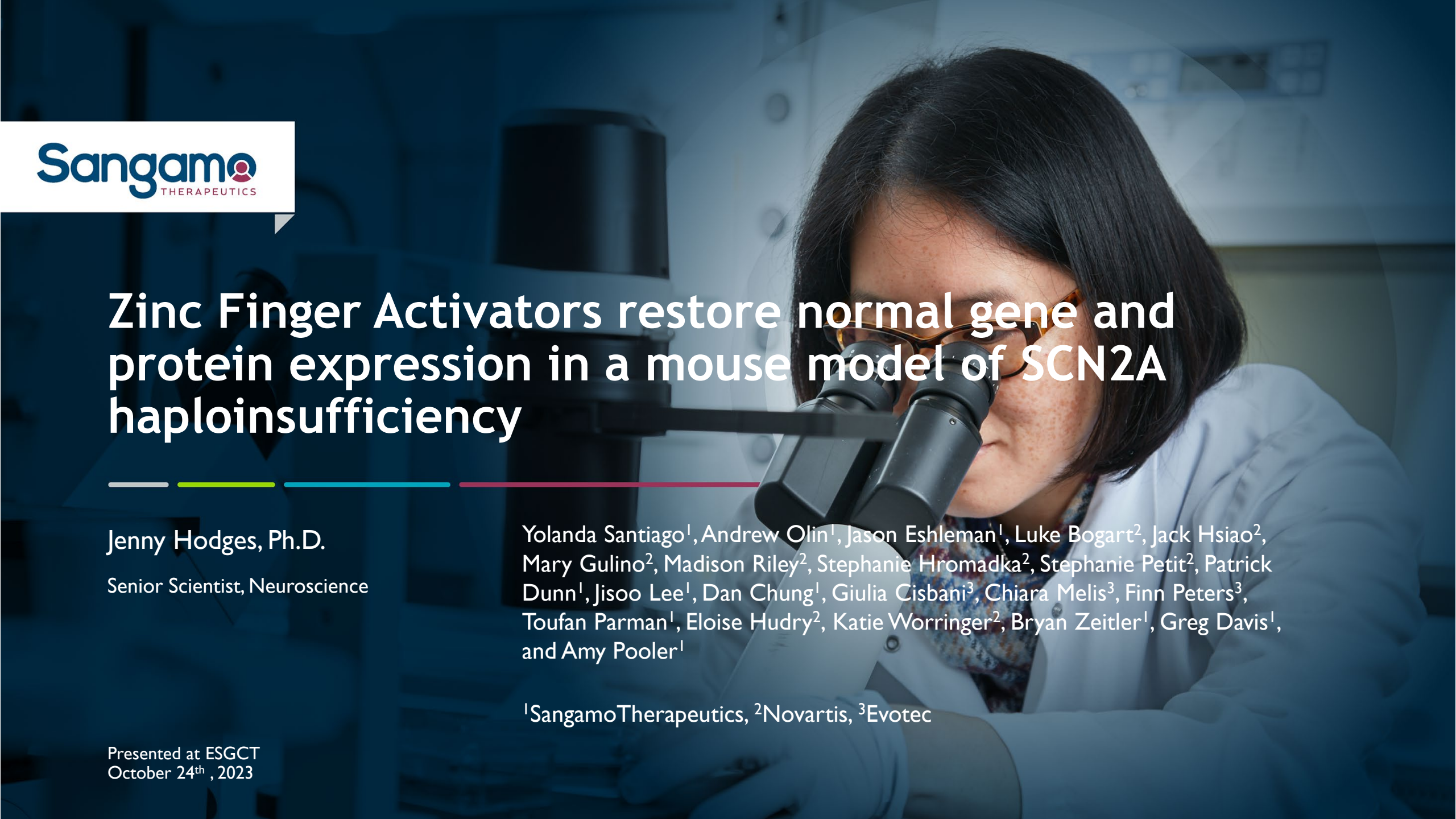


Zinc Finger Activators restore normal gene and protein expression in a mouse model of SCN2A haploinsufficiency

Jenny Hodges, Ph.D.

Senior Scientist, Neuroscience

Yolanda Santiago[1], Andrew Olin[1], Jason Eshleman[1], Luke Bogart[2], Jack Hsiao[2], Mary Gulino[2], Madison Riley[2], Stephanie Hromadka[2], Stephanie Petit[2], Patrick Dunn[1], Jisoo Lee[1], Dan Chung[1], Giulia Cisbani[3], Chiara Melis[3], Finn Peters[3], Toufan Parman[1], Eloise Hudry[2], Katie Worringer[2], Bryan Zeitler[1], Greg Davis[1], and Amy Pooler[1]

[1]SangamoTherapeutics, [2]Novartis, [3]Evotec

Presented at ESGCT
October 24th , 2023

Disclosure

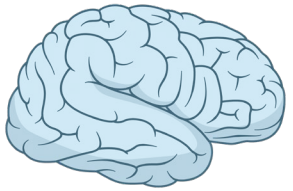
I am a full-time employee of Sangamo Therapeutics

SCN2A haploinsufficiency

de novo mutations in *SCN2A* causing loss of function in one allele

Incidence of **1:10,000-20,000** people

Comprises 1.1% of Autism Spectrum Disorder patients & 1% with intellectual disability

