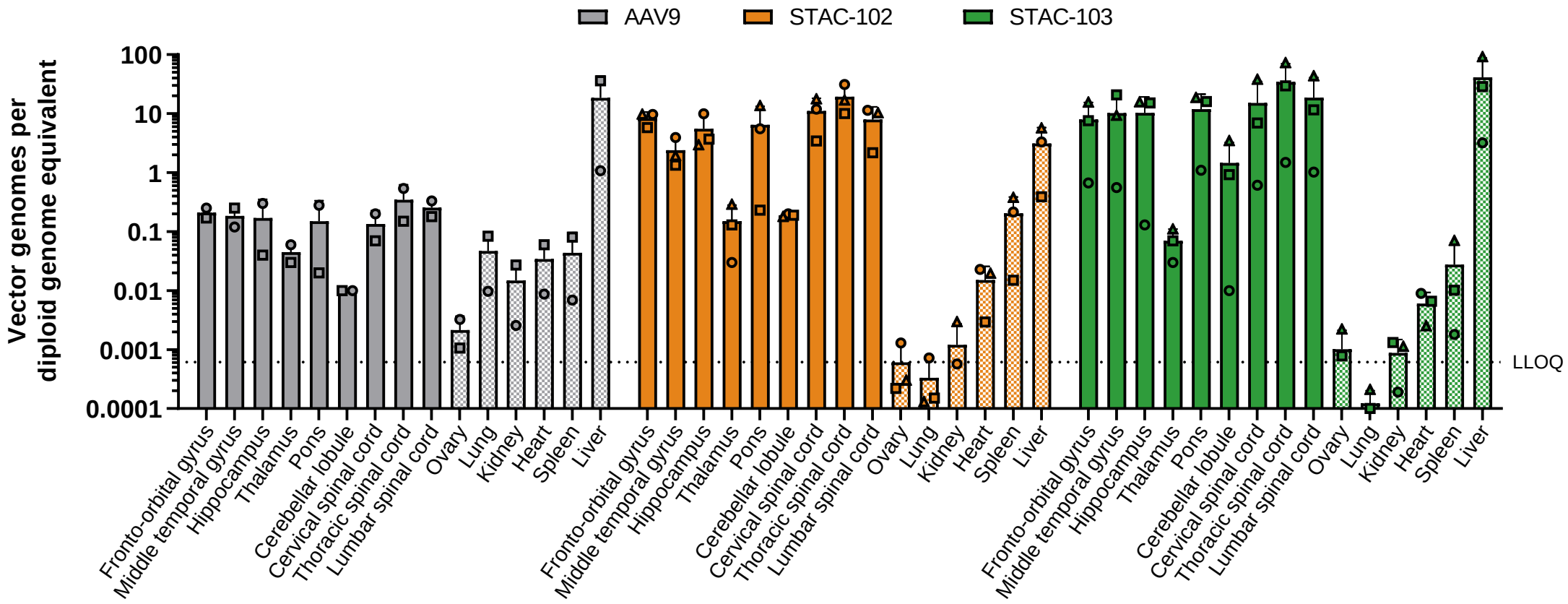
STAC-103 exhibits improved ZF-TF expression compared to AAV9



Fold change in ZF-TF expression versus AAV9



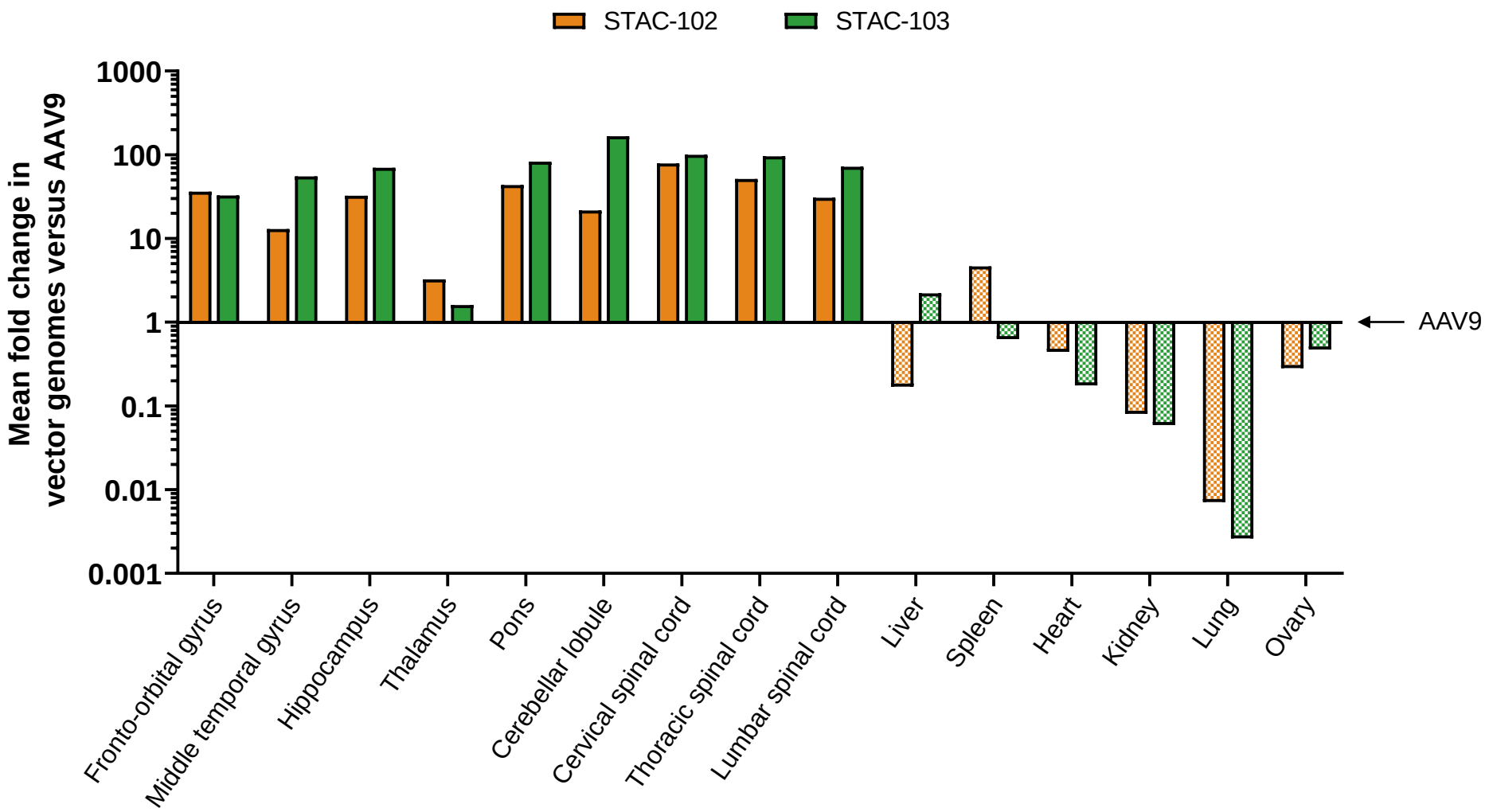
DNA vector genome biodistribution



LLOQ = Lower limit of quantification

○ NHP #1
□ NHP #2
△ NHP #3 (AAV9 NHP #3 exhibited no ZF-TF expression and was removed from the analysis and all fold change calculations)

