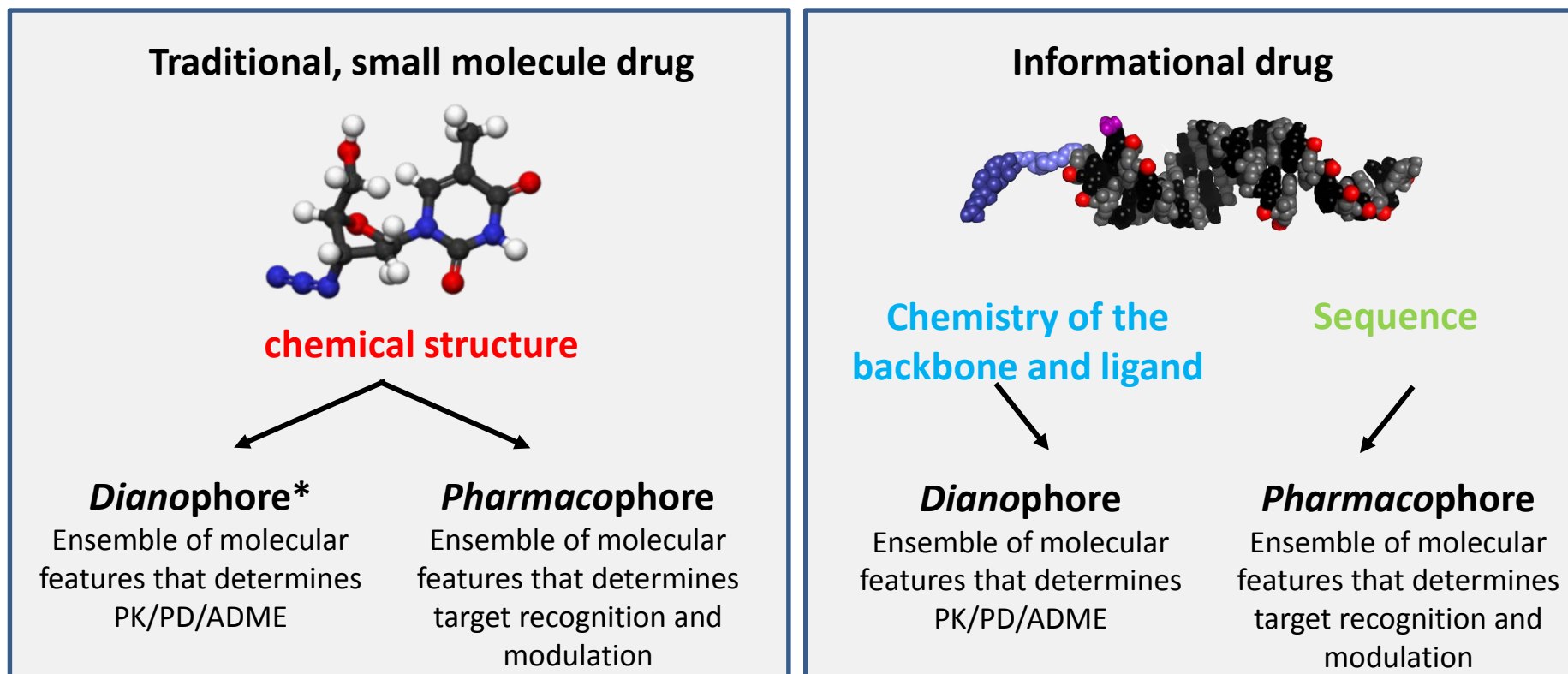


Modulation of Gene Expression In CNS by Chemically Modified Oligonucleotides

Anastasia Khvorova,
RNA Therapeutic Institute, UMASS Medical School

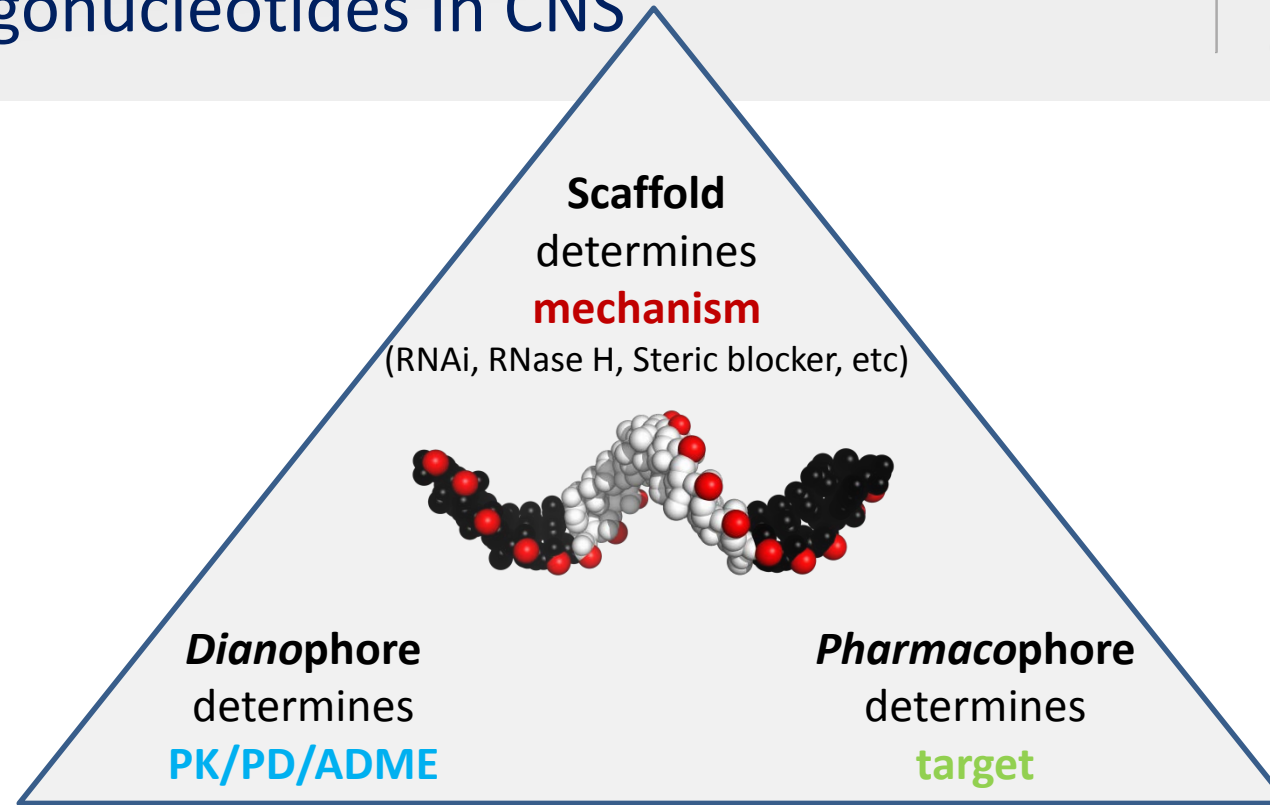


Informational Drugs: Optimizing Chemical Architecture for Delivery is a Key



***Dianophore**—from the Greek “διανομή-dianomi” for distribution or delivery

Three Classes of Oligonucleotides In CNS



Increasingly complex requirements for oligonucleotide chemistry

SPINRAZA®
(Nusinersen)