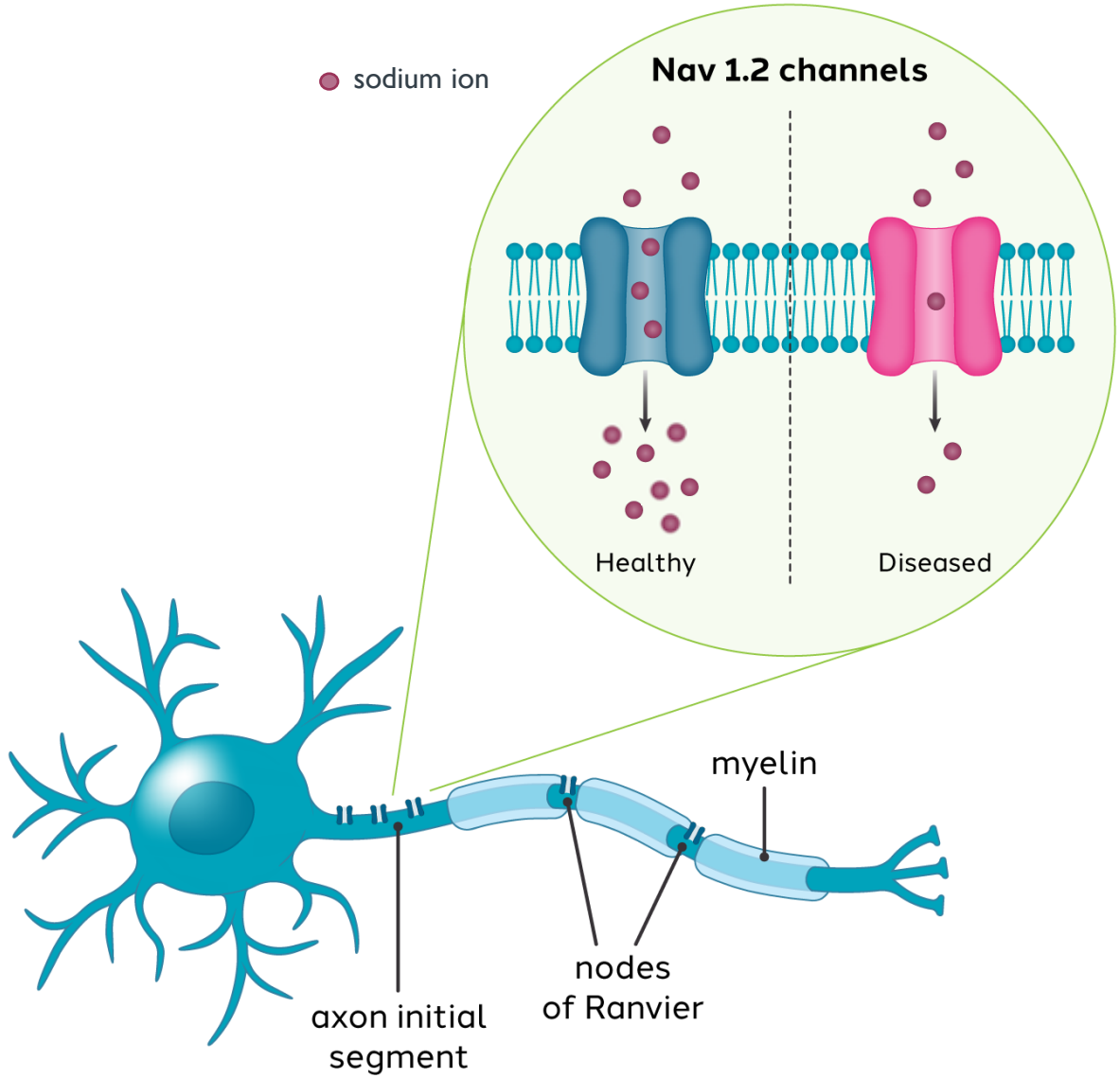


~1/3 of patients have early childhood seizures

No cure exists

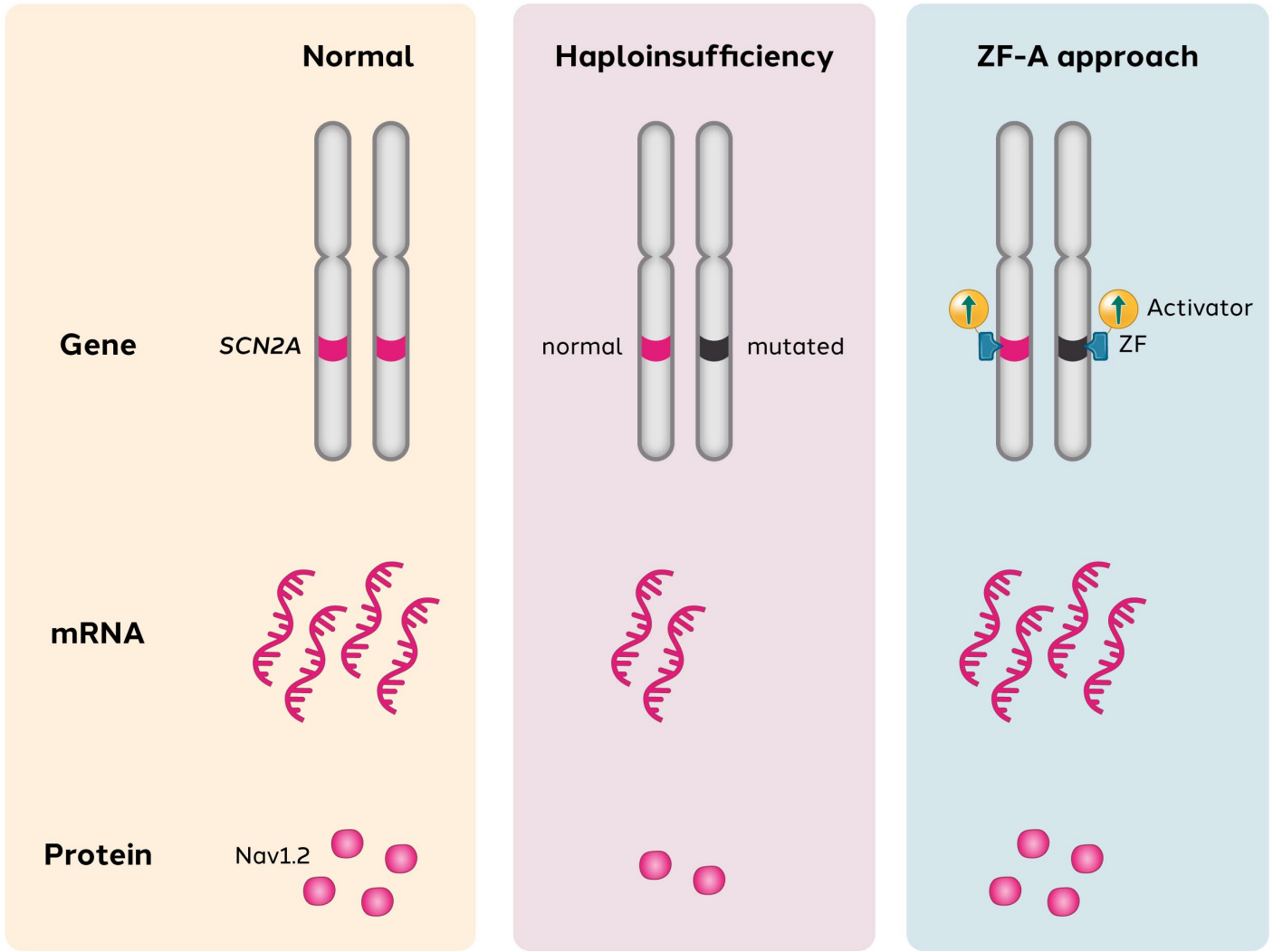
Loss of function mutations in the *SCN2A* reduce $Na_v1.2$ expression and alter neuronal excitation

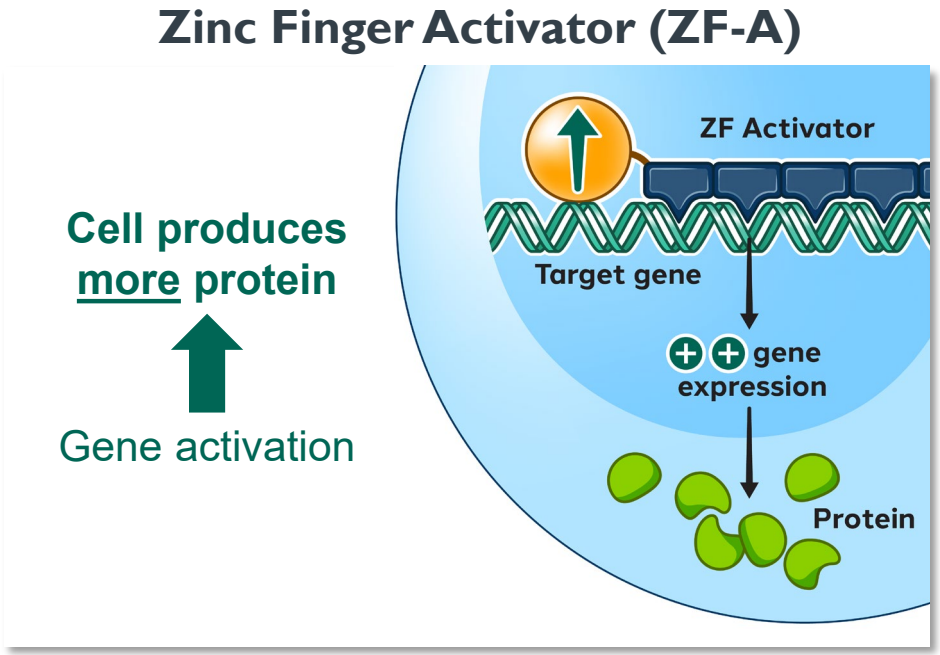
- $Na_v1.2$ is a voltage gated sodium channel expressed primarily in excitatory neurons in the brain
- Reduced $Na_v1.2$ expression impairs neuronal excitation and synaptic plasticity
- Restoring normal levels of Nav1.2 is expected to correct neuronal function