Fold change in vector genome delivery versus AAV9



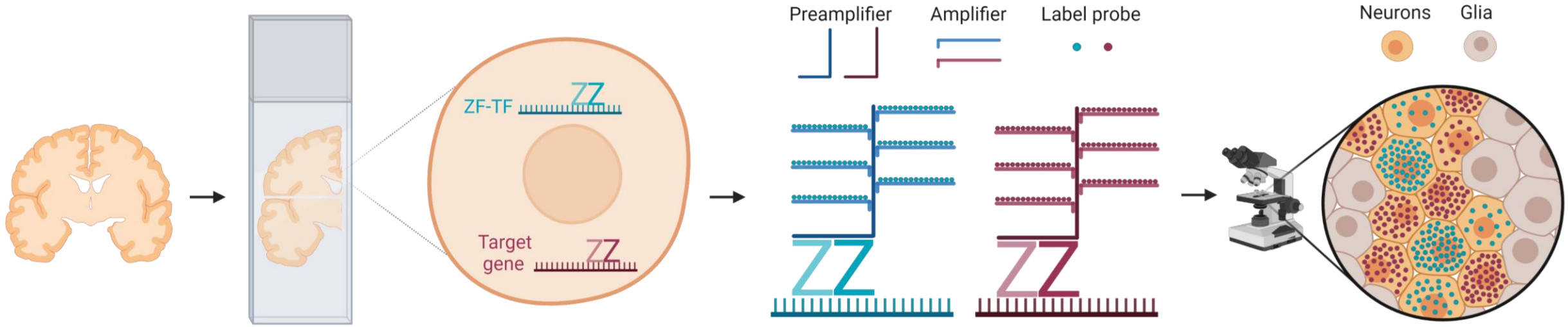
Multiplexed RNAscope in macaque brain sections links ZF-TF expression and target engagement with single cell resolution

① **Fix brain hemispheres, mount sections and permeabilize**

② **Hybridize target-specific RNAscope probes**

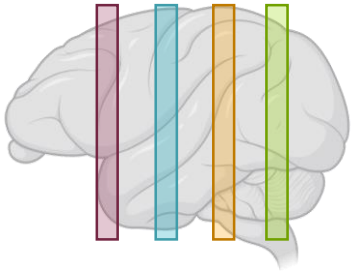
③ **Amplify signal through sequential hybridization of amplifier and label probes**

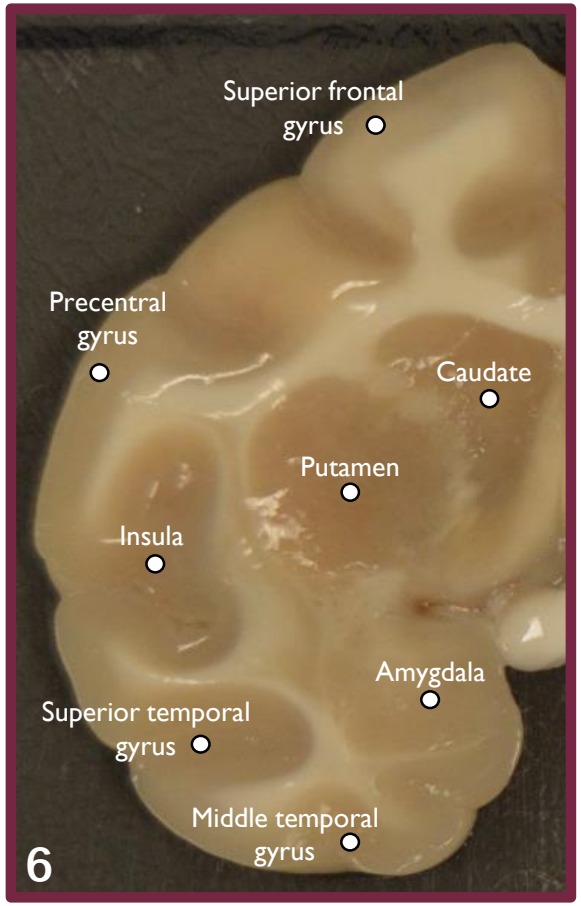
④ **Detect fluorescent labels**

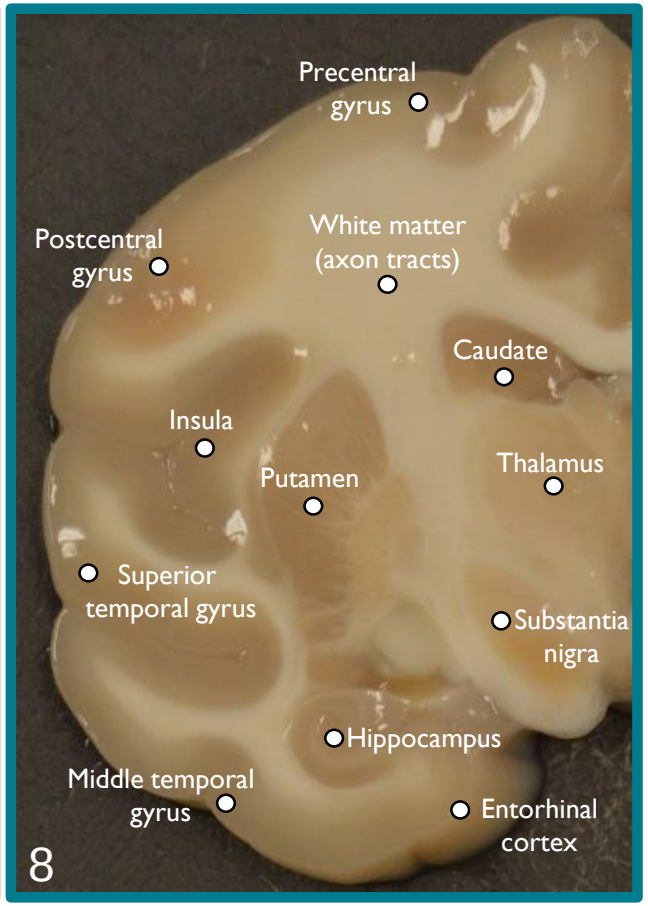


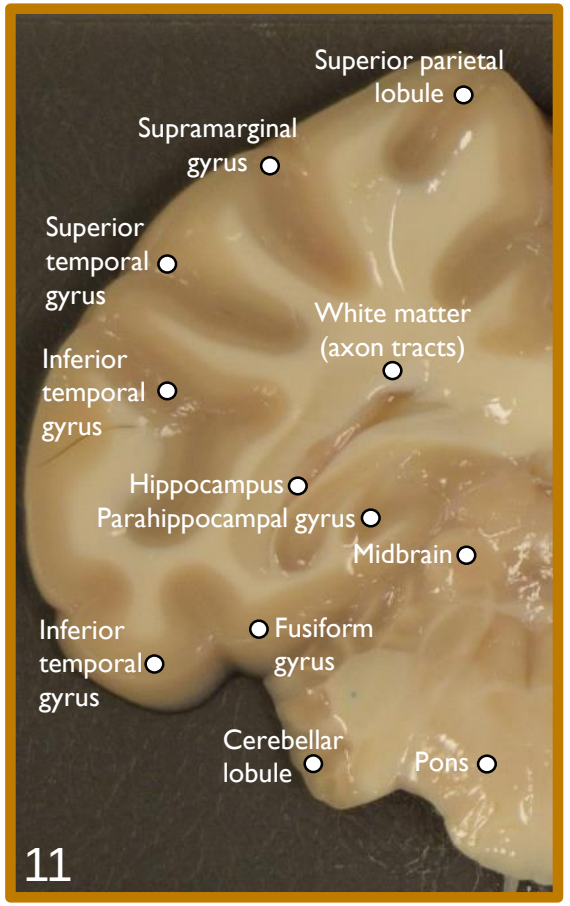
ZF-TF expression and target gene repression is restricted to neurons