ASO for single
patient with
Batten (Dr Yu)

Steric blocker ASO
Nuclease resistance
Binding affinity
Bioavailability

Wave-HTT
IONIS-HTT, TAU, C9orf72

RNase H ASO
Nuclease resistance
Binding affinity
Bioavailability
RNase H compatibility
Specificity of cleavage

RNAi
Nuclease resistance
Bioavailability
AGO2 compatibility
Specificity

CNS-RNAi-HTT

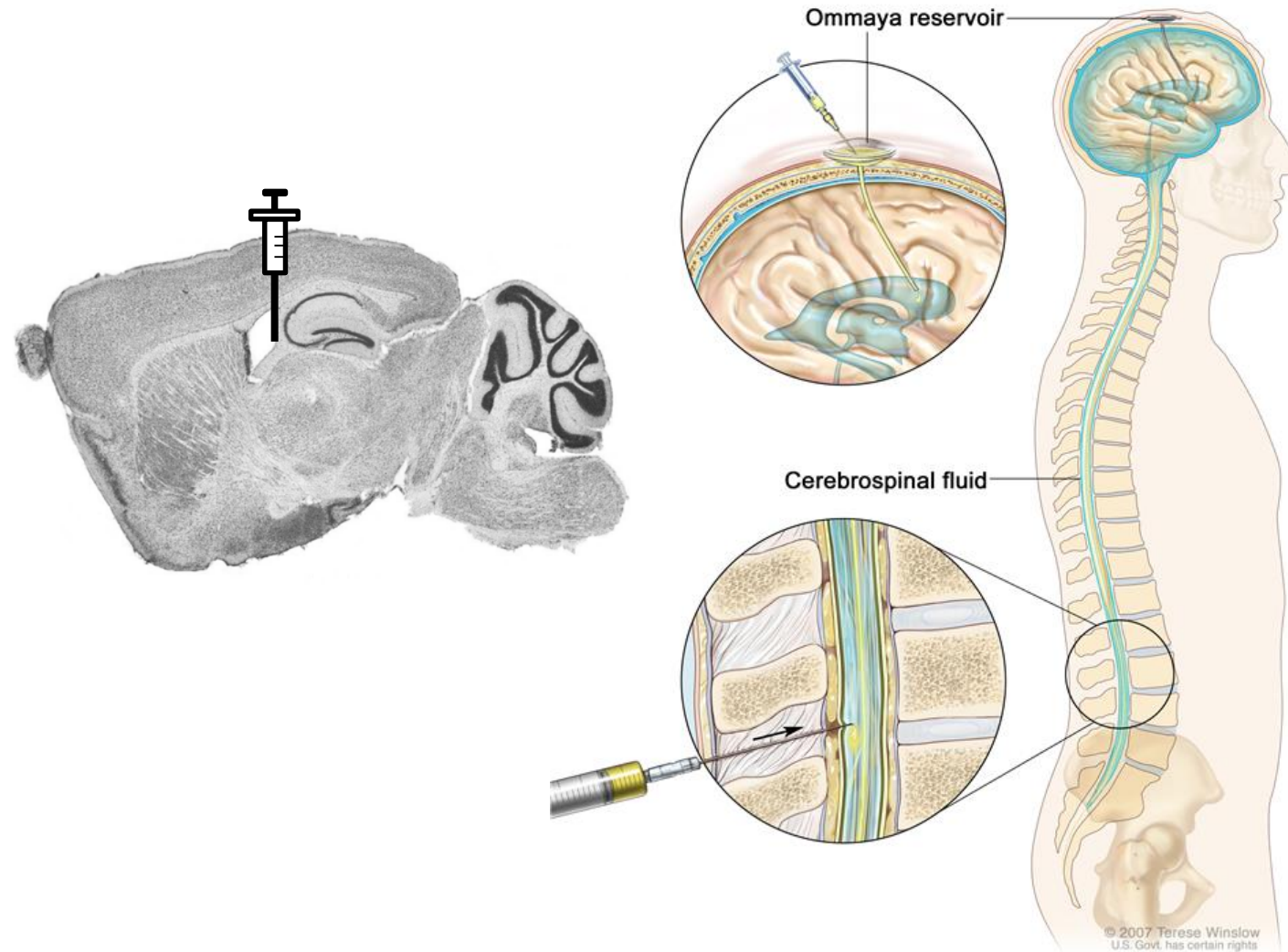
Oligonucleotides Do Not Cross BBB, Need to Be Delivered to CSF

Ommaya Reservoir

- Requires one surgery (<1 hour with local anesthesia and sedation) to implant a catheter
- Routinely use for pain and cancer management
- Enable safe, non-invasive, painless, robust, repetitive administration
- Preferred method for repetitive CSF delivery
- Long-term use, no reported clogging issues with ICV placement
- Clinical recruitment might be limited to medically advanced countries

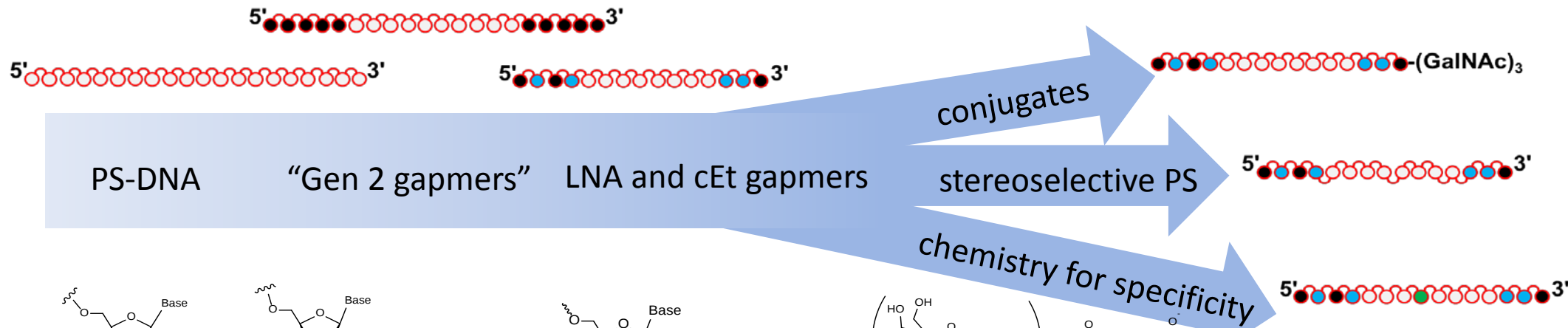
IT Injection

- No surgery required
- Routinely used procedure (< 1 hour with local anesthesia and optional sedation)
- 80% of patients report Post-lumbar puncture syndrome, back pain and headaches
- Repetitive administrations might be problematic
- Clinical recruitment is easier with more sites available



