A Zinc Finger Activator Platform to Restore SCN1A Gene Expression In Vivo and in Cellular Models of Dravet Syndrome

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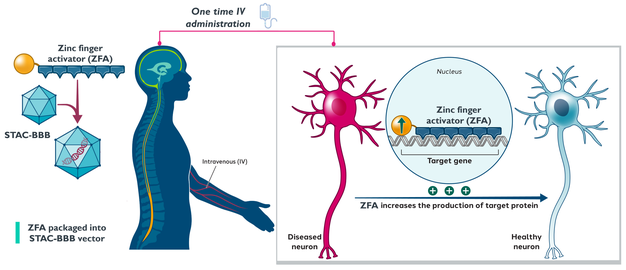
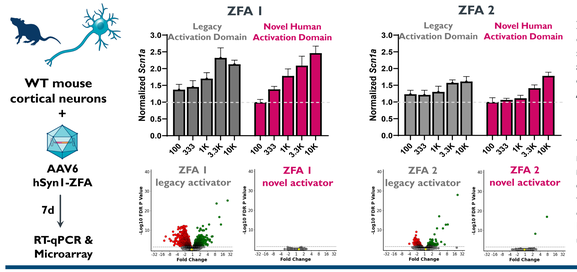
Poster #642

Zinc finger-mediated activation of *SCN1A* as a potentially potent and specific therapeutic approach for treating Dravet syndrome

- Dravet Syndrome is a severe neurodevelopmental disorder and epileptic encephalopathy characterized by intractable seizures, intellectual disability, motor deficits, and a 10% to 20% rate of premature death.
- Dravet Syndrome is caused by *de novo* loss-of-function mutations in the *SCN1A* gene resulting in insufficient gene expression and abnormally low levels of its encoded protein, neuronal voltage-gated type I sodium channel, $Na_v1.1$.
- Our proposed therapeutic approach to treating Dravet Syndrome involves targeting a zinc finger protein tethered to a novel transcriptional activator domain, called a zinc finger activator (ZFA), to the *SCN1A* gene to drive transcriptional upregulation of the healthy allele and thereby restore normal $Na_v1.1$ levels.
- Our ZFA platform consists of human-derived components naturally expressed in the brain (see poster #1609), which was demonstrated to be functional and safe *in vivo* in adult wildtype (WT) mice
- We engineered a proprietary activation domain that is functional *in vivo* and increased *Scn1a* expression in single, infected neurons by more than two-fold in adult WT mice. ZFA administration was well-tolerated in WT mice as no clinical or cellular signs of toxicity were observed (see poster #1609).
- We identified several ZFAs that mediate selective and specific upregulation of *SCN1A* in human iPSC-derived neurons and in disease-model *Scn1a*$^{+/-}$ mouse cortical and hippocampal neurons; demonstrating proof-of-concept for our ZFA platform as a potential therapeutic approach to treating Dravet syndrome.



Our proprietary activation domain tethered to zinc fingers targeting *Scn1a* is highly selective and specific in mouse cortical neurons



ZFs tethered to the novel activation domain upregulate *Scn1a* expression slightly more than the legacy activation domain tethered ZFs. RT-qPCR data normalized to mean of *Atp5b* and *Eif4a2*.

Transcriptome analysis shows the novel activation domain mediates minimal to no off-target effects in contrast to the same ZFs fused to the legacy activation domain. Red dots indicate downregulated gene(s); FDR p-val<0.05. Green dots indicate upregulated gene(s); FDR p-val<0.05.

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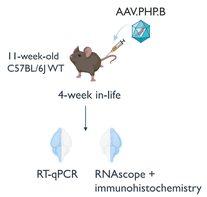
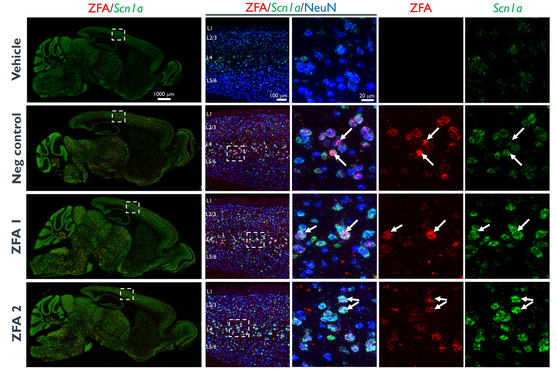
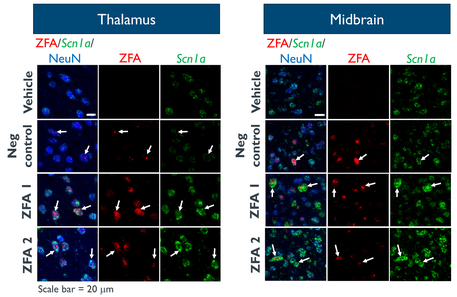
Our proprietary activation domain fused to zinc fingers is functional *in vivo* and upregulates *Scn1a* expression in neurons in WT mice

