

Restoration of Normal Gene and Protein Expression in Mouse and Human Disease Models of SCN2A Haploinsufficiency Using Zinc Finger Activators

Sangamo THERAPEUTICS

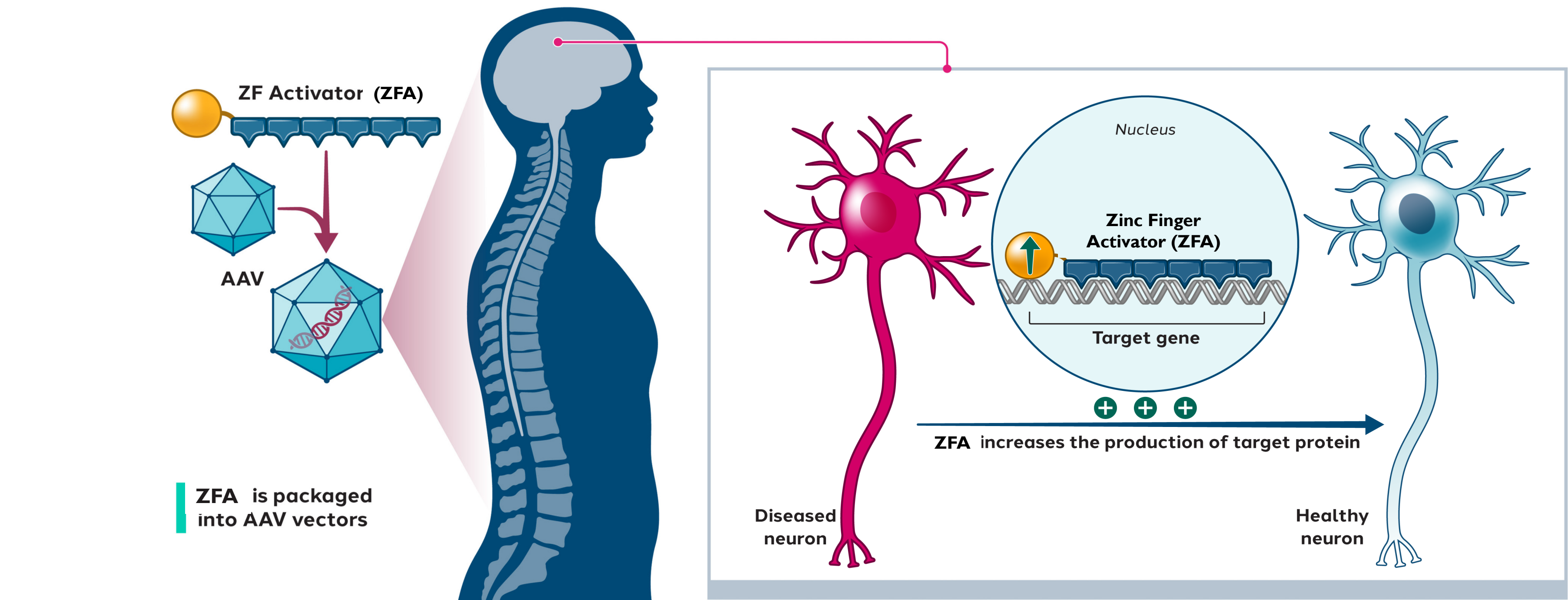
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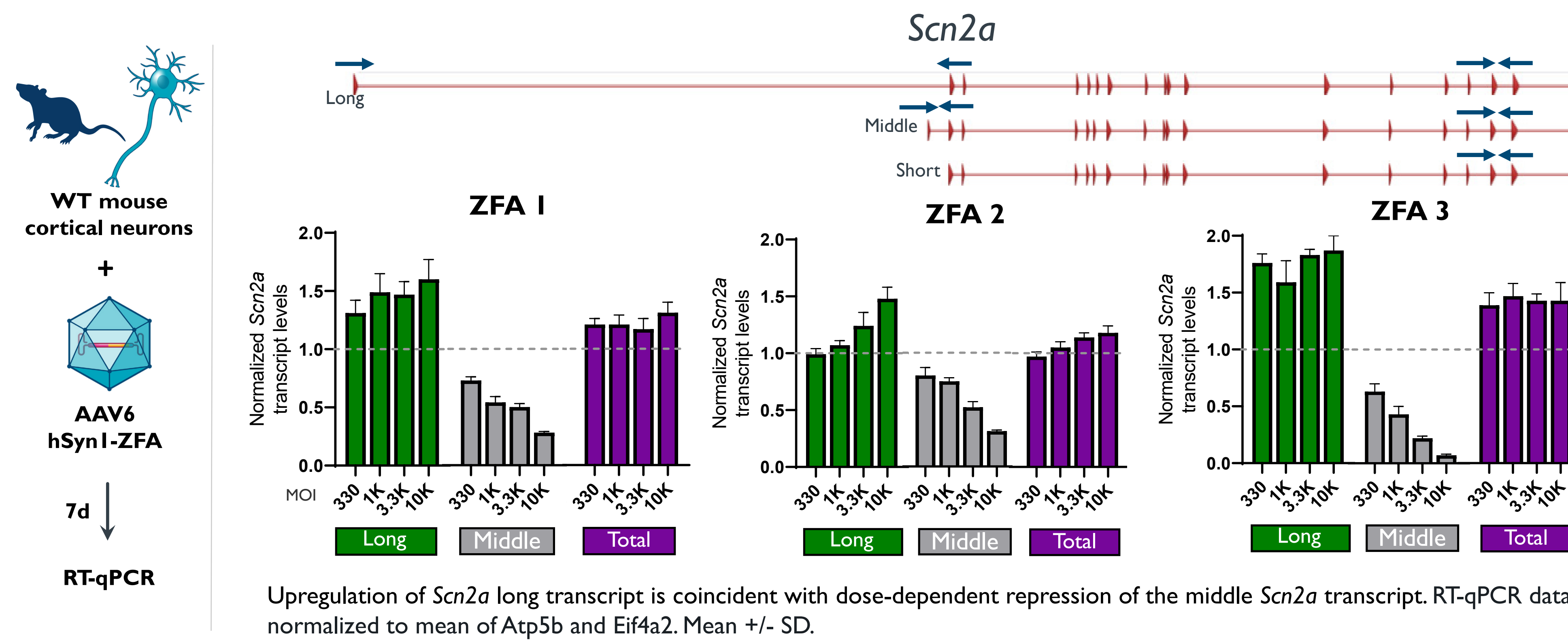
Poster #636