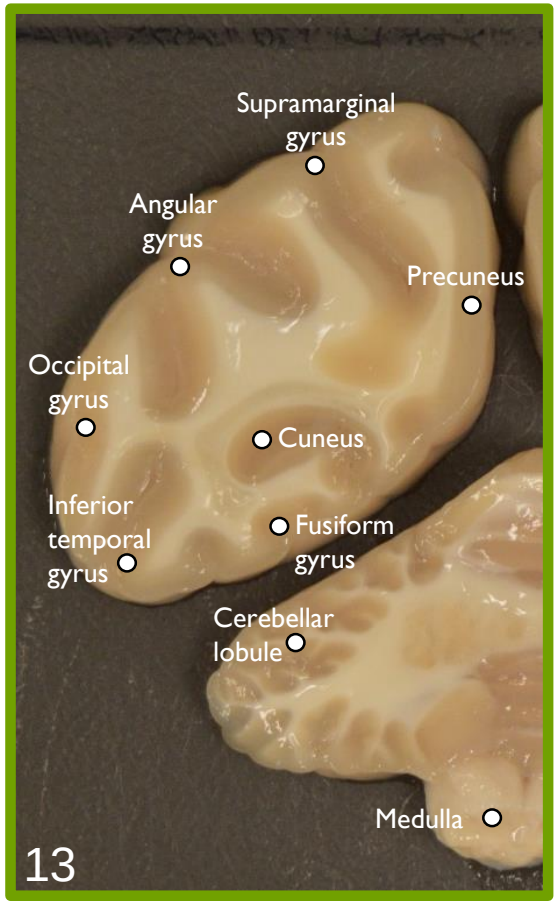
Overview of brain levels analyzed and key structures