Chemistry Defines Distribution, Duration of Effect and Efficacy

ASO technology maturation



siRNA technology maturation



CNS-RNAi Shows Widespread Distribution with CSF Delivery

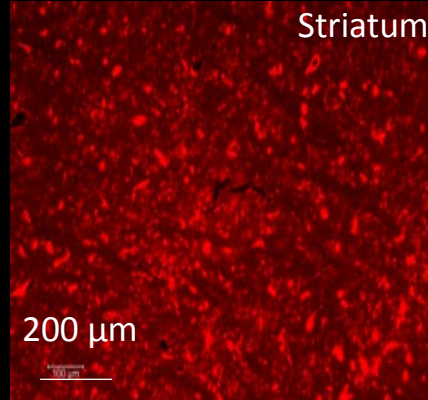
CSF

CNS-RNAi-siRNA



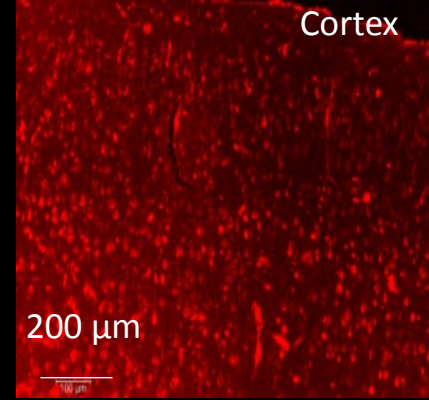
Striatum

200 μ m



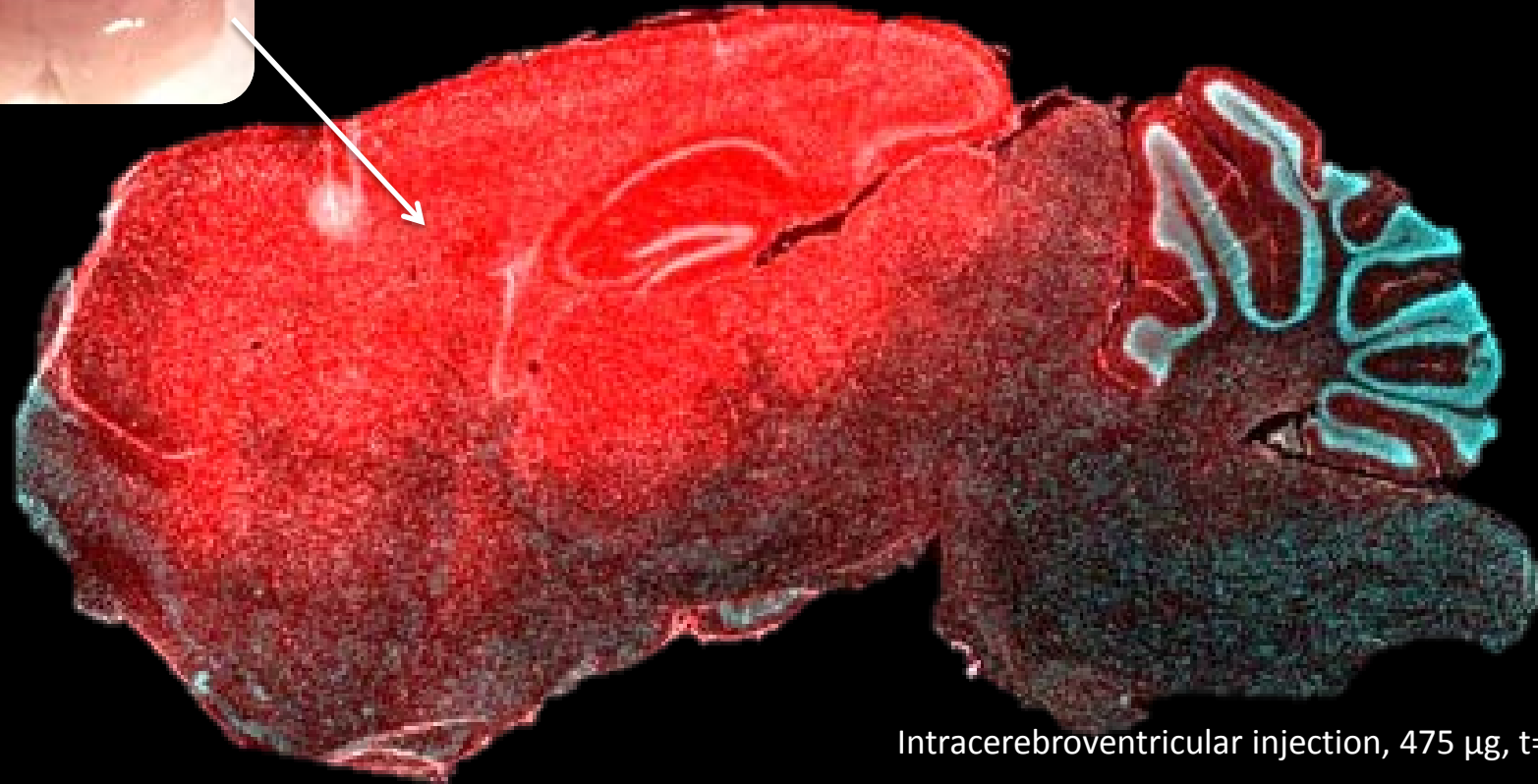
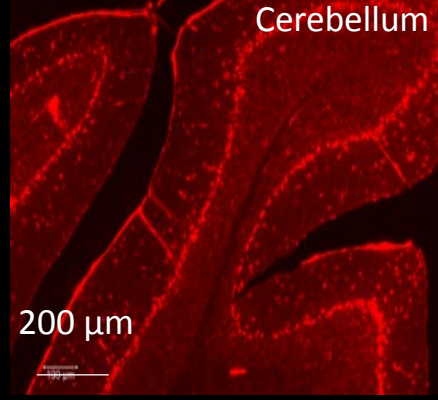
Cortex