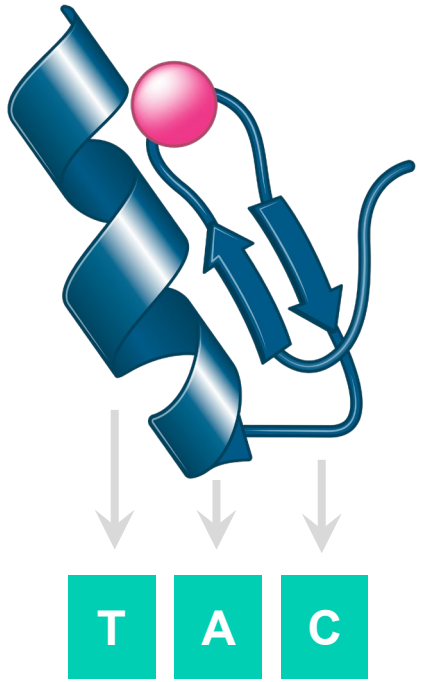
Images made with Biorender.com

Zinc Finger Activator approach to treating haploinsufficiency disorders

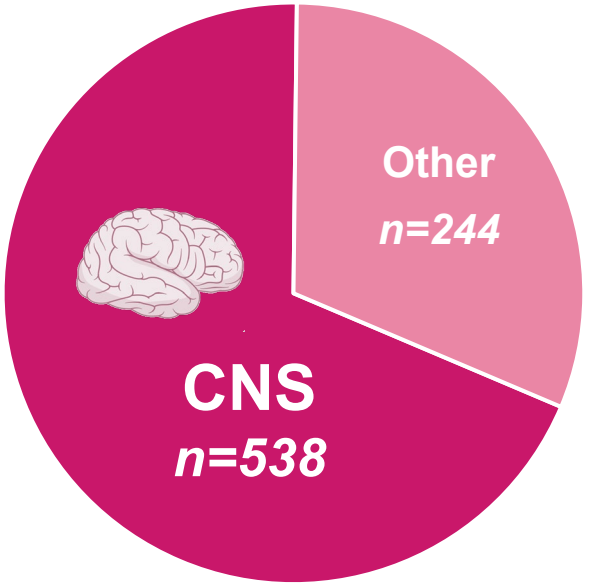




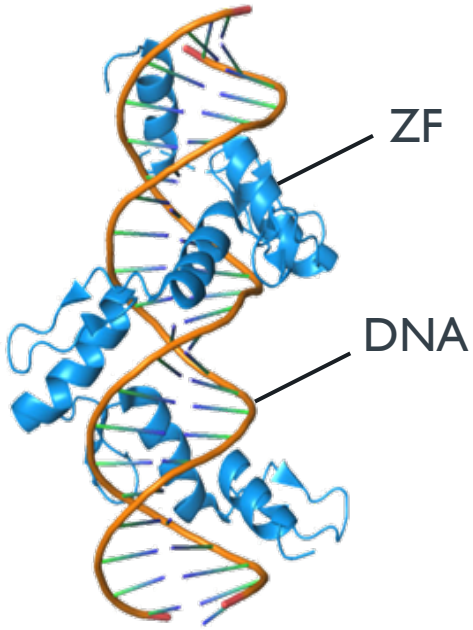
Zinc fingers are nature's solution for highly specific DNA binding



Zinc Fingers are
natural proteins
that bind DNA sequences
with high specificity

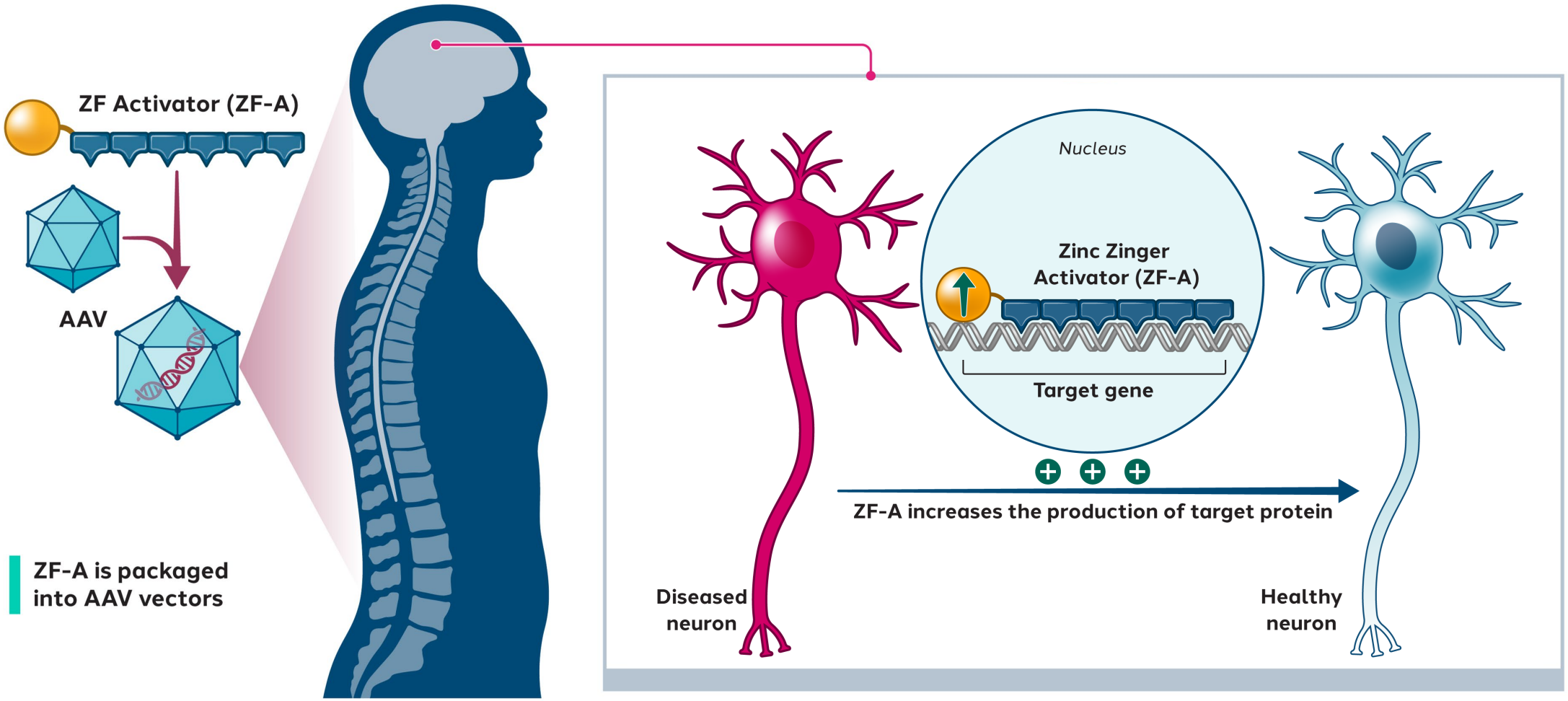


At least 782 human genes
encode for Zinc Finger
Proteins



Most natural Zinc Finger
Proteins function to
regulate the epigenetic state
of other genes

Zinc finger-mediated activation of Nav1.2 as a potent and specific therapeutic avenue for treating *SCN2A* haploinsufficiency

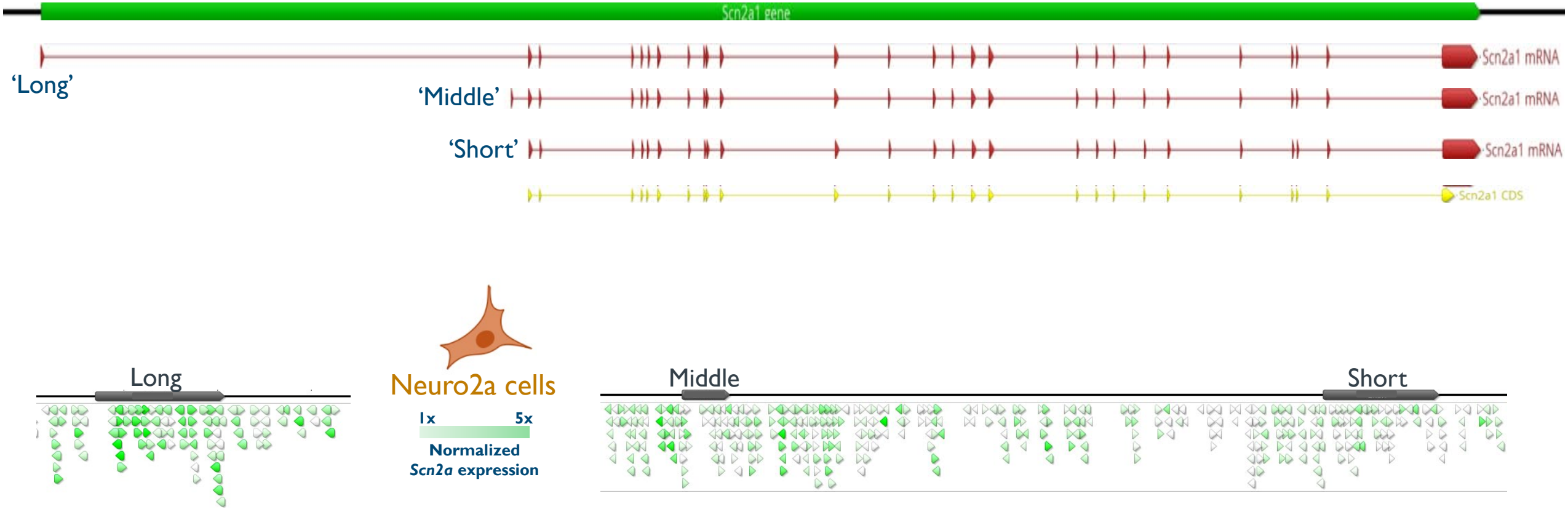


ZF-A is packaged into AAV vectors

Several potential routes of administration, including IV and CSF

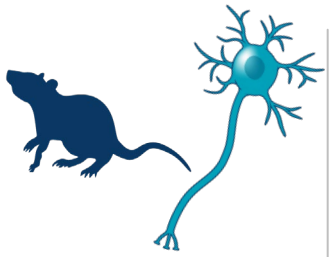
AAVs deliver ZF-As to neurons. ZF-As activate the targeted gene and restore protein levels.