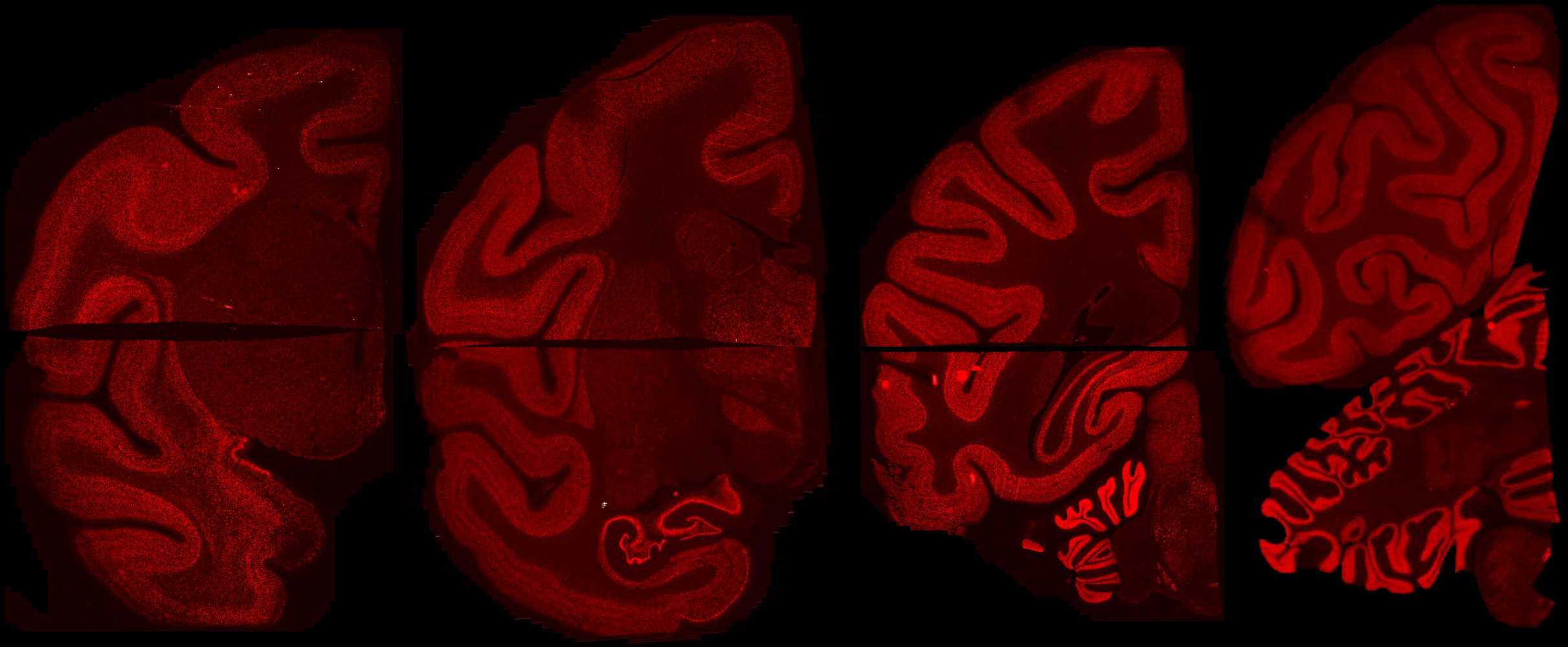




Representative 3 mm brain slices, actual images vary slightly for each animal

Control brain tissue

Level 6 Level 8 Level 11 Level 13

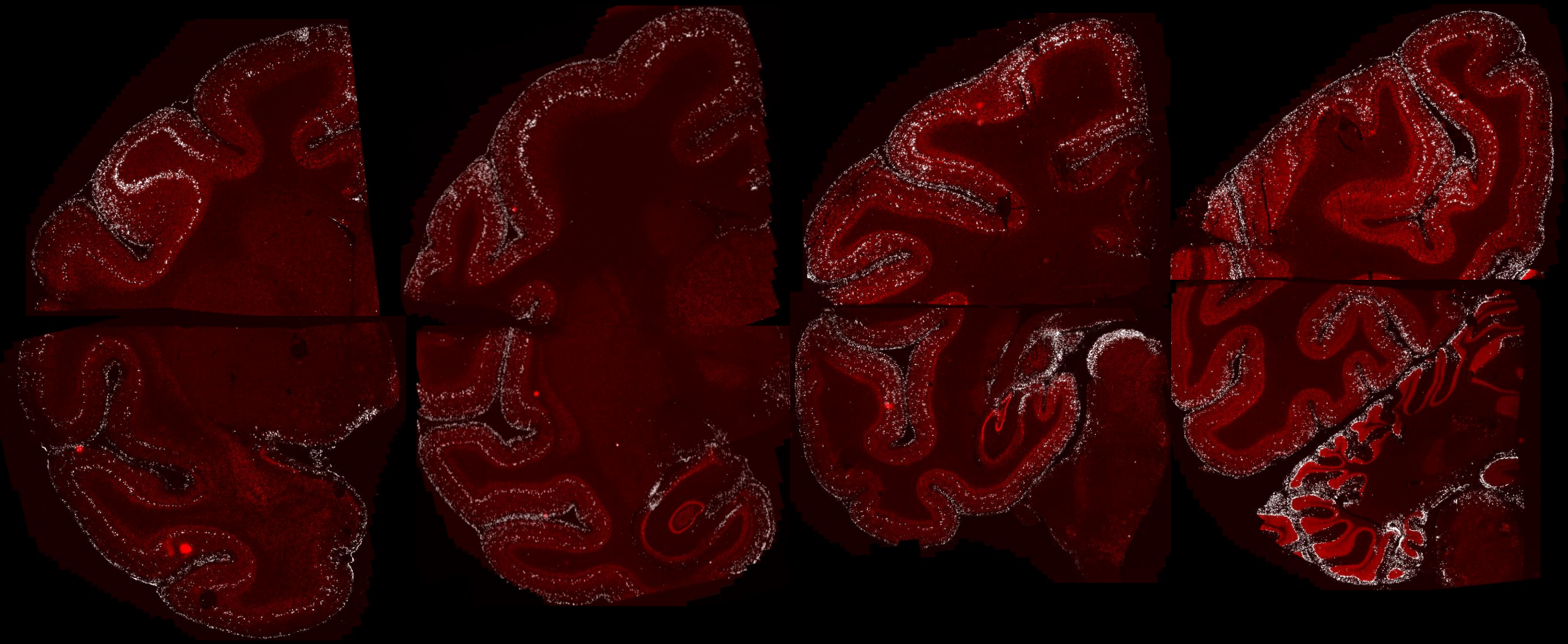


Minimal background signal detected with ZF-TF probe

ZF-TF
Target gene

STAC-103 NHP 2

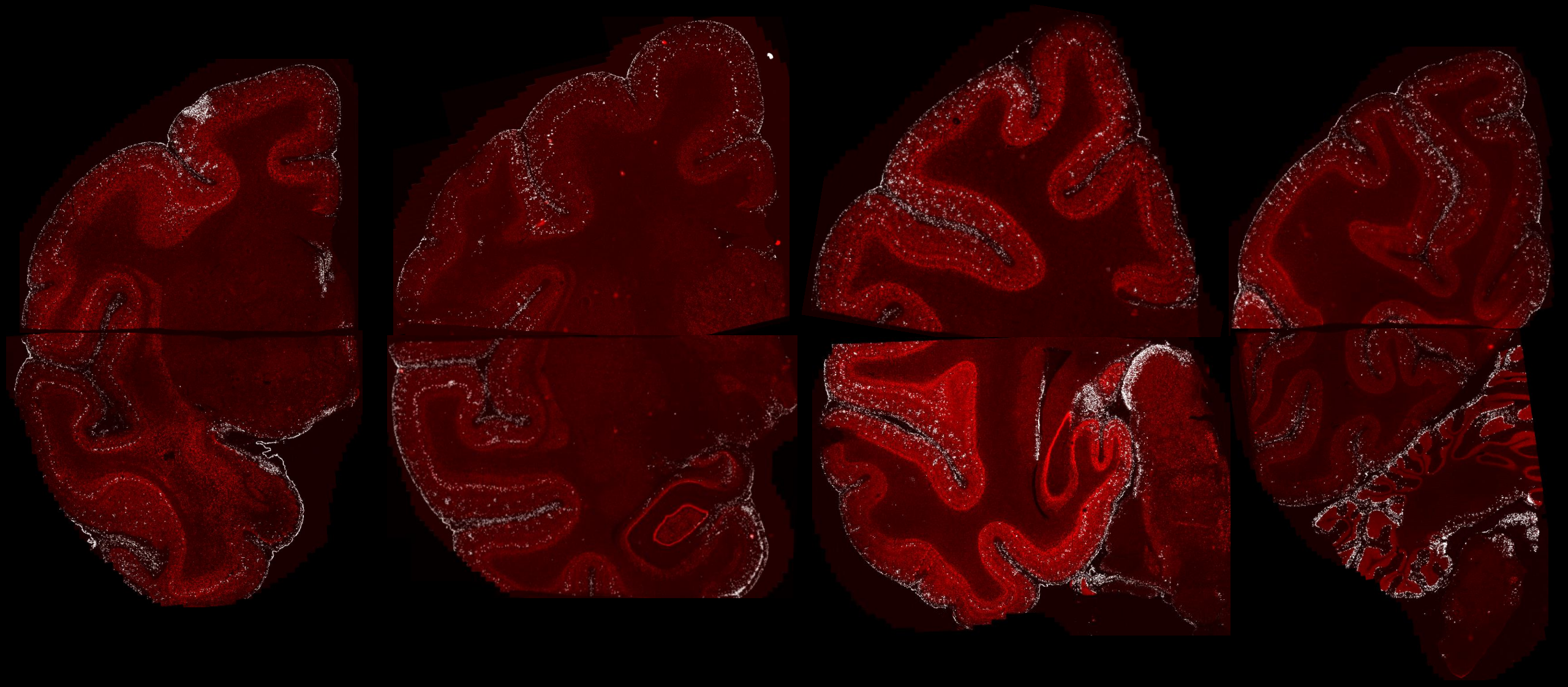