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

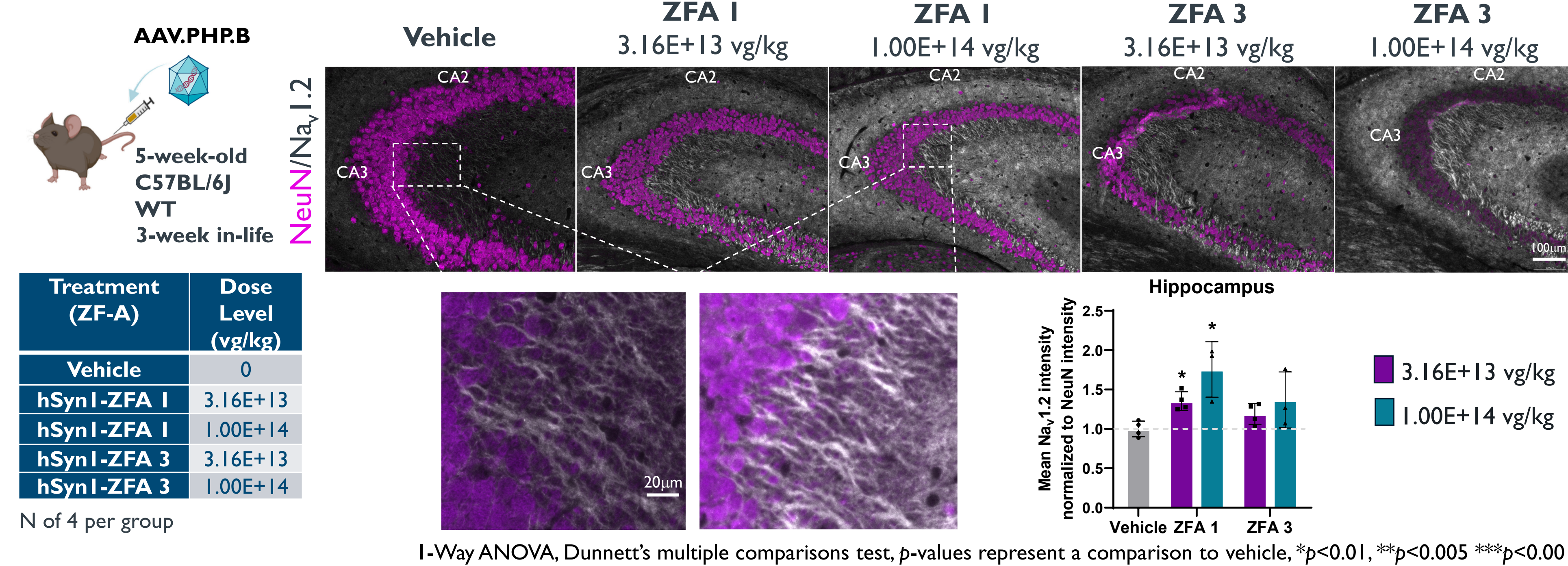
- SCN2A haploinsufficiency is a common monogenetic cause of autism spectrum disorder (ASD) and intellectual disability (ID), for which no effective treatment currently exists.
- SCN2A haploinsufficiency is caused by de novo loss-of-function mutations in the SCN2A gene resulting in insufficient gene expression and abnormally low levels of its encoded protein, neuronal voltage-gated sodium channel Na_v1.2.
- Our therapeutic approach to treating SCN2A haploinsufficiency involves targeting a zinc finger protein tethered to a transcriptional activator, a zinc finger