200 μ m



Cerebellum

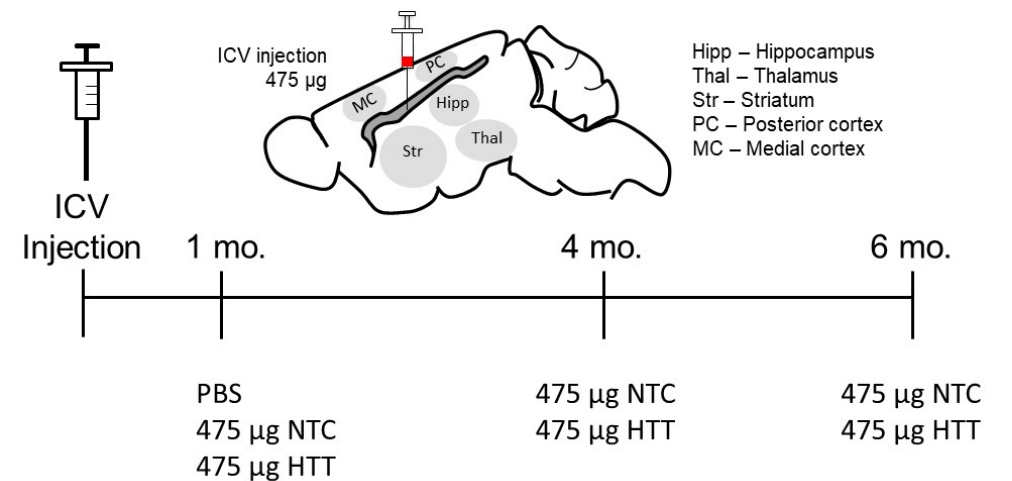
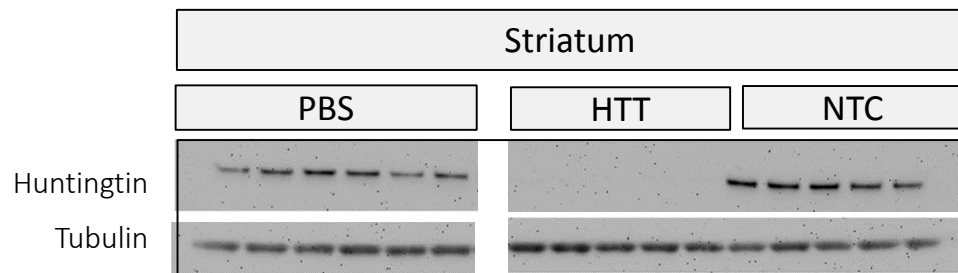
200 μ m



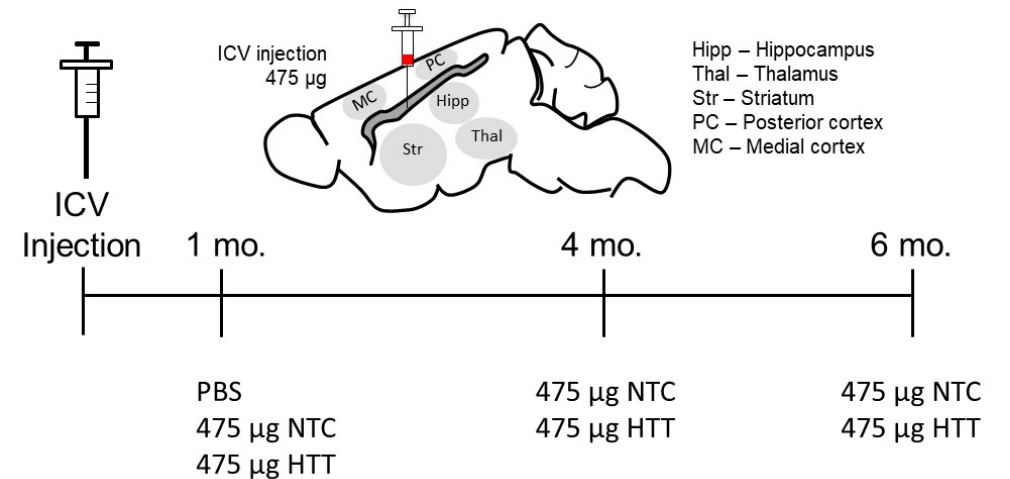
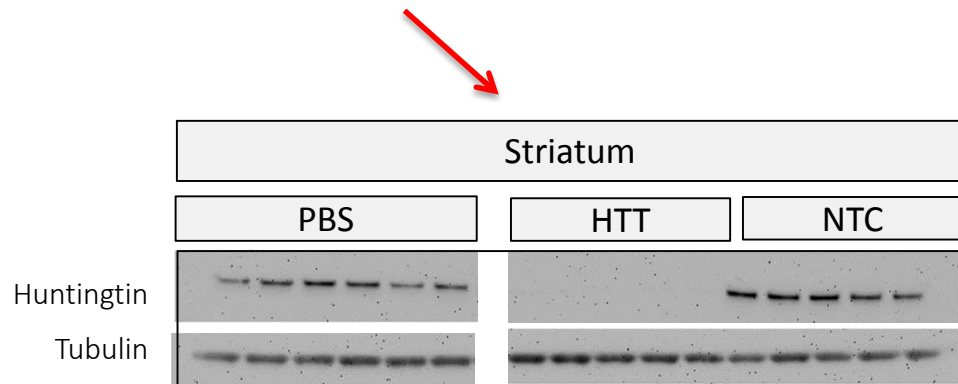
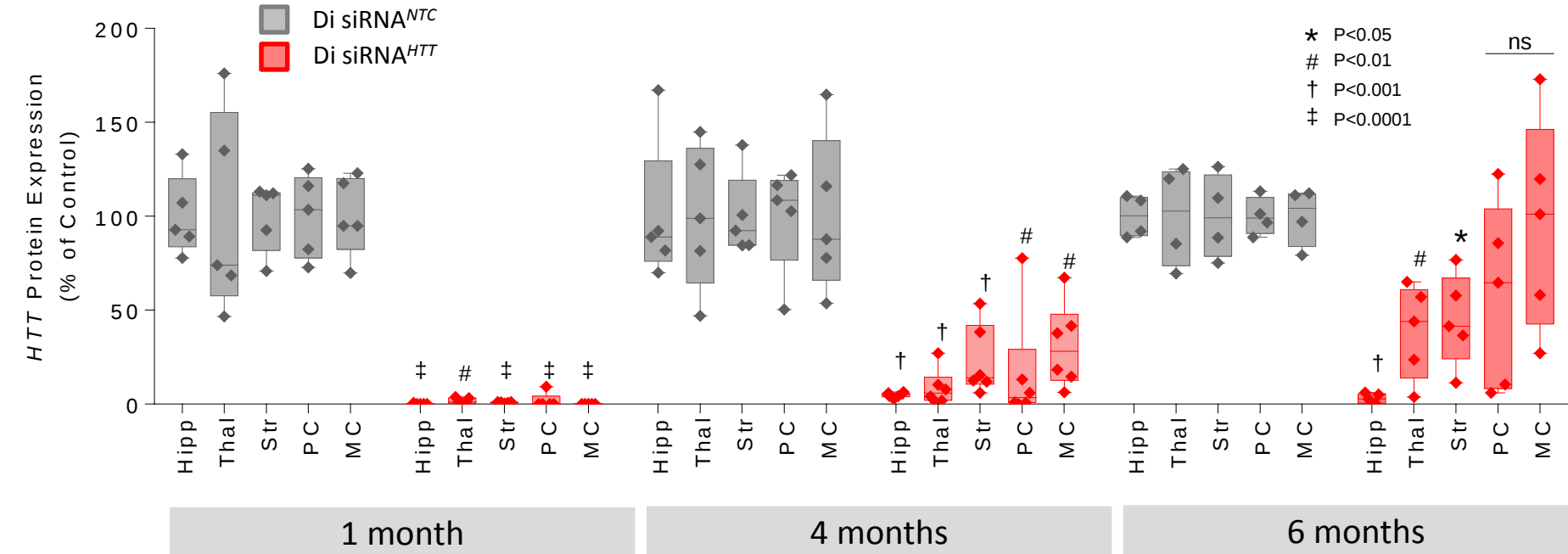
CNS-RNAi
DAPI