Level 6 | Level 8 | Level 11 | Level 13



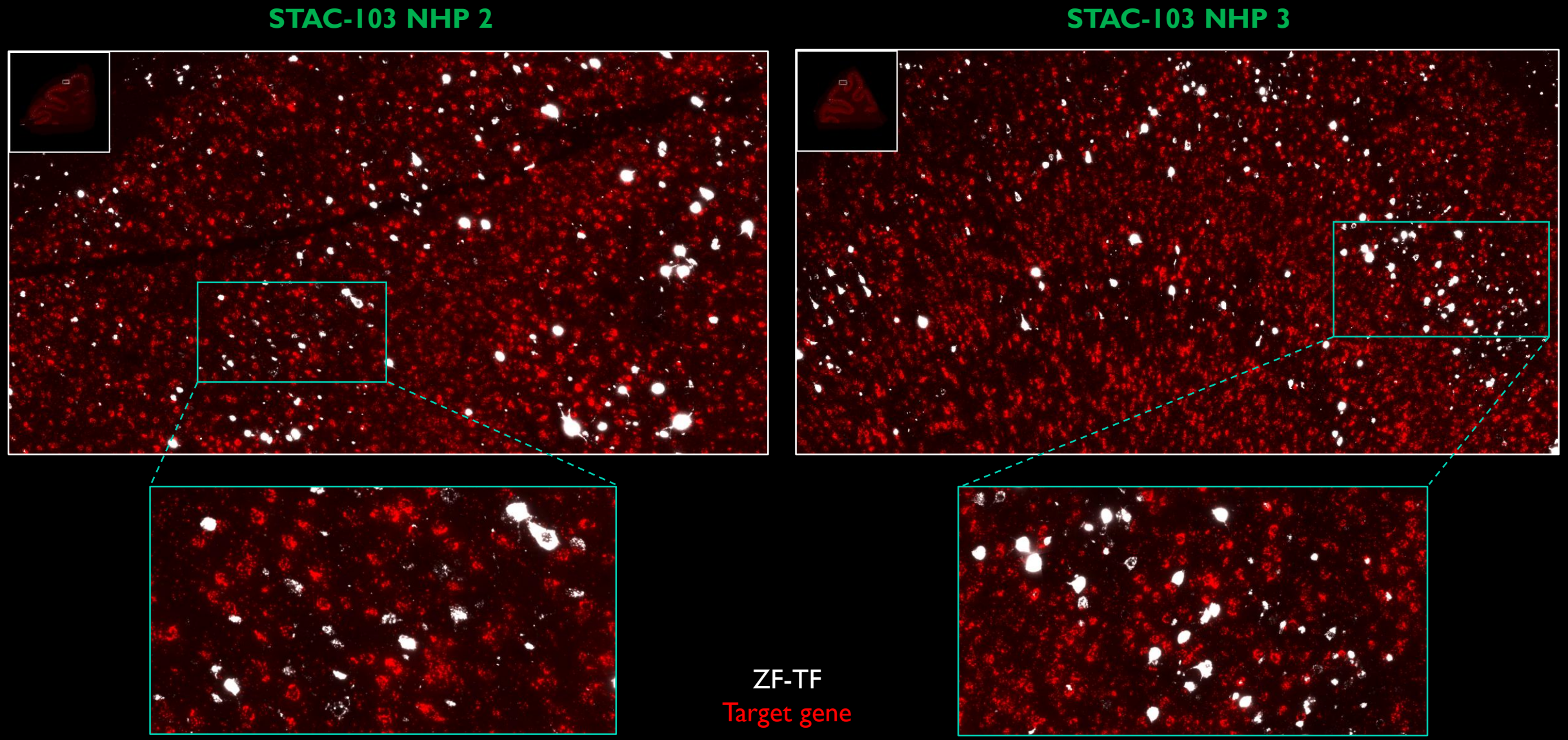
ZF-TF

Target gene

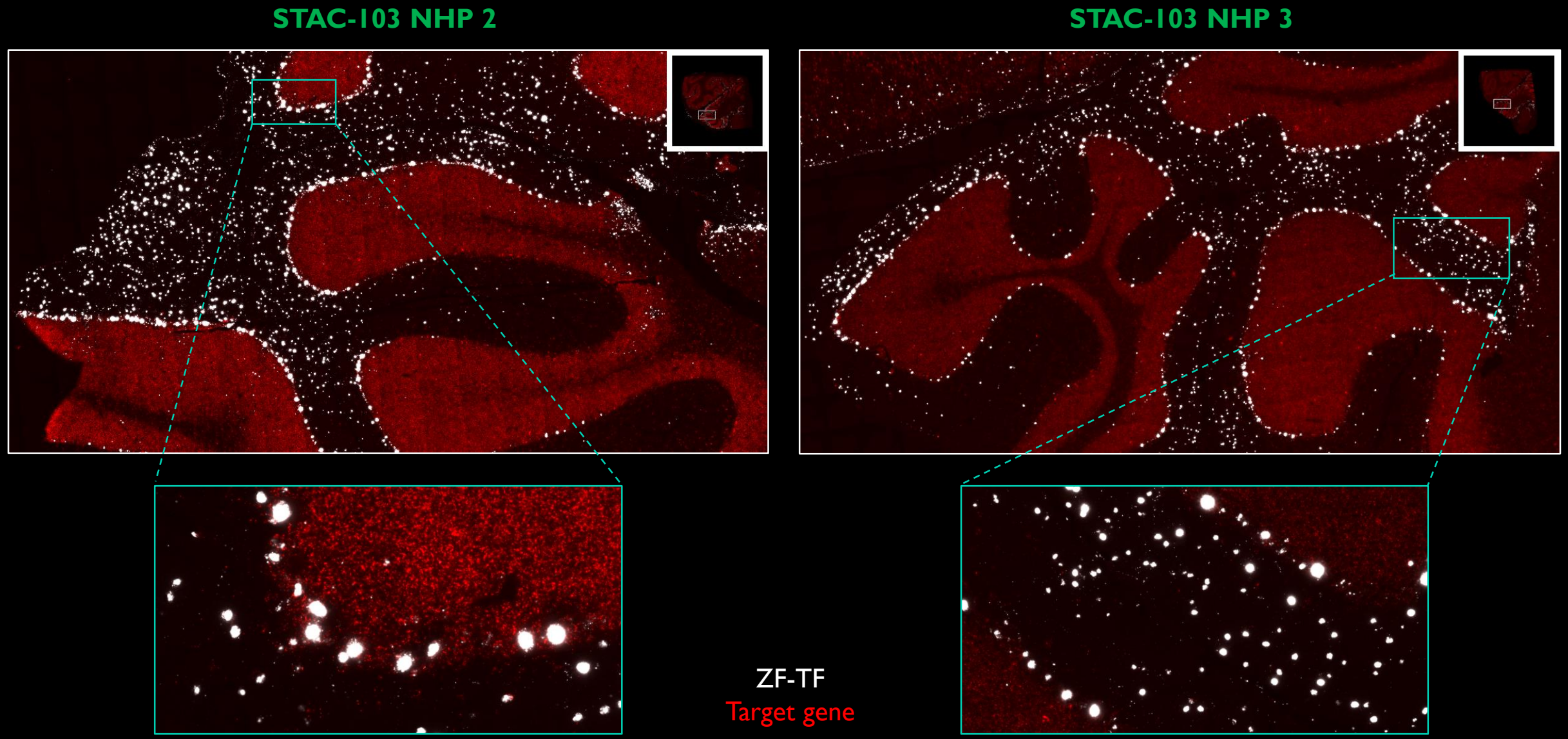
STAC-103 NHP 3



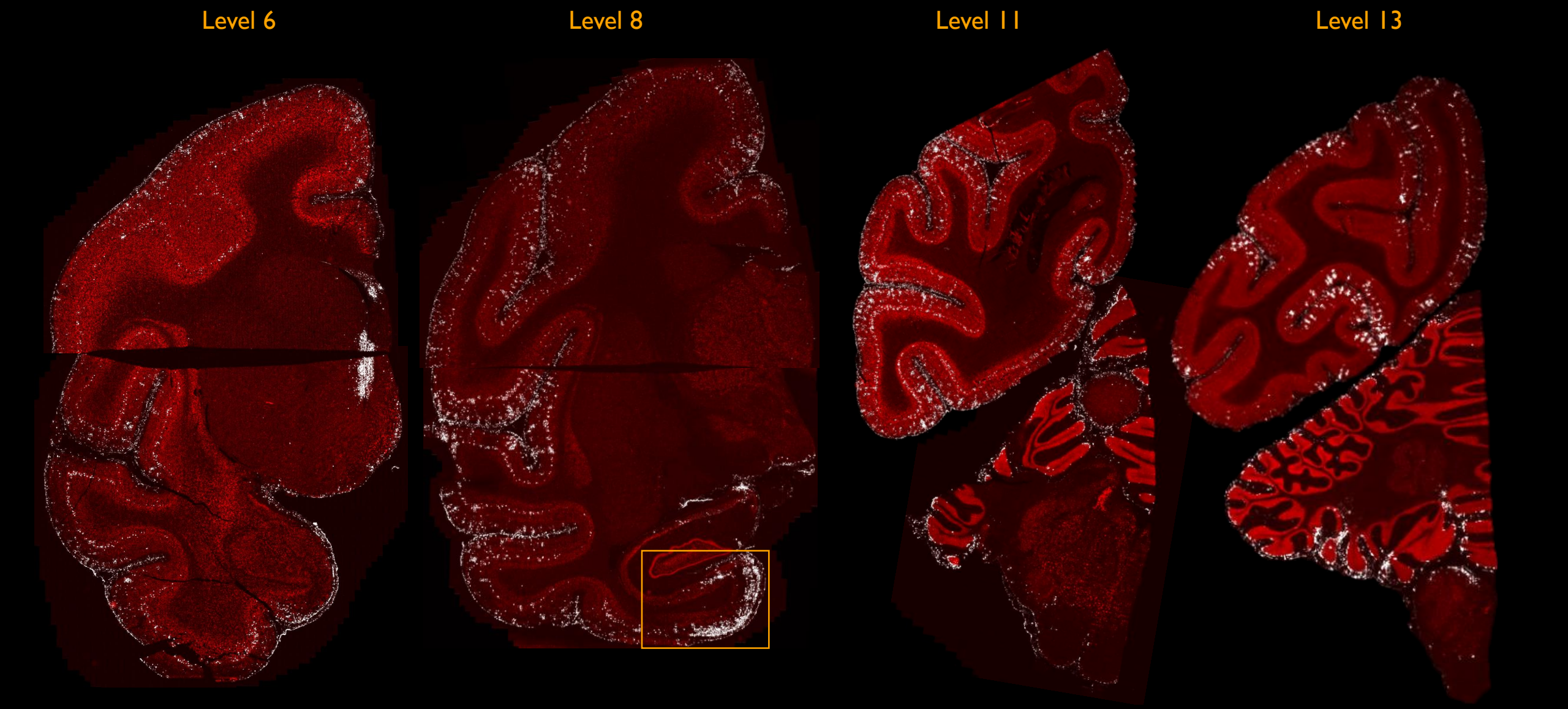
STAC-103 mediates neuronal transduction throughout the cortex



STAC-103 mediates neuronal transduction in the molecular and Purkinje layers of the cerebellum