activator (ZFA), to the SCN2A gene to drive transcriptional upregulation of the healthy allele and thereby restore normal protein levels.
- We utilized our proprietary ZFA platform to upregulate *Scn2a* and Na_v1.2 expression in neurons of wildtype (WT) and *Scn2a*^{+/-} mice *in vitro* and *in vivo*.
- We also demonstrate proof of concept in human neurons whereby highly specific human ZFAs increased SCN2A levels in engineered human NGN2 neurons harboring a common mutation modeling SCN2A haploinsufficiency.



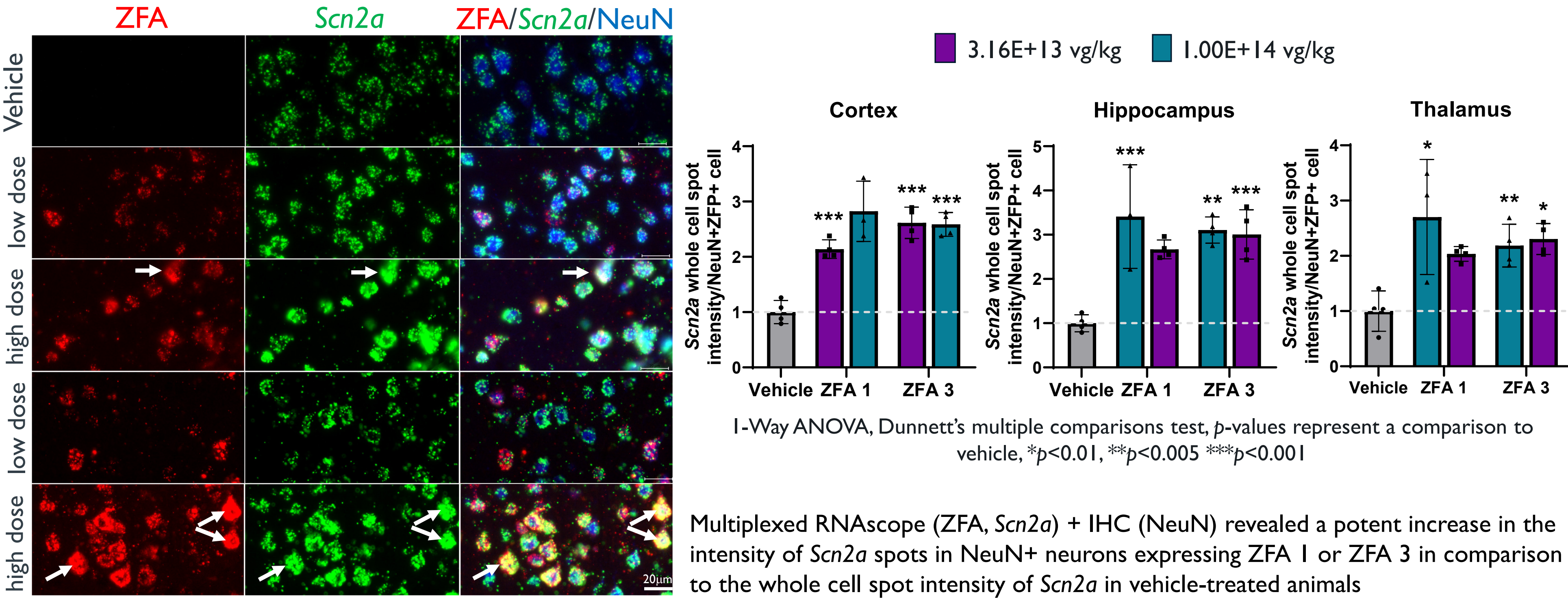
Mouse ZFAs upregulate *Scn2a* long transcript in a dose-dependent manner in WT mouse cortical neurons