Intracerebroventricular injection, 475 μ g, t=48 h, FVB/NJ mice (n=3)

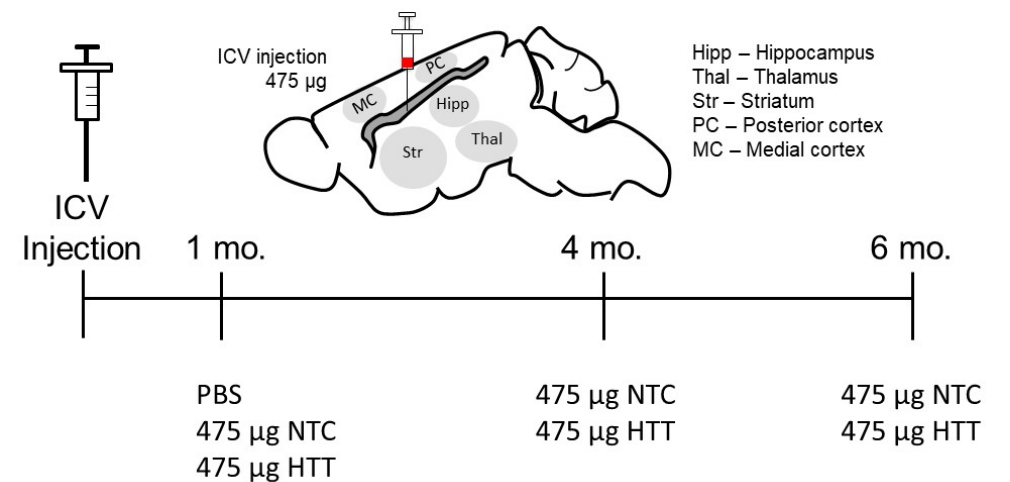
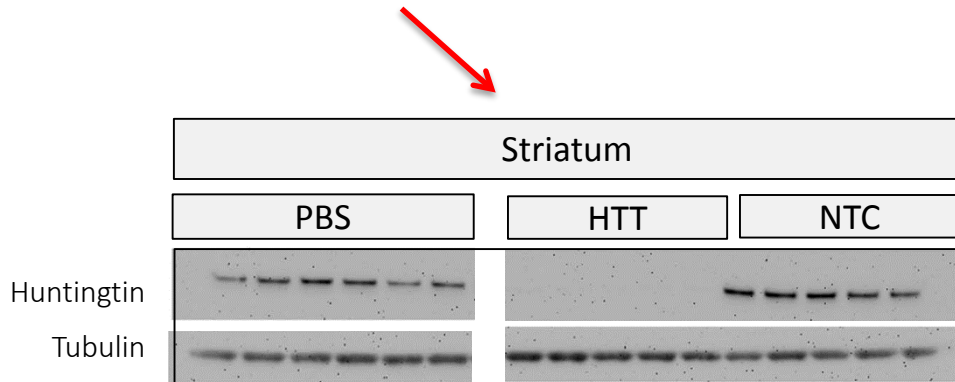
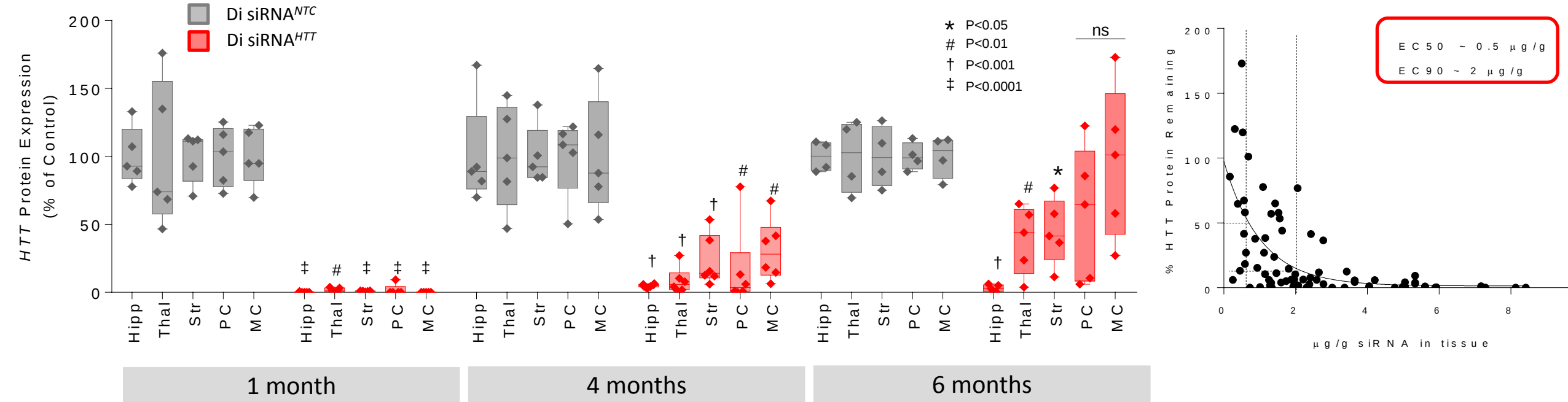
CNS-RNAi Shows Significant **Protein** Silencing for up to 6 Months after a Single Injection