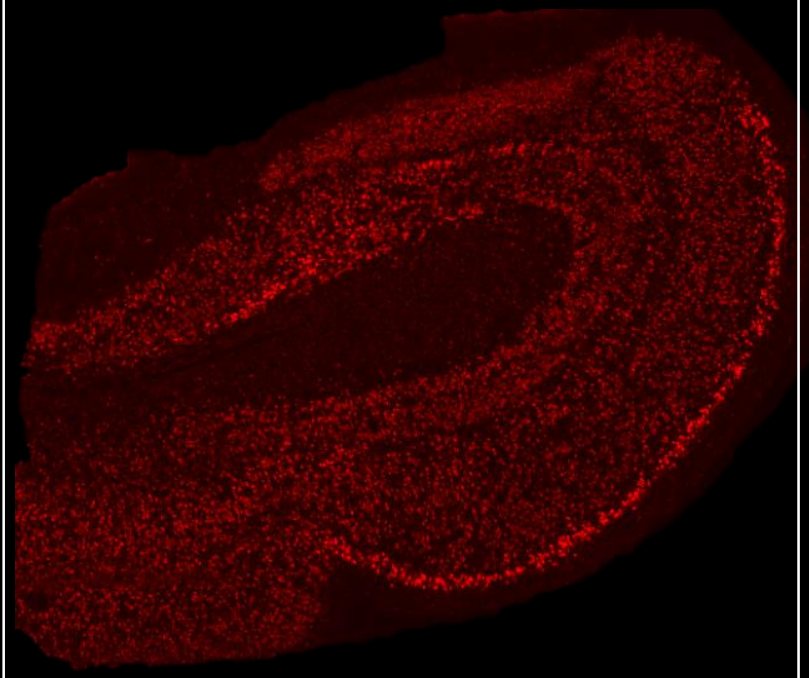
STAC-102 NHP 3



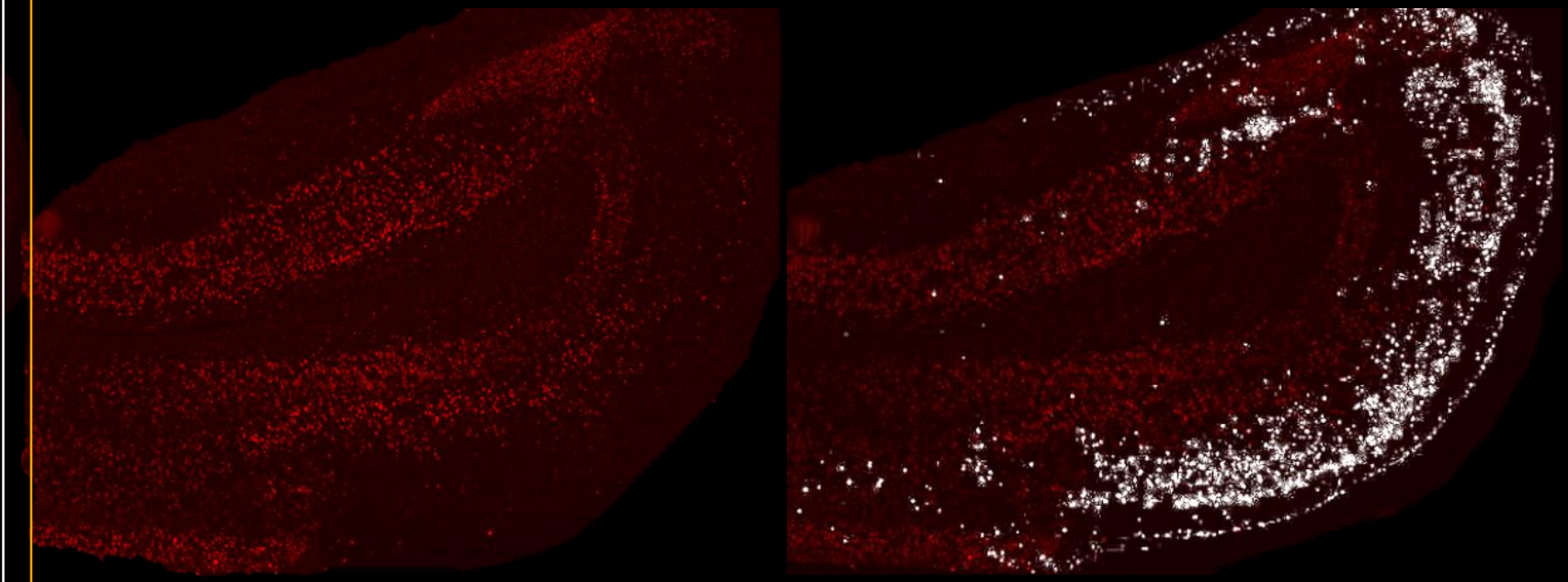
ZF-TF
Target gene

STAC-102 delivery and ZF-TF expression mediates repression of targeted gene in the entorhinal cortex

Control tissue – Entorhinal cortex



STAC-102 NHP 3 – Entorhinal cortex

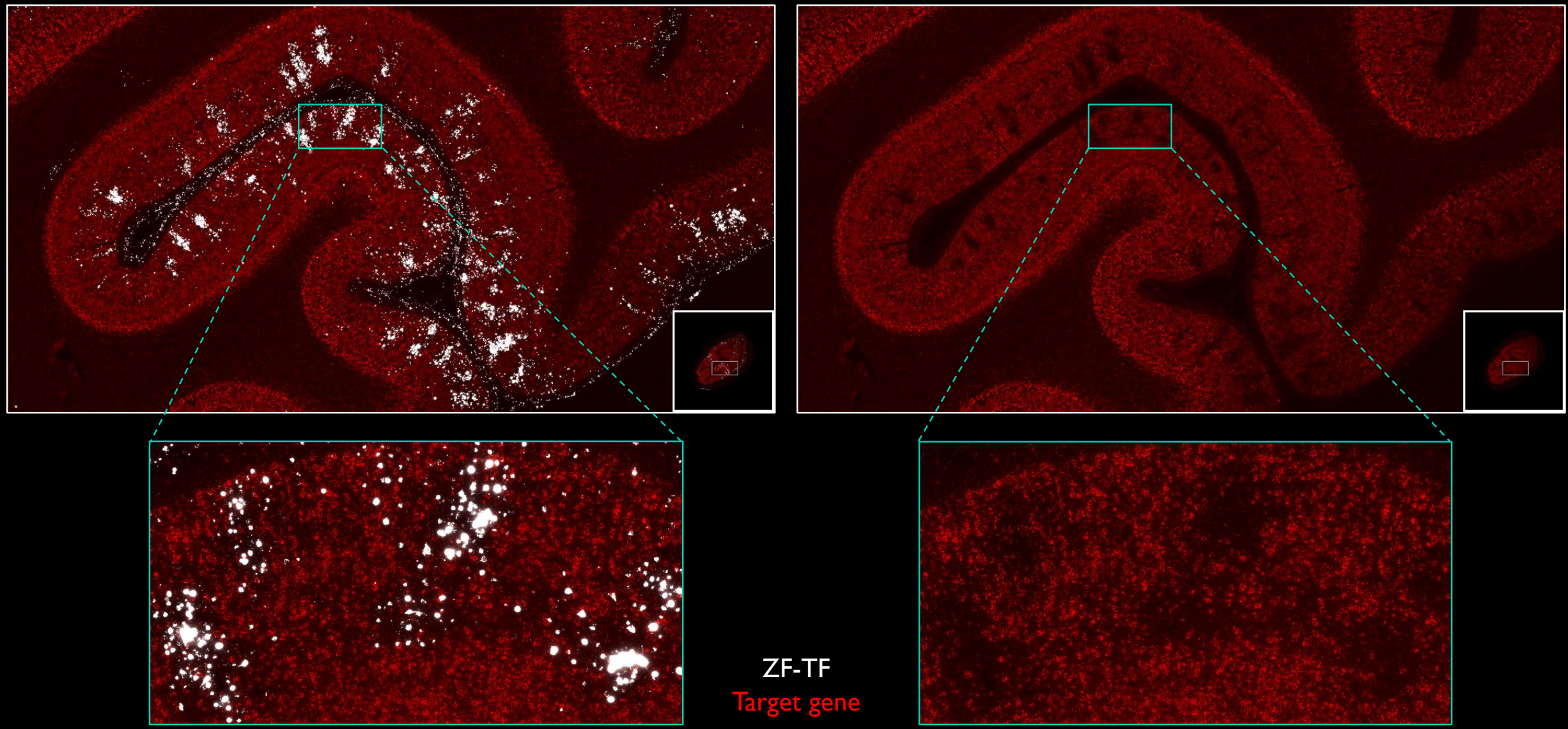


ZF-TF

Target gene

STAC-102 delivery and ZF-TF expression mediates repression of targeted gene in the visual cortex