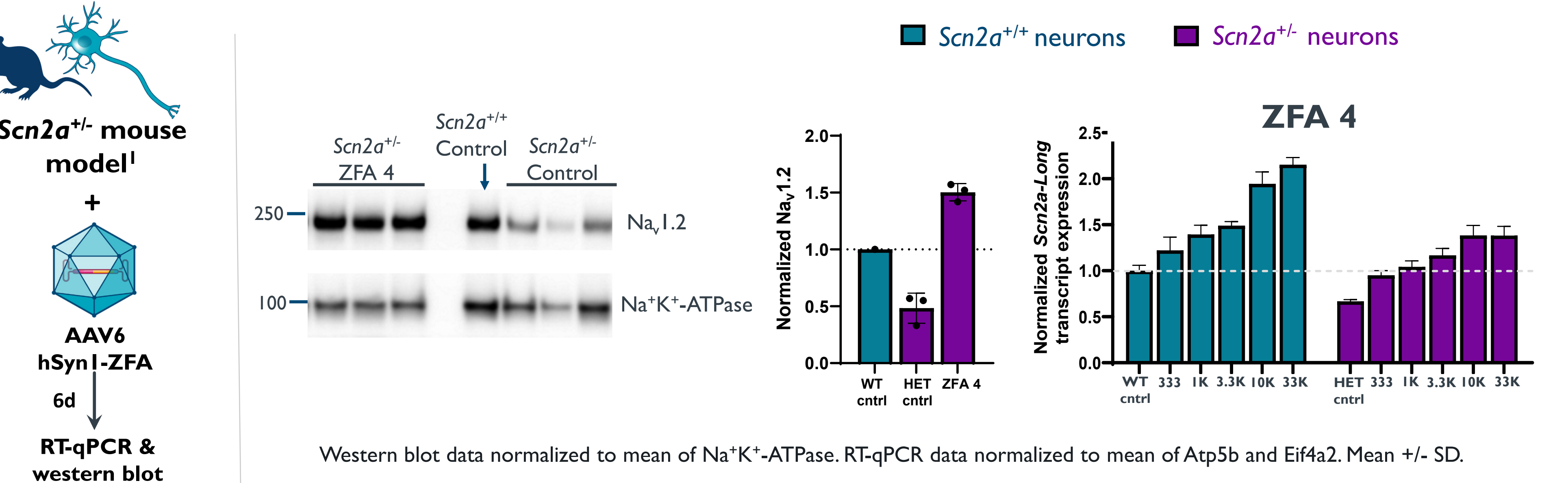
Upregulation of the long *Scn2a* transcript elicits a dose-dependent increase in Na_v1.2 expression *in vivo*



