AWARD NUMBER: W81XWH-21-1-0323

TITLE: RNA-Directed Therapy for C9ORF72-Linked ALS Using Engineered Zinc Finger Nucleases

PRINCIPAL INVESTIGATOR: Yeo, Eugene, PhD MBA, Professor

CONTRACTING ORGANIZATION: The Regents of the University of California San Diego
La Jolla, CA

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RNA-Directed Therapy for C9ORF72-Linked ALS Using Engineered Zinc Finger Nucleases

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6. AUTHOR(S)

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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14. ABSTRACT

We demonstrate that an RNA-targeting zinc finger nuclease fusion system (Z1-PIN) eliminates G4C2 RNA expansions in mutant C9ORF72 RNA in C9-ALS patient-derived iPSC-derived spinal cord organoids, with minimal transcriptomic off-target effects. We demonstrate that Z1-PIN delivered via an adeno-associated viral vector to the central nervous system of a BAC transgenic mouse model of C9-ALS alleviates signs of neurodegeneration without gross adverse effects, providing proof-of-principle that a member of a new class of zinc-finger RNA-targeting effectors can potentially treat C9-ALS, with implications for other neurodegenerative diseases such as frontotemporal dementia.

15. SUBJECT TERMS

ALS, C9ALS, C9ORF72, spinal organoids, zinc finger, RNA foci

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES
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19a. NAME OF RESPONSIBLE PERSON	19b. PHONE NUMBER *(Include area code)*
USAMRDC	

TABLE OF CONTENTS

1. INTRODUCTION:

GGGGCC hexanucleotide repeat expansions ($G_4C_2^{EXP}$) in the first intron of the *C9ORF72* gene is the most common known cause of familial and sporadic ALS (C9-ALS) and have been linked to RNA-mediated pathogenesis due to the formation of toxic RNA foci formed from both sense $G_4C_2^{EXP}$ and antisense $C_4G_2^{EXP}$ transcripts. We engineered an RNA-targeting zinc finger nuclease fusion system (Z1-PIN) that targets both forms of mutant RNA *in vitro* and eliminates each, simultaneously, within C9-ALS patient-derived iPSC-derived spinal cord organoids, with minimal transcriptomic off-target effects. We also demonstrate that delivery of Z1-PIN via an adeno-associated viral vector to the central nervous system of a BAC transgenic mouse model of C9-ALS alleviates signs of neurodegeneration without gross adverse effects. Overall, we have successfully completed the proposed project that demonstrates proof-of-principle that a member of a new class of zinc-finger RNA-targeting effectors can potentially treat C9-ALS, with implications for other neurodegenerative diseases such as frontotemporal dementia. The results of this study are currently under review at *Science*.

2. KEYWORDS:

C9ALS, C9ORF72, spinal organoids, zinc finger, RNA foci

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1. Evaluation of ZNF fusion proteins to eliminate repeat expansion RNA in patient-specific spinal organoids.

Major Task 1: Generation and characterization of spinal organoids from a panel of C9ALS patient iPSCs and neurotypical controls (6 months).

Major Task 2: Validation of an ZNF fusion proteins for targeted cleavage of G_4C_2 and C_4G_2-containing RNAs in C9-ALS spinal organoid models (9 months).

Aim 2. *In vivo* safety studies of the RNA-targeting ZNF fusion protein system.

Major Task 3: Subpial injection of AAV9-packaged ZNF fusion protein system into mice (Timeline 12 months)

Aim 3: *In vivo* efficacy studies of the scAAV9-delivered ZNF fusion proteins in C9-ALS mouse models.

Major Task 4: Generate AAV9 titers that express RNA transcript containing 66 sense and antisense C_4G_2 repeats.

Major Task 5: Evaluation of AAV9-delivered ZNF fusion protein to reduce RAN dipeptide repeat protein and RNA foci in C9-ALS mouse model.

What was accomplished under these goals?

Relevant to Aim 1 ***(Evaluation of ZNF fusion proteins to eliminate repeat expansion RNA in patient-specific spinal organoids)***: As illustrated in **Fig. 1**, in C9-ALS patient-derived spinal cord organoids (**Fig. 1a,b**) our lead candidate Z1-PIN, delivered by lentiviral vectors, reduces the fraction of cells with $G_4C_2^{EXP}$ foci by >60%, $C_4G_2^{EXP}$ foci by >70%, and poly-GA dipeptide foci by >70%. Transcriptome-wide bulk RNA-seq analysis utilizing the DESeq2 package identified 1409 differentially expressed genes (DEGs; FDR-adjusted p-value <0.01) that distinguish our C9-ALS spinal cord from control organoids (**Fig. 2**). Gene ontology (GO) biological process analyses revealed that these DEGs were enriched for terms associated with C9orf72-linked ALS including "extracellular matrix", "cell adhesion", "negative regulation of protein kinase", and "RNA metabolic process.". As spinal cord organoids consist of a heterogeneous population of cell-types, we next subjected our C9-ALS spinal cord organoids (C9-ALS$^{900(1)}$) and controls to single-cell RNA-seq (scRNA-seq) analysis to determine the effects of Z1-PIN on cell-type specific gene expression changes. Unsupervised clustering was implemented on the combined dataset of 12,617 cells from control and C9-ALS spinal cord organoids that consisted of ~3,337 cells per organoid line and ~22,515 average reads per cell to identify genotype- and treatment-specific clusters at 60 days post-differentiation. In comparing Z1-PIN or PIN-treated C9-ALS organoids, we determined that Z1-PIN-treated C9-ALS cells clustered closer to control cells within single cell UMAPs compared to untreated C9-ALS cells while, 66.3%