STAC-102 NHP 3



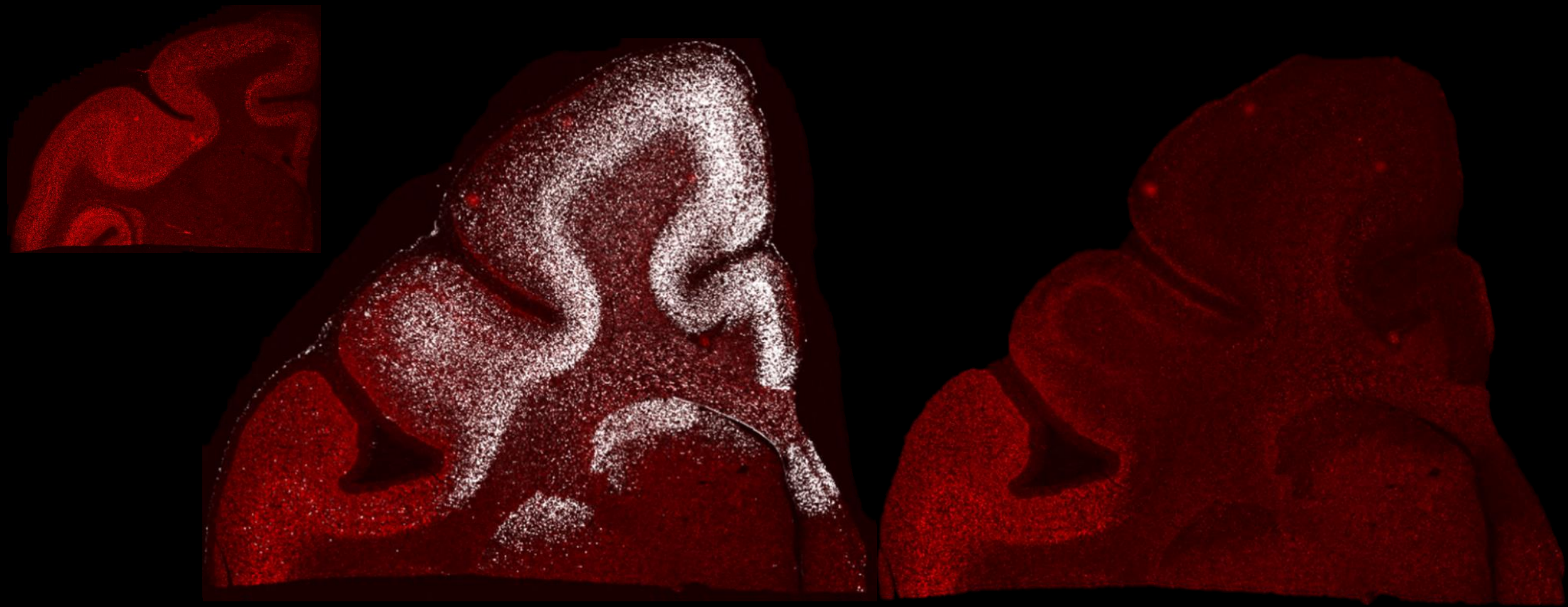
ZF-TF

Target gene

ZF-TF expression and target repression observed in the area of ICV injection backflow