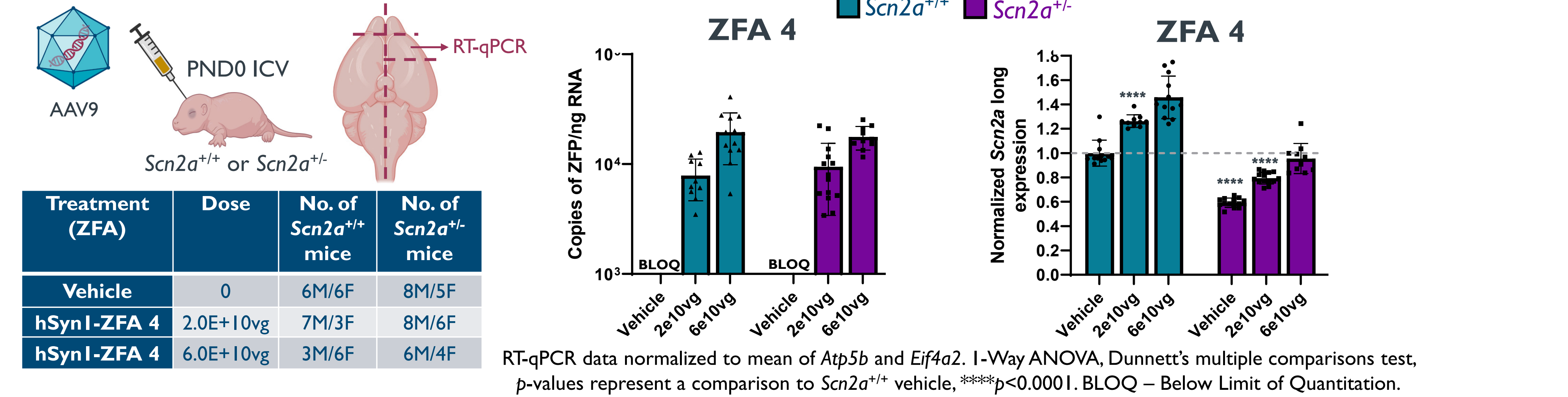
RNAscope shows ZFAs increase *Scn2a* levels by more than two-fold in single neurons of several brain regions in WT mice



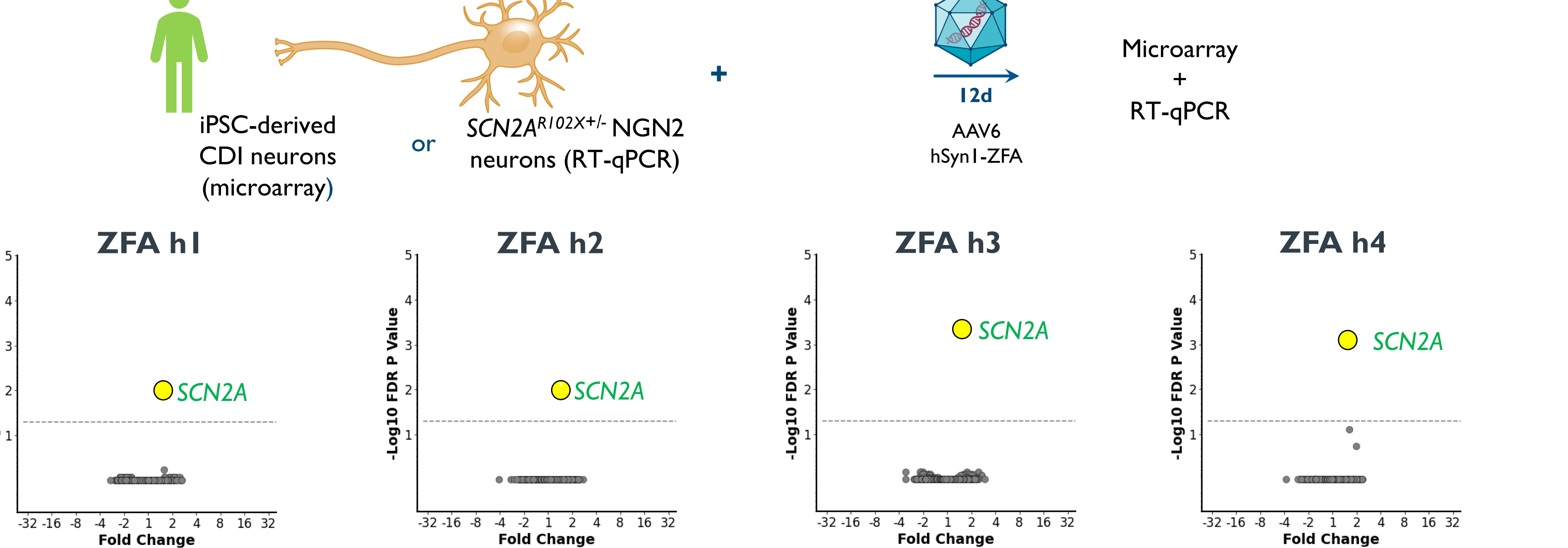
Lead ZFAs increase *Scn2a* expression and restore Na_v1.2 levels to normal in *Scn2a* haploinsufficient mouse cortical neurons