Active ZF-As were found at all three *Scn2a* transcription start sites



- **123/550** ZF-As >1.5-fold *Scn2a* activation in Neuro2a cells

ZF-As upregulate *Scn2a* long transcript in a dose-dependent manner in WT mouse cortical neurons



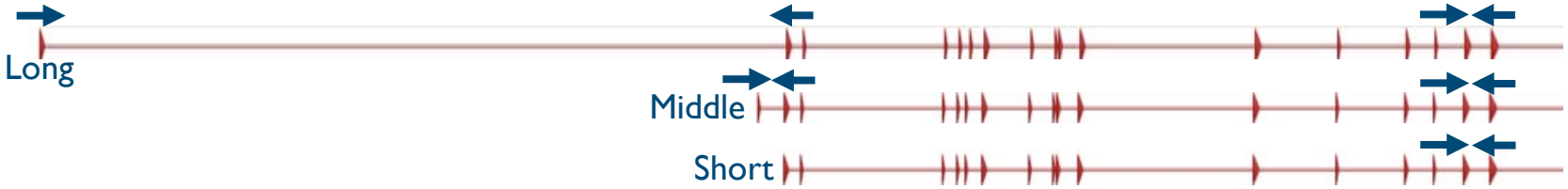
WT mouse cortical neurons

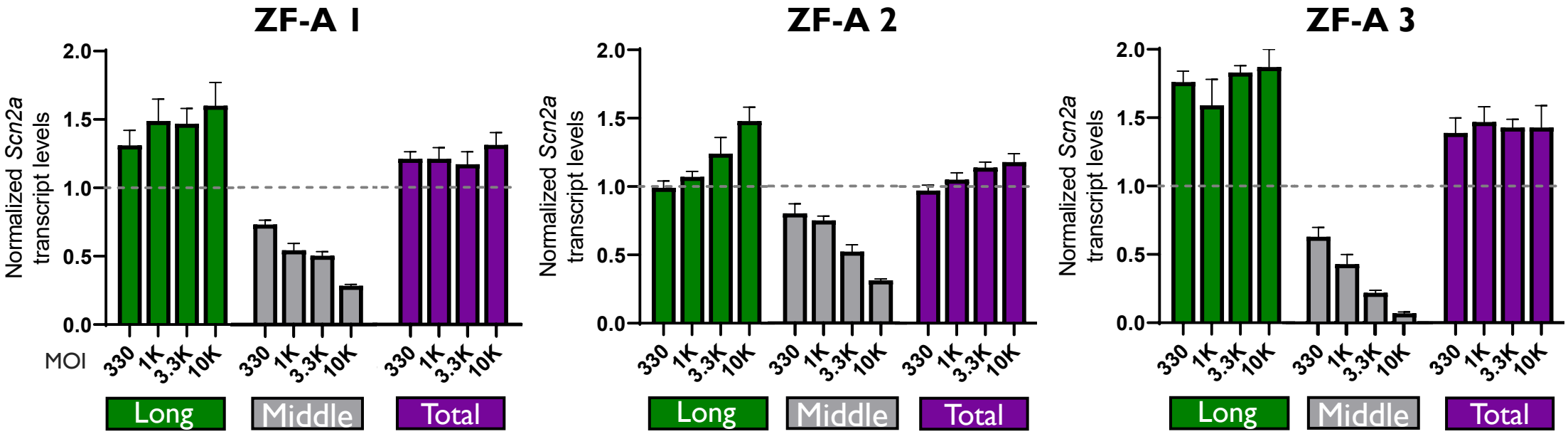
+

AAV6
hSyn1-ZF-A

7d

RT-qPCR

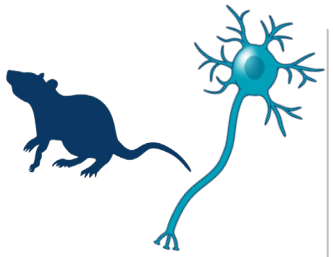




- Upregulation of *Scn2a* long transcript causes a dose-dependent repression of the middle *Scn2a* transcript

Mouse primary cortical neurons were harvested 7 d after AAV infection; Normalized to mean of Atp5b and Eif4a2. Mean +/- SD.

ZF-A mediated activation of the long *Scn2a* transcript increases $Na_v1.2$ in WT mouse cortical neurons



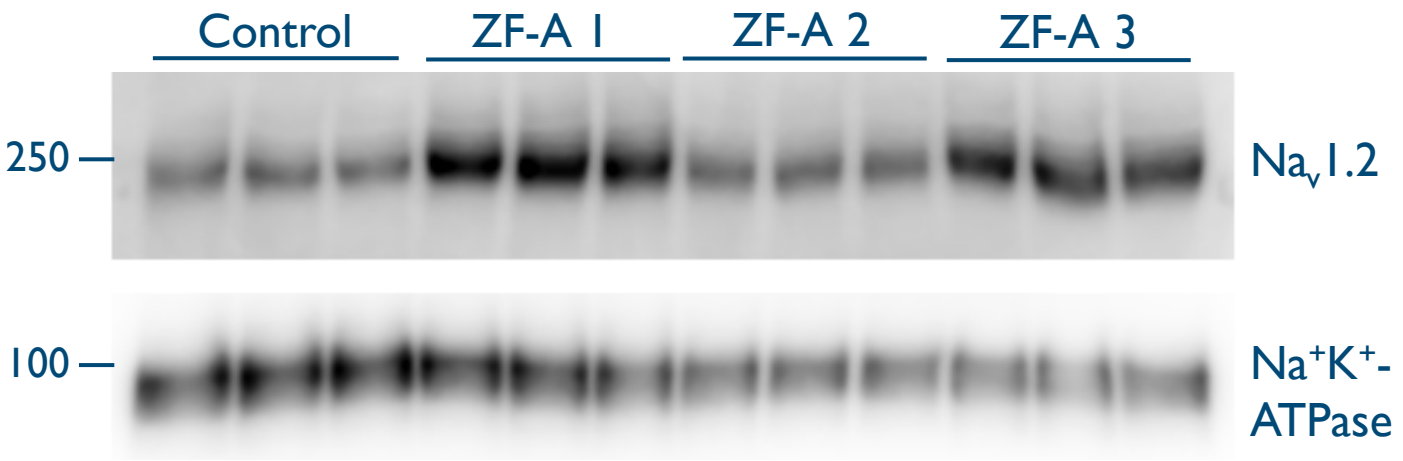
WT mouse cortical neurons

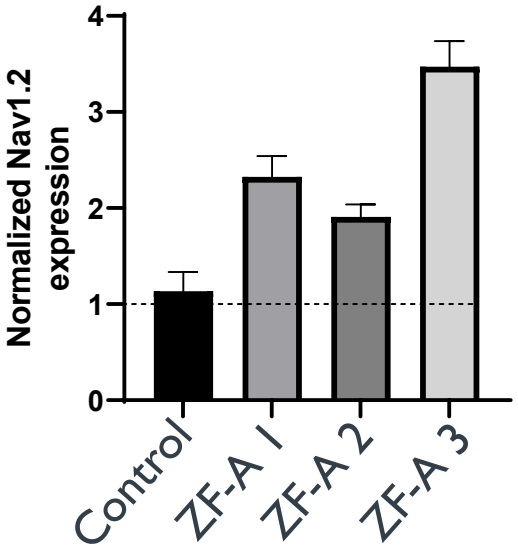
+

AAV6
10K MOI

10d

Western blot

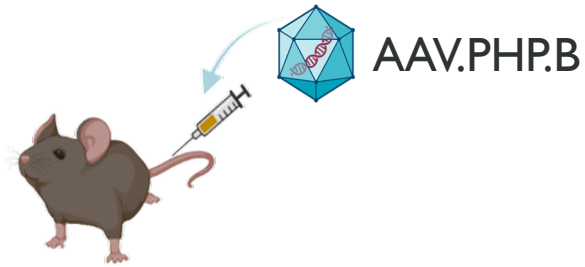




➢ **Do these *in vitro* findings translate *in vivo*?**

Mouse primary cortical neurons were harvested 8 d after AAV infection;
Normalized to Na^+K^+-ATPase. Mean +/- SD.