(396/598) of C9-ALS signatures identified in astrocytes, 43.7% (136/311) within oligodendrocytes, and 58.7% (33/55) within excitatory neurons were partially or fully reversed in C9-ALS spinal organoid treated with Z1-PIN.Thus (**Fig. 3**), we demonstrate partial reversal of C9-ALS patient-specific molecular biomarkers on the bulk cell and cell-type resolved levels. Taken together, these data suggest that ***Z1-PIN can alleviate both cell-autonomous and non-cell autonomous C9-ALS pathological pathways in multiple cell-types within the human spinal cord.***

Relevant to Aims 2 (*In vivo safety studies of the RNA-targeting ZNF fusion protein system*) and **3 (*In vivo* efficacy studies of the scAAV9-delivered ZNF fusion proteins in C9-ALS mouse models):** We cloned Z1-PIN and PIN-only into self-complementary AAV vectors with PHP.eB capsid variants (AAV-PHP.eB) (**Fig. 4a,b**). We performed a bilateral intracerebroventricular (ICV) injection of each AAV-PHP.eB vector to 24-week-old C9-BAC-500 mice and age-matched wildtype littermate controls (WT). At four weeks post injection, we observed successful expression of Z1T and PIN-only in mouse CNS, indicated by GFP fluorescent signals from the GFP protein tag fused to each construct **(Fig. 5)**. Next, we performed western blot analysis with protein isolated from the cerebral hemisphere using antibodies against established markers of neurodegeneration: neuronal nuclei (NeuN), Glial fibrillary protein (GFAP), and cleaved Caspase-3 (clCASP3). Excitingly, we determined that Z1-PIN delivered by AAV-PHP.eB can prevent the mutant C9ORF72-dependent decrease of NeuN protein, suggesting our approach can ameliorate neuronal less in the cerebral cortex (**Fig. 4c**; quantified in **4d**). Western blot analysis with antibodies against GFAP and clCASP3 also revealed Z1-PIN treatment improves neuronal apoptosis and reduced reactive gliosis (Fig. 5c; quantified in 5d). These results are consistent with slowing neurodegeneration and potentially reversal of neuronal dysfunction. Z1 consists of two naturally linked C_2H_2 ZFDs in tandem. Interestingly,

reducing Z1 to only one of its ZFDs (referred to as Z1.1-PIN) maintains its effect *in vivo*, supporting our RNA-targeting zinc-finger-endonuclease strategy. Thus, we show that ***Z1-PIN treatment can largely stall the progression of neurodegeneration in vivo in an established mouse model of C9-ALS.***