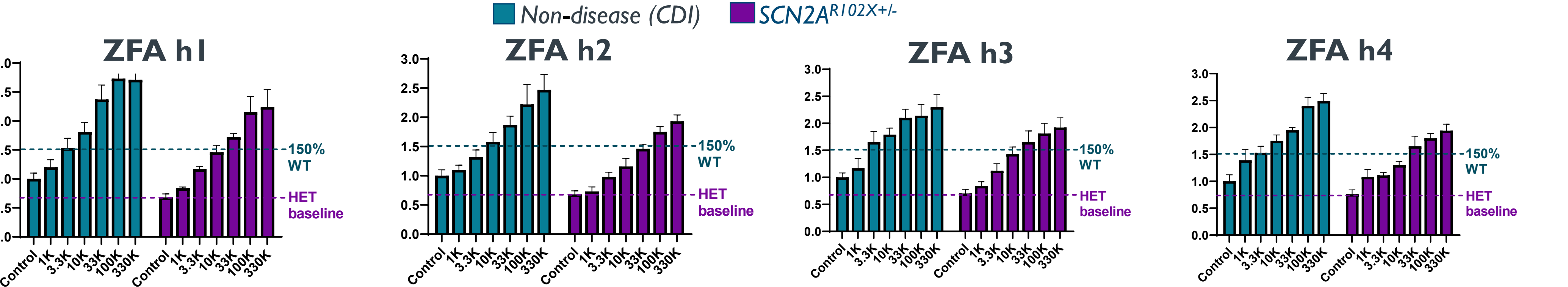
Lead candidate mouse ZFA restores *Scn2a* expression to normal levels in *Scn2a*^{+/-} mice *in vivo*



Human ZFAs increase SCN2A levels in healthy and disease-model human neurons with no detectable off-targets



Transcriptome analysis using a Human Clariom S HT GeneTitan microarray (N=4-6) shows ZFAs selectively and specifically increase SCN2A levels in comparison to neurons expressing a negative control ZFA. Neurons were transduced with an MOI of 100K.
FDR p-val < 0.05; Downregulated gene(s). FDR p-val < 0.05; Upregulated gene(s).



Conclusions

- Mouse ZFAs potentially upregulate *Scn2a* expression at the bulk and single-cell level in cultured neurons and the brain.
- Highly specific human ZFAs increase *SCN2A* expression in healthy and *SCN2A*^{R102X+/-} engineered NGN2 neurons.
- ZFA-mediated upregulation of *SCN2A* in disease models restores target mRNA and Nav1.2 protein to normal WT levels, demonstrating proof-of-concept for our ZFA approach to potentially treat *SCN2A* haploinsufficiency disorder.

We thank everyone in the Screening Core, Neurology & Technology Teams, Vector Core, Nonclinical Operations and Facilities at Sangamo Therapeutics. We would also like to thank our collaborators at Novartis. Select images generated using biorender.com

¹Planells-Cases et al., *Biophysical Journal* (2000).