Early ZF-A leads were evaluated *in vivo* for *Scn2a* upregulation

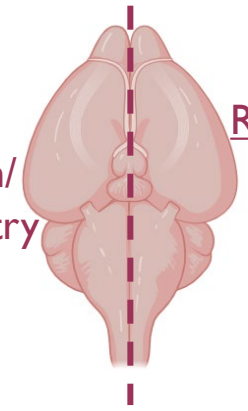


5-week-old
C57BL/6J WT

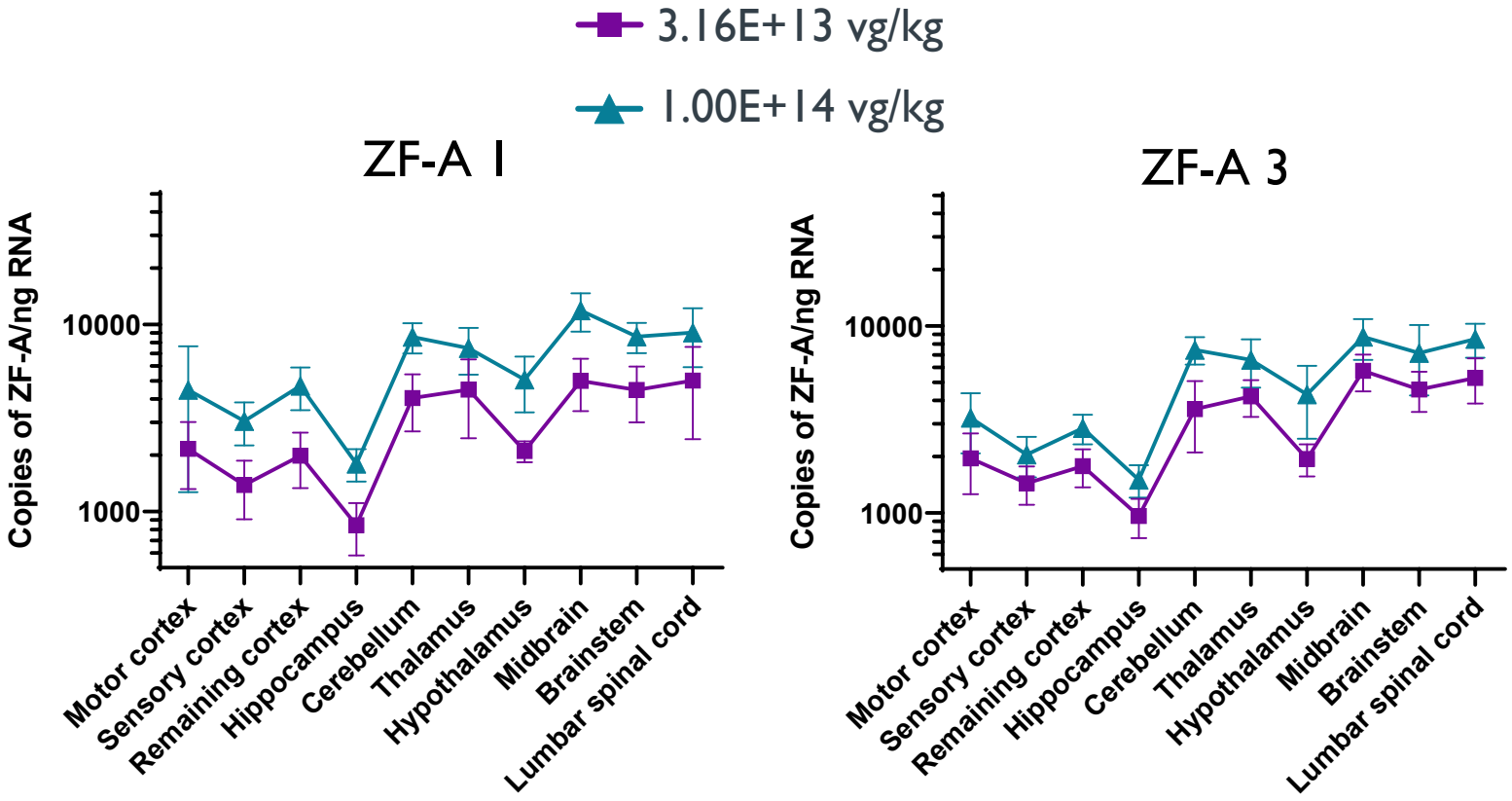
Treatment (ZF-A)	Dose Level (vg/kg)	No. of Mice
Vehicle	0	8
hSyn1-ZF-A 1	3.16E+13	8
hSyn1-ZF-A 1	1.00E+14	8
hSyn1-ZF-A 3	3.16E+13	8
hSyn1-ZF-A 3	1.00E+14	8

3-week in-life

Left Hemisphere
In-situ hybridization/
Immunohistochemistry

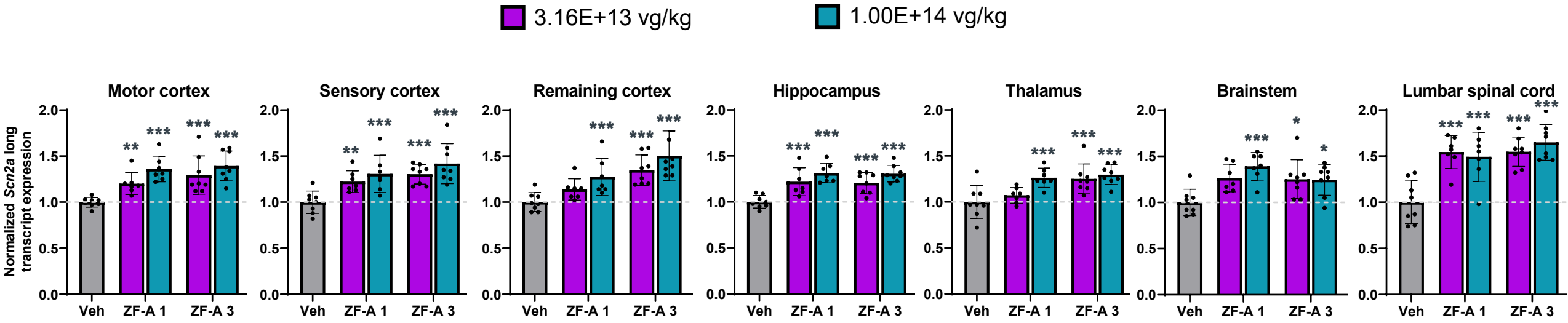


Right Hemisphere
micro-dissected
for RT-qPCR

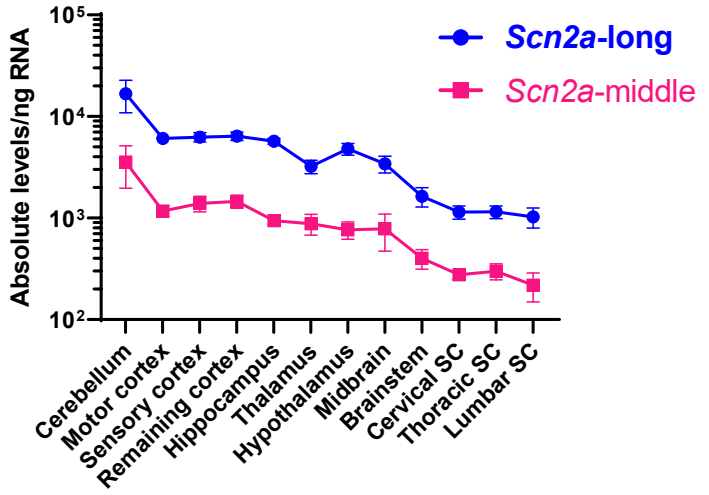


- Transgene is expressed throughout the CNS 3 weeks post-administration

ZF-As upregulate *Scn2a* long transcript in several brain regions and spinal cord