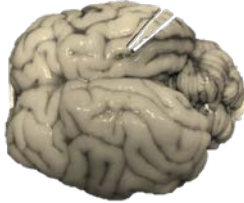
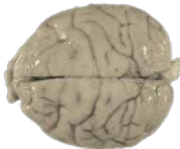
CNS-RNAi Shows Significant **Protein** Silencing for up to 6 Months after a Single Injection



CNS-RNAi Shows Significant **Protein** Silencing for up to 6 Months after a Single Injection

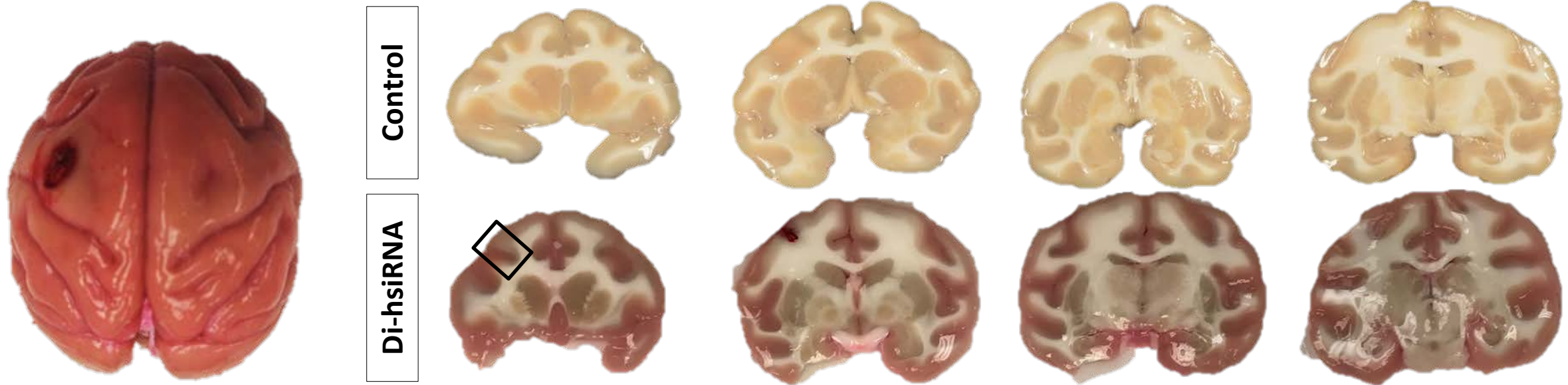


Relative Mammal Brain Sizes

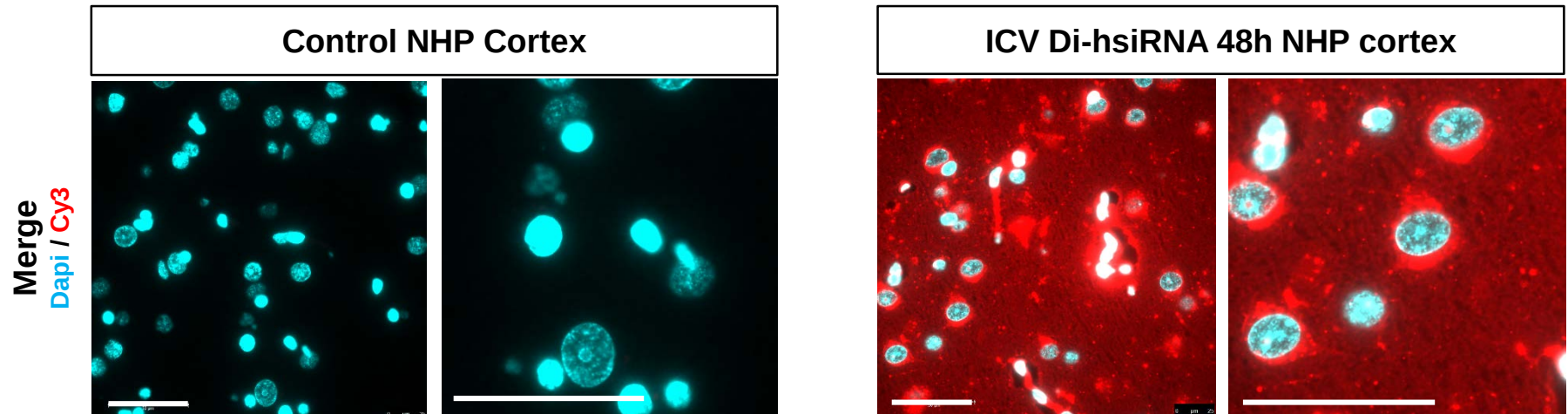