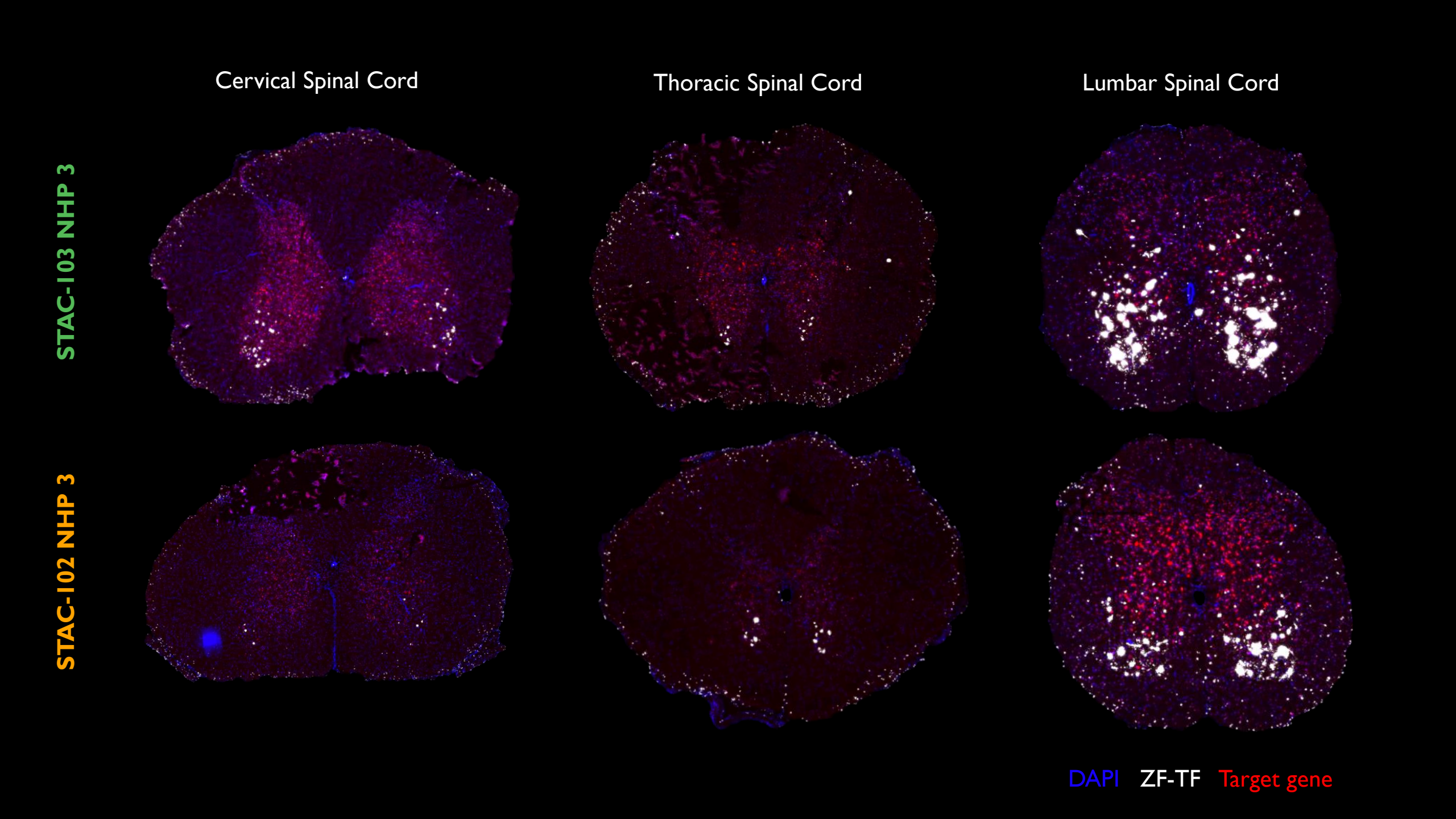
Control tissue – Level 6 Dorsal Section

STAC-102 NHP 2 - Level 6 Dorsal Section



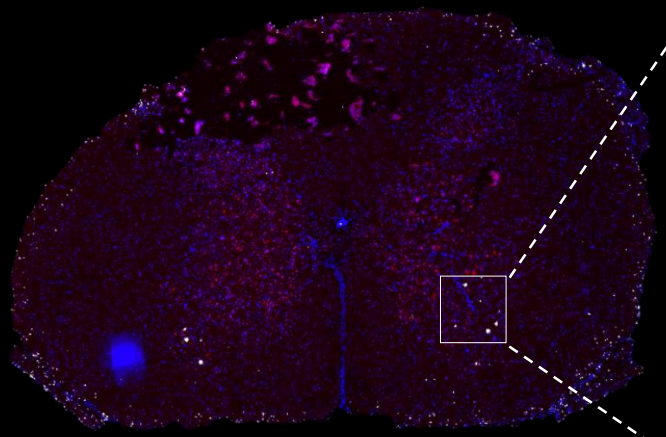
ZF-TF

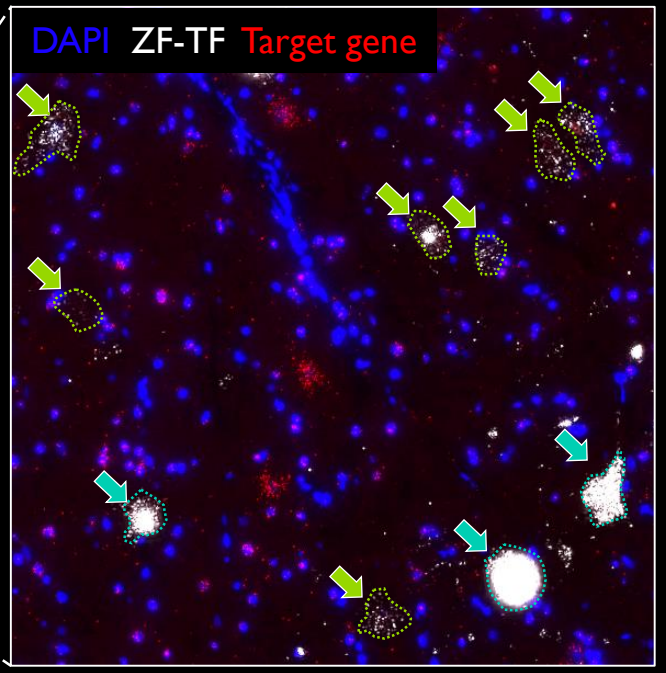
Target gene


Cervical Spinal Cord
Thoracic Spinal Cord
Lumbar Spinal Cord
STAC-103 NHP 3
STAC-102 NHP 3
DAPI
ZF-TF
Target gene

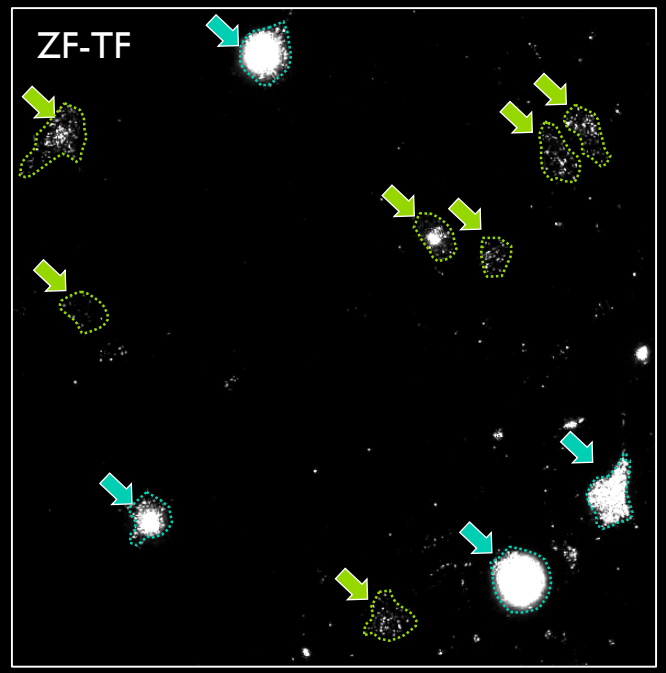
STAC-102 NHP 3

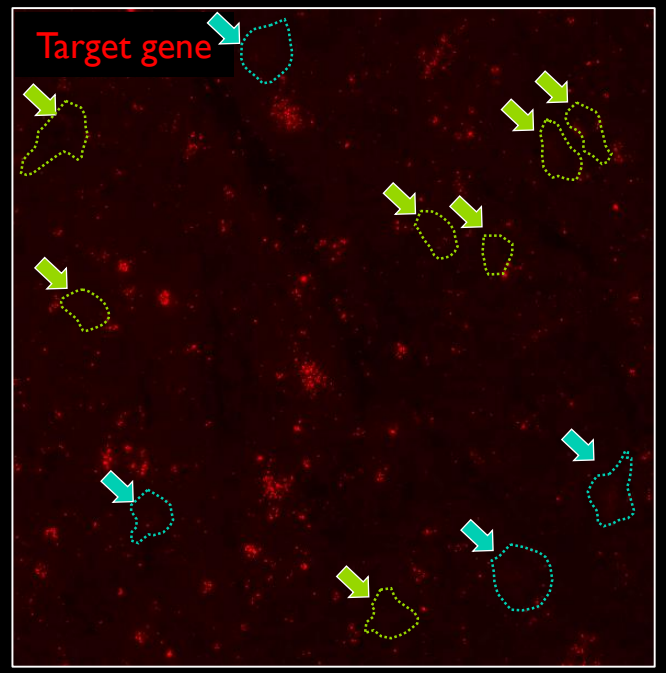
Cervical Spinal Cord

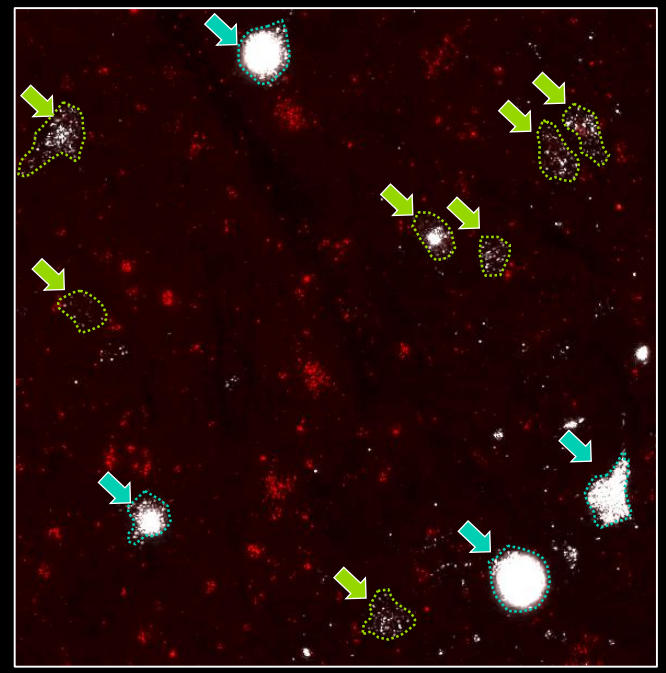




- Cells with high ZF-TF expression are visible in low magnification view
- Many more cells with lower ZF-TF expression are present and these low levels of expression are sufficient to drive potent target repression







Summary

SIFTER platform

Proprietary method for multiplexed transcription-dependent screen

Custom bioinformatic pipeline for analysis of screening rounds

Parallel screen ongoing for BBB-penetrant capsids

NHP evaluation results

Novel capsids STAC-102 and STAC-103 exhibit improved CNS delivery relative to AAV9

AAV delivery and ZF-TF expression result in efficient repression of the targeted gene

Capsids and ZF-TF payload were well tolerated for duration of 8-week study

Next steps

Additional cell-type marker staining and quantification

Fitness maturation of STAC-102 and STAC-103 capsids to further improve performance

Larger scale manufacturing and application in CNS pipeline

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Thank you