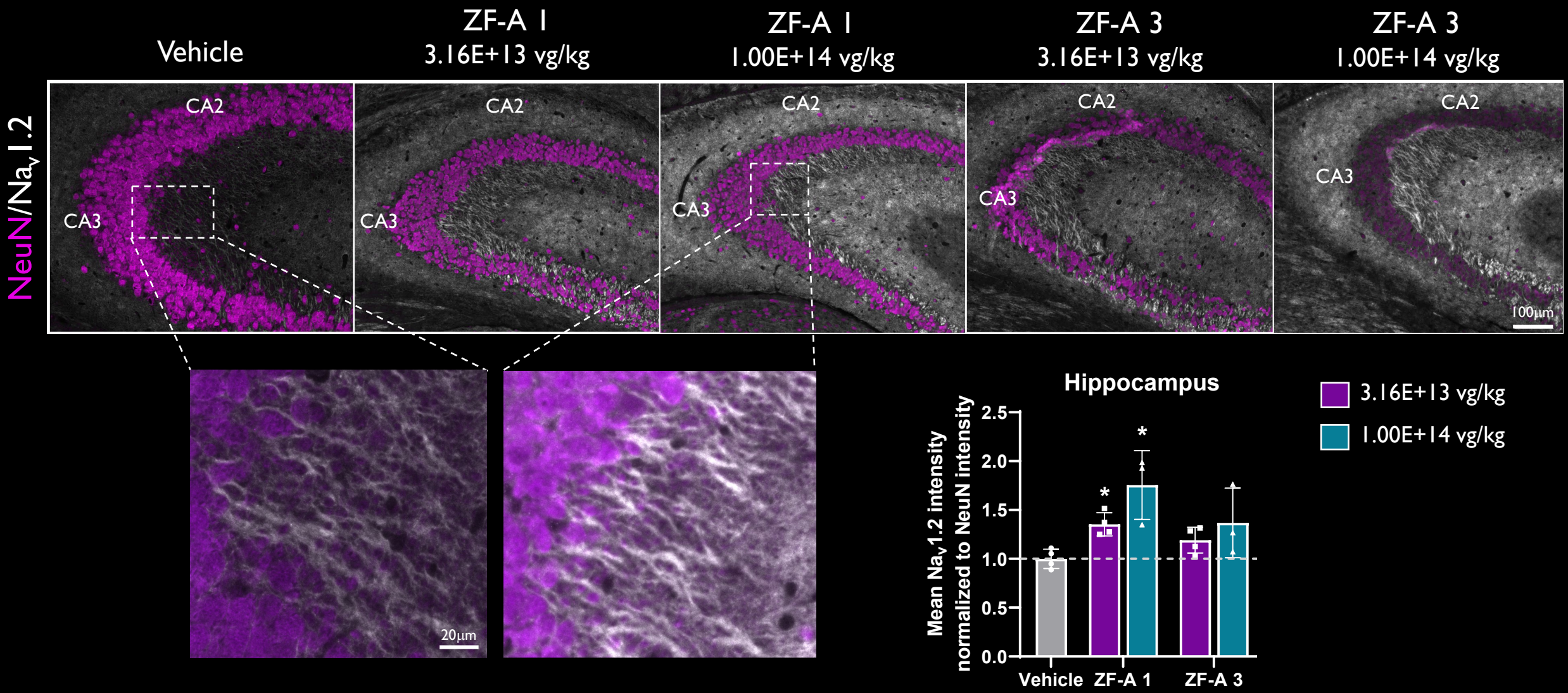


- ZF-A-mediated dose-dependent repression of the middle *Scn2a* transcript was observed in all brain regions except the cerebellum
- Long *Scn2a* transcript is expressed at 5-fold higher absolute levels than the middle *Scn2a* transcript in all brain regions of vehicle treated animals



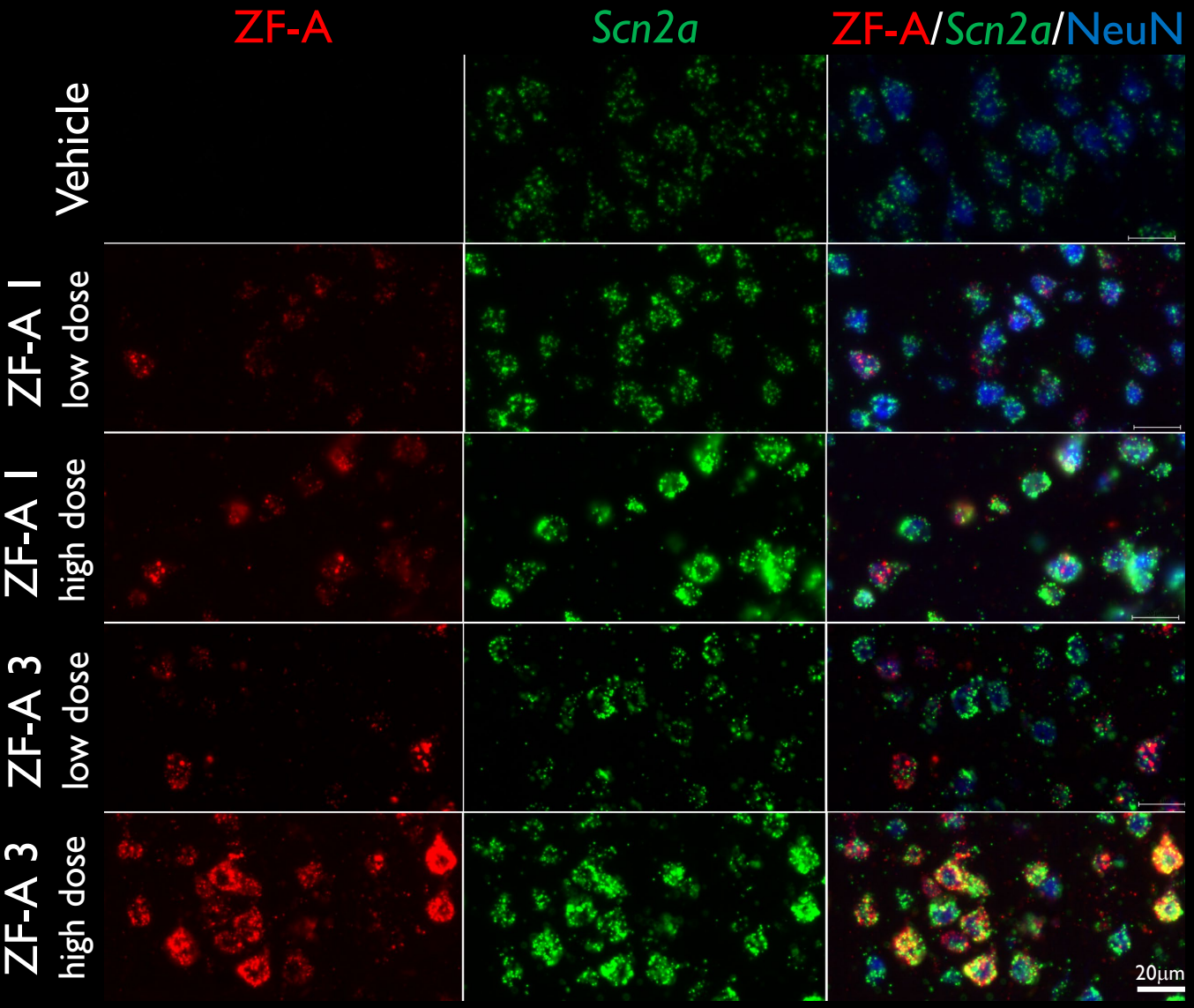
1-Way ANOVA, Dunnett's multiple comparisons test, *p*-values represent a comparison to vehicle, **p*<0.05, ***p*<0.01 ****p*<0.001