Human	Sheep	Monkey	Mouse
			
1300-1400g	140g	70-100g	0.4g

CNS-RNAi Shows Broad Distribution in NHP Brain

Cynomolgus Macaque Brain

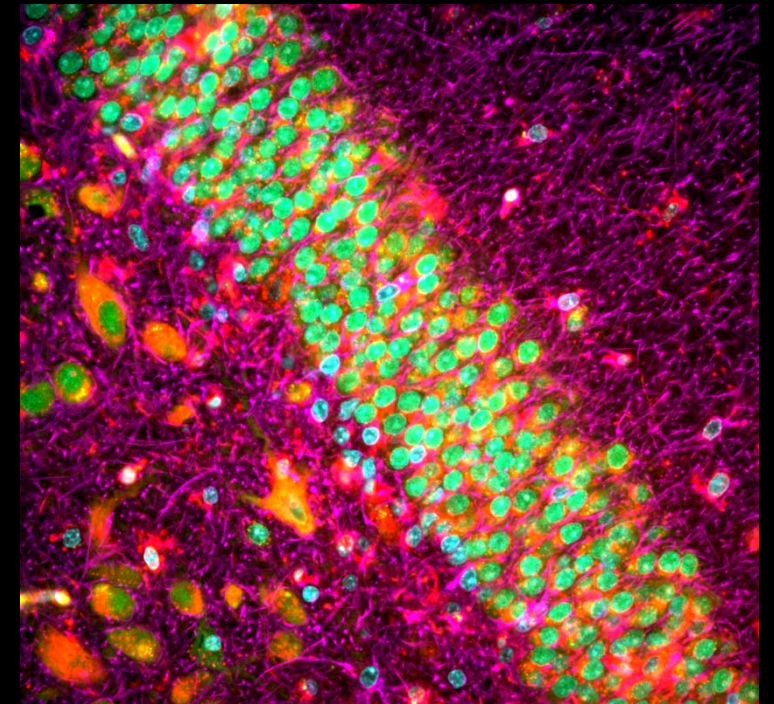
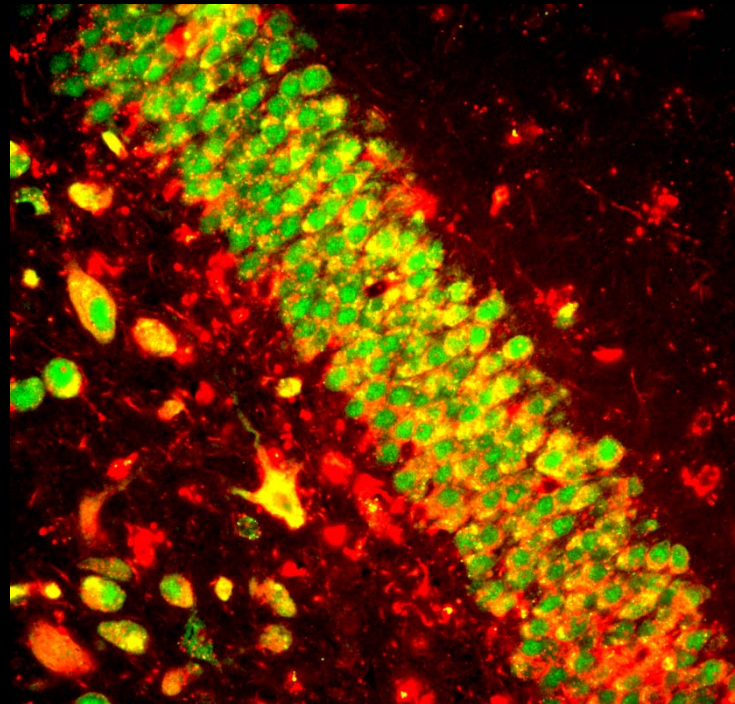
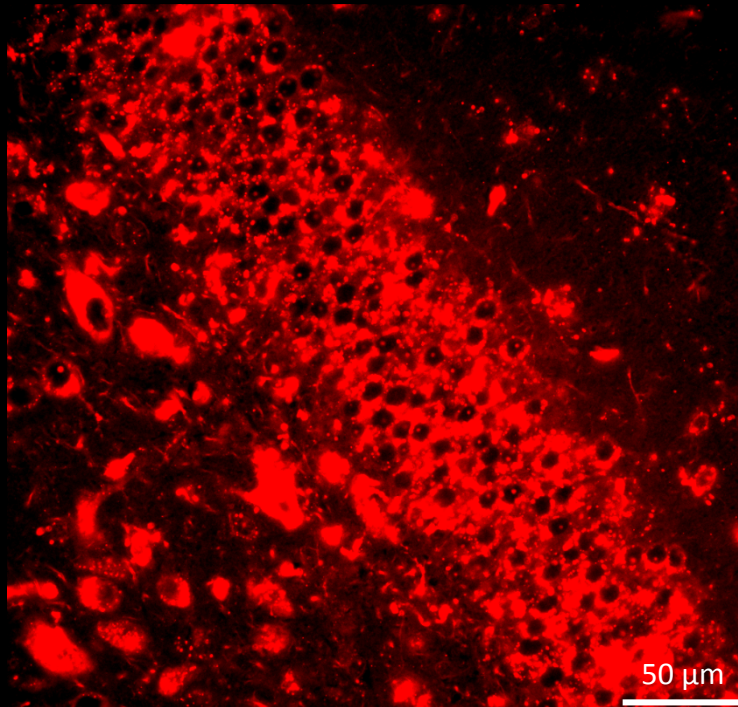


ICV injection (25 mg) in Cynomolgus Macaque (Male). t = 48 hours. n = 1.
DAPI (**Blue**), siRNA (**Red**).
Scale bar = 50 μ m.