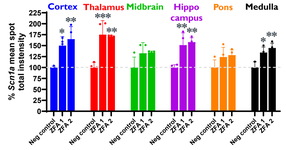


Test Article	Dose (vg/kg)	Animal No.
Vehicle	NA	4
Neg Control (hSyn1-ZFA)	1.0E+14	4
hSyn1-ZFA 1	1.0E+14	4
hSyn1-ZFA 2	8.95E+13	4



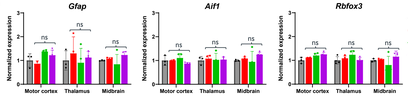


Scale bar = 20 µm



Scn1a RNAscope is reported as spot total intensity in the cell soma (nucleus + cytosol) of NeuN+/ZFA+ cells. Data is normalized to the negative control treatment. For each brain region, 1-way ANOVA, Dunnett's multiple comparisons test, p-values represent a comparison to negative control; *p<0.05, **p<0.005, ***p<0.001. ZFA 1 and ZFA 2 transduction increased Scn1a expression by 1.3 to 2-fold in individual ZFP+/NeuN+ neurons in several brain regions.

RT-qPCR analysis on RNA extracted from each brain region showed no significant differences in neuroinflammatory markers (*Gfap* and *Aif1*) or neuronal health (*Rbfox3*) compared to vehicle treated animals. 2-way ANOVA, Dunnett's multiple comparisons test; ns is non-significant in comparison to vehicle in same brain region. Also see poster #1609.



Acknowledgments

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References

Miller AR, et al., Kearney JA. Mapping genetic modifiers of survival in a mouse model of Dravet syndrome. *Genes Brain Behavior* (2014).

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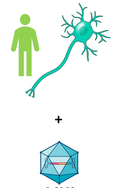
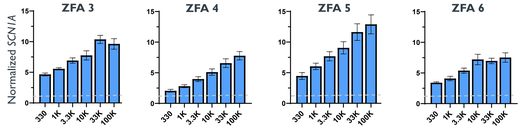
ZFAs mediate selective and specific upregulation of *SCN1A* in iPSC-derived human neurons with minimal to no detectable off-targets

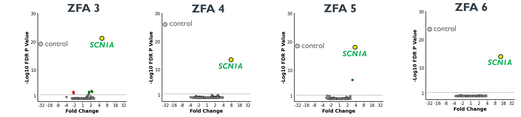
ZFAs targeting conserved regions of the *SCN1A* promoter were screened in human and mouse neuroblastomas; hundreds of ZFAs were identified that increased both human *SCN1A* and mouse *Scn1a* expression by more than 2-fold above baseline levels. Select ZFAs were manufactured into AAV for testing in human iPSC-derived neurons.



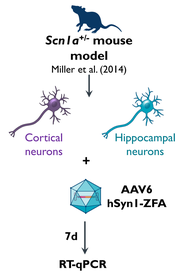
ZFAs increase *SCN1A* expression in an MOI-dependent manner, 8-14-fold higher than baseline levels established by transfection with a negative control ZFA. RT-qPCR data normalized to mean of *ATP5b* and *Eif4a2*.

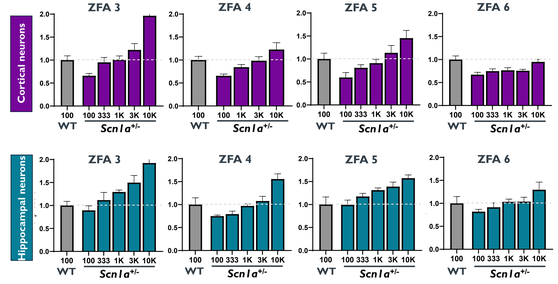


Transcriptome analysis shows ZFAs targeting *SCN1A* selectively and specifically increase *SCN1A* mRNA levels in comparison to neurons expressing a negative control ZFP. Neurons were transduced with an MOI of 100K. Control (grey circle) is a non-targeting control ZFA. *FDR p-val < 0.05; Downregulated gene(s), FDR p-val < 0.05; Upregulated gene(s).*



Scn1a expression is restored to normal levels in disease model *Scn1a*$^{+/-}$ mouse cortical and hippocampal neurons expressing ZFAs





ZFAs increase *Scn1a* expression in an MOI-dependent manner and restore *Scn1a* expression to or above normal WT levels. RT-qPCR data normalized to mean of *ATP5b* and *Eif4a2*. WT neurons were transduced with a negative control ZFA at 100 MOI (grey bar).

Conclusions

- We identified several ZFAs targeting conserved regions of *SCN1A* that mediate selective and specific upregulation of *SCN1A* in human iPSC-derived neurons and in disease-model *Scn1a*$^{+/-}$ mouse neurons.
- ZFAs potently increase *Scn1a* levels in infected neurons *in vivo* demonstrating proof-of-concept for our ZFA platform as a potential therapeutic approach to treating Dravet syndrome.

Presented at ASGCT 2024