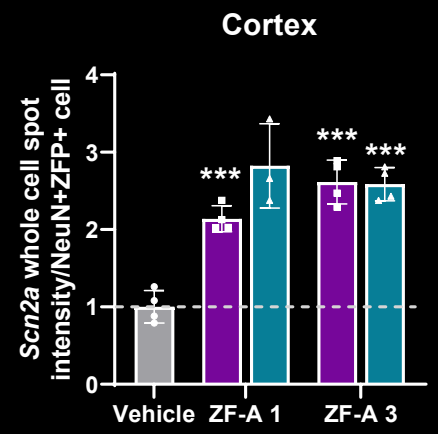
ZF-As elicit a dose-dependent increase in $Na_v1.2$ expression

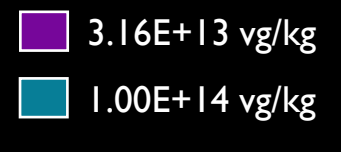


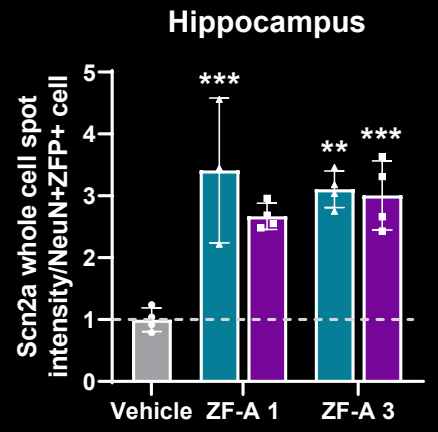
1-Way ANOVA, Dunnett's multiple comparisons test, *p*-values represent a comparison to vehicle, **p*<0.01, ***p*<0.005 ****p*<0.001

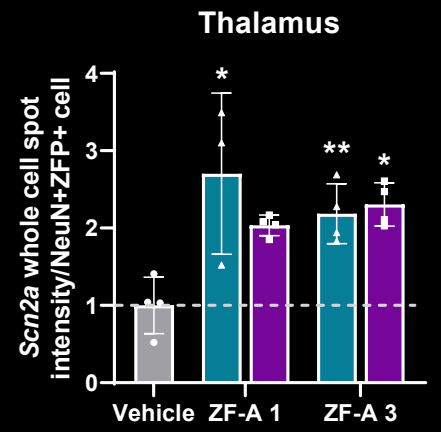
RNAScope shows ZF-As increase *Scn2a* levels by more than two-fold in single neurons







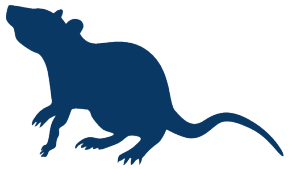




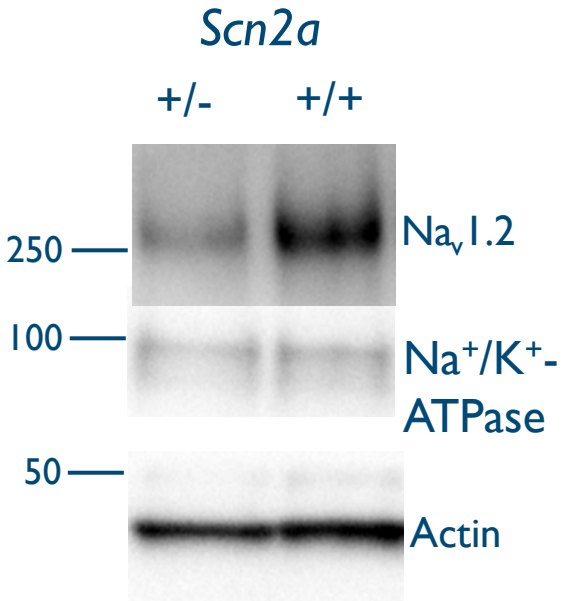
Sangamo THERAPEUTICS

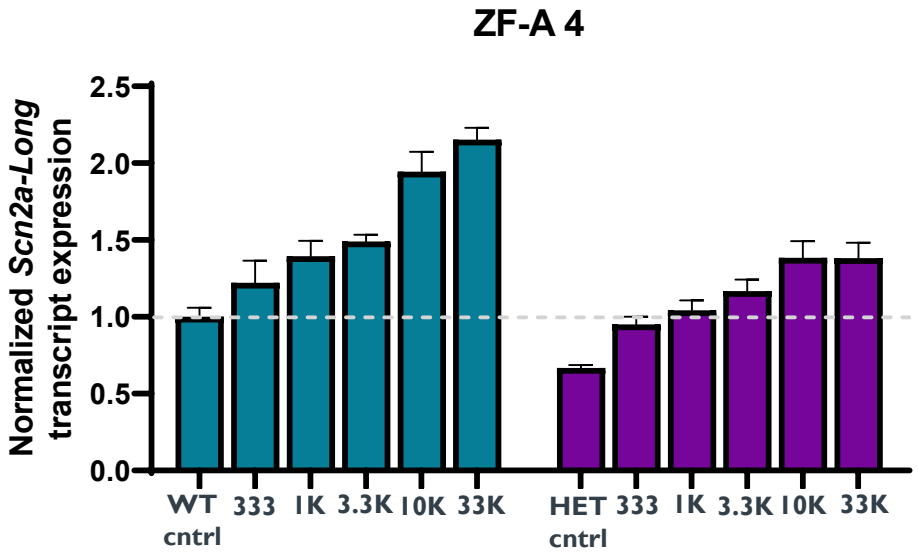
1-Way ANOVA, Dunnett's multiple comparisons test, *p*-values represent a comparison to vehicle, **p*<0.01, ***p*<0.005 ****p*<0.001

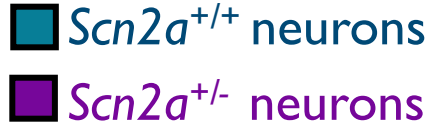
Lead ZF-As candidates increase *Scn2a* expression and restore $Na_v1.2$ levels in *Scn2a* haploinsufficient mouse cortical neurons

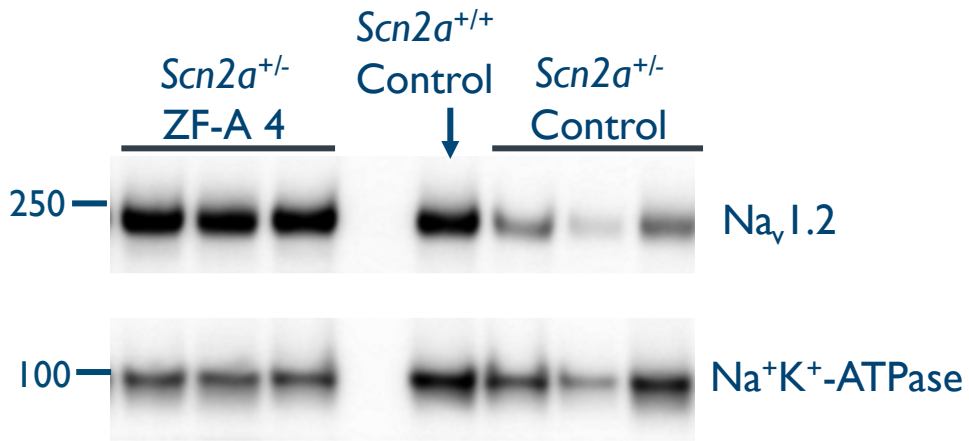


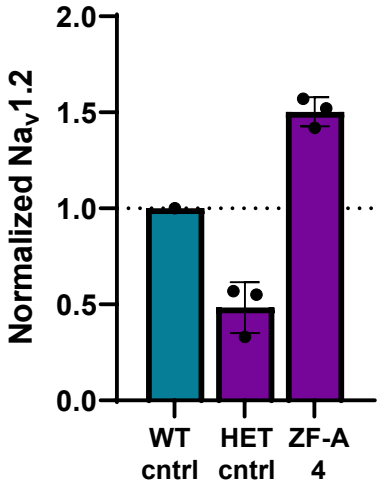
Scn2a$^{+/-}$ mouse model
(Planells-Cases et al., 2000)