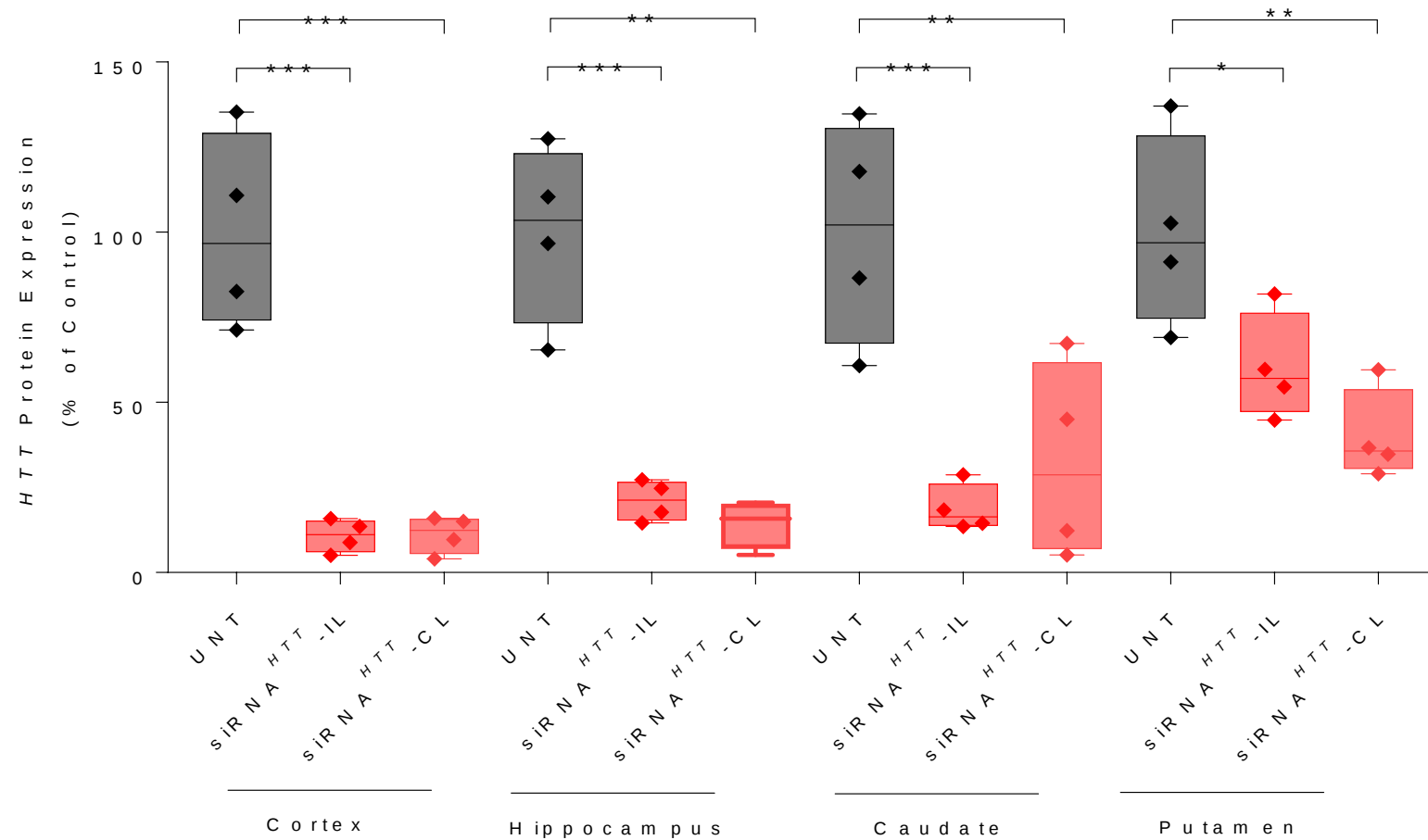


Distribution to Hippocampus (Dentate Gyrus)

CNS-RNAi NeuN- Neurons GFAP- Glia Dapi- Nucleus

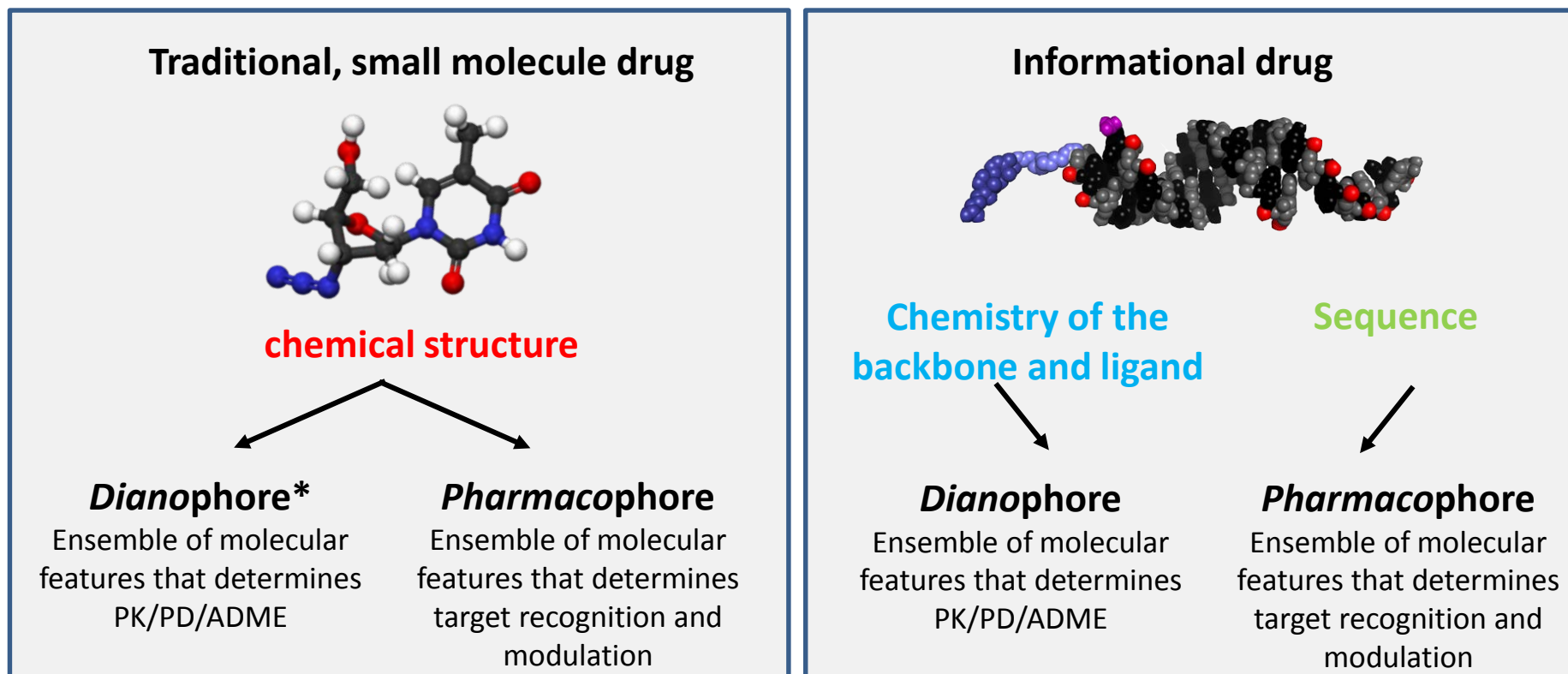


Single CSF Injection of CNS-RNAi Efficiently Silence **HTT** Protein Expression (n=4)



NHP, 25 mg, t=1 m, (n=4), Western, Normalized to Tubulin

One-way ANOVA with Bonferonni correction, *** p<0.001, ** p<0.01, * p<0.05



****Dianophore***—from the Greek “διανομή-dianomi” for distribution or delivery

Nusinersen (~ 20 years)

ASO for single patient with Batten (Dr Yu)
(~ 9 month)

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Julia Alterman

Matthew Hassler

Bruno Godinho

Chantal Ferguson

Dimas Echeverria

Socheata Ly

James Gilbert

Sarah Davis

Ken Yamada

Marie Didiot

Emily Knox

Jacquelyn Sousa

Sam Hildebrand

Lorence Vangiely

Annabelle Biscans

Yann Thillier

Former Lab Members

Maire Osborn (Dicerna)

Loic Roux (Evovx)

Havisha Karnam (Medical School)

Mehran Nikan (Ionis)

Anton Turanov (Dicerna)

Andrew Coles (Alexicon)

Reka Haraszti (Germanv/Residency)

Pai LAB

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Aronin LAB

Faith Conroy

Rachael Miller

Lauren Hall

Kathryn Chase

Marian DiFiglia

Ellen Sapp

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Suzanne Wheeler

Samer Jaber

George DeMarco

Miguel Estevez

Robert King