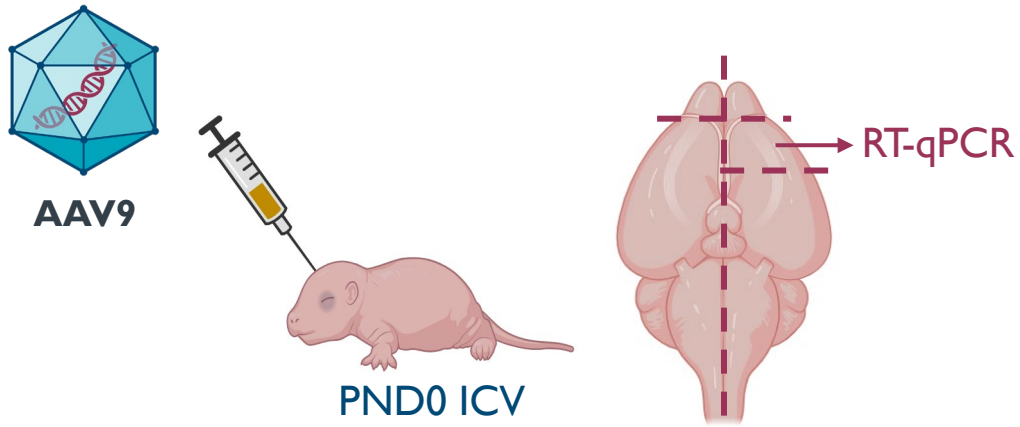






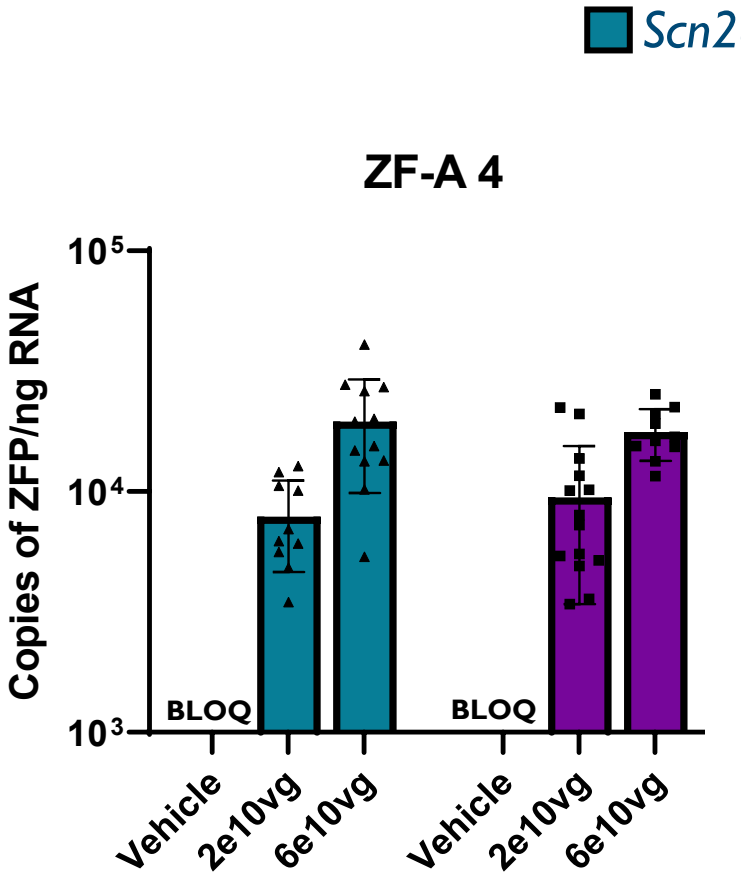
Neurons were transduced on day 2 and harvested 6 days later for RT-qPCR and western blot. RT-qPCR data normalized to mean of Atp5b and Eif4a2. Western blot data normalized to mean of Na^+K^+-ATPase. Mean +/- SD.

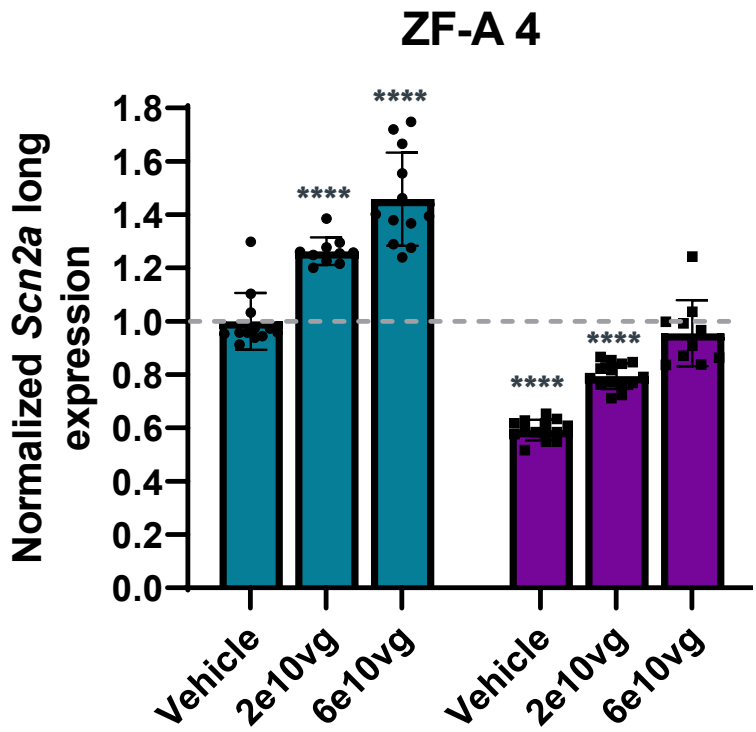
Lead candidate ZF-A restores *Scn2a* expression to normal in *Scn2a*$^{+/-}$ mice *in vivo*



Treatment (ZF-A)	Dose	No. of *Scn2a*$^{+/+}$ mice	No. of *Scn2a*$^{+/-}$ mice
Vehicle	0	6M/6F	8M/5F
hSyn1-ZF-A 4	2.0E+10vg	7M/3F	8M/6F
hSyn1-ZF-A 4	6.00E+10vg	3M/6F	6M/4F

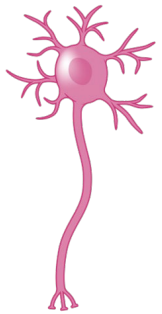
4-week in-life





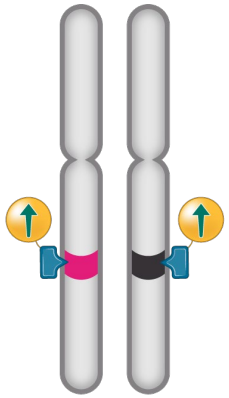
RT-qPCR data normalized to mean of Atp5b and Eif4a2. 1-Way ANOVA, Dunnett's multiple comparisons test, *p*-values represent a comparison to *Scn2a*$^{+/+}$ vehicle, ****p<0.0001. BLOQ – Below Limit of Quantitation.

Conclusion



- ZF-As targeting mouse *Scn2a* increase *Scn2a* long transcript expression and $Na_v1.2$ levels in *Scn2a*$^{+/+}$ and *Scn2a*$^{+/-}$ mouse cortical neurons *in vitro*

- *In vitro* to *in vivo* translation achieved with ZF-As targeting *Scn2a*
- $Na_v1.2$ levels are elevated in several brain regions of WT mice following ZF-A treatment
- Lead ZF-A restores normal *Scn2a* long transcript levels in the brains of *Scn2a*$^{+/-}$ mice



- Demonstrated proof-of-concept that ZF-As can be utilized to potentially to treat diseases caused by haploinsufficiency
- PoC also achieved for ZF-As targeting mouse *Shank3* as a therapeutic approach for treating Phelan-McDermid syndrome. See Daniel Chung's poster – P415 (Wednesday 5pm and Thursday 830pm)

Sangamo THERAPEUTICS

Thank you

Jenny Hodges

Senior Scientist, Neuroscience

Sangamo Therapeutics Inc.