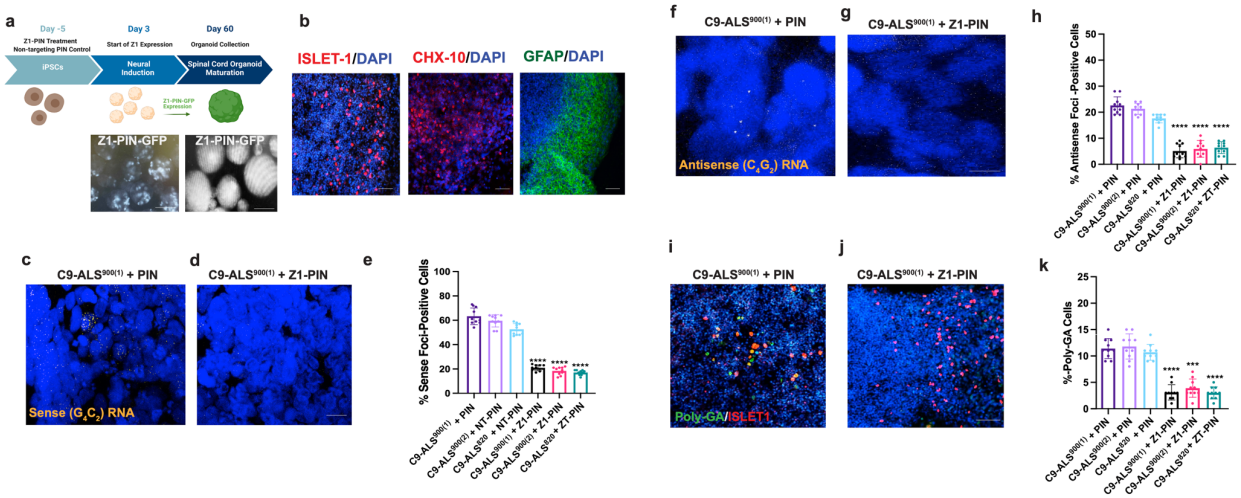

Figure 1. Z1-PIN reduces $G_4C_2^{EXP}$ and $C_4G_2^{EXP}$ RNA foci and poly-GA protein aggregates in C9-ALS patient-derived spinal cord organoids. (A) Schematic of the differentiation protocol used for iPSC-derived spinal cord organoids treated with a lentiviral system expressing Z1-PIN fused to a GFP fluorescent tag. **(B)** Representative immunofluorescence images of day 60 control spinal cord organoids showing cells positive for ISLET-1 (left), CHX-10 (middle), and GFAP (right). Scale bar = 50 µm at 20x magnification. **(C,D)** Images of RNA-FISH with antisense probe recognizing $G_4C_2^{EXP}$ foci in C9-ALS spinal cord organoids, with and without introduction of the Z1-PIN system. Scale bar = 50 µm at 20x. **(E)** Bar plot summarizing the frequency of $G_4C_2^{EXP}$ foci-positive cells across C9-ALS organoid lines. Graph shows mean ± SEM (n= 10 individual organoids per line; *N* = 2;one-way ANOVA, Tukey's Multiple Comparison Test; **** p < 0.0001). **(F,G).** Images of RNA-FISH with antisense probe recognizing $C_4G_2^{EXP}$ foci in C9-ALS spinal cord organoids, with and without introduction of the Z1-PIN system. Scale bar = 50 µm at 20x. **(H)** Bar plot summarizing the frequency of $C_4G_2^{EXP}$ foci-positive cells across C9-ALS organoid lines. Graph shows mean ± SEM (n= 10 individual organoids per line; *N* = 2;one-way ANOVA, Tukey's Multiple Comparison Test; **** p < 0.0001). **(I,J)** Images of poly-GA immunofluorescence in C9-ALS spinal cord organoids, with and without introduction of the Z1-PIN system. Scale bar = 50 µm at 20x. **(K)** Bar plot summarizing the frequency of poly-GA-positive cells across C9-Als organoid lines. Graph shows mean ± SEM (n= 10 individual organoids per line; *N* = 2;one-way ANOVA, Tukey's Multiple Comparison Test; *** p < 0.001, **** p < 0.0001).

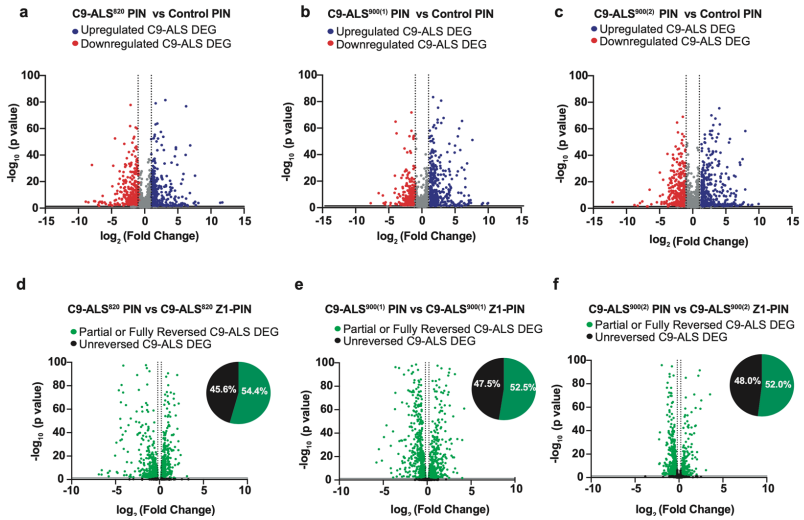

Figure 2. Z1-PIN partially reverses molecular biomarkers of C9-ALS in patient iPSC-derived spinal cord organoids. (A-C) Scatter plots of a common list of 1,409 upregulated and downregulated DEGs of within C9-ALS-800, C9-ALS-900(1), and C9-ALS-900(2) transduced with PIN lentiviral vector compared to Controls 1-3("Control"). C9-ALS-associated DEGs were defined by a two-fold change from Control and a FDR-adjusted p-value <0.01. **(D-F)** Volcano plots of C9-ALS DEGs between C9-ALS organoid lines treated with Z1-PIN vs PIN. Fold change is relative to C9-ALS PIN. Significance cutoffs include a Fold change > +/- 20% and FDR-adjusted p-value < 0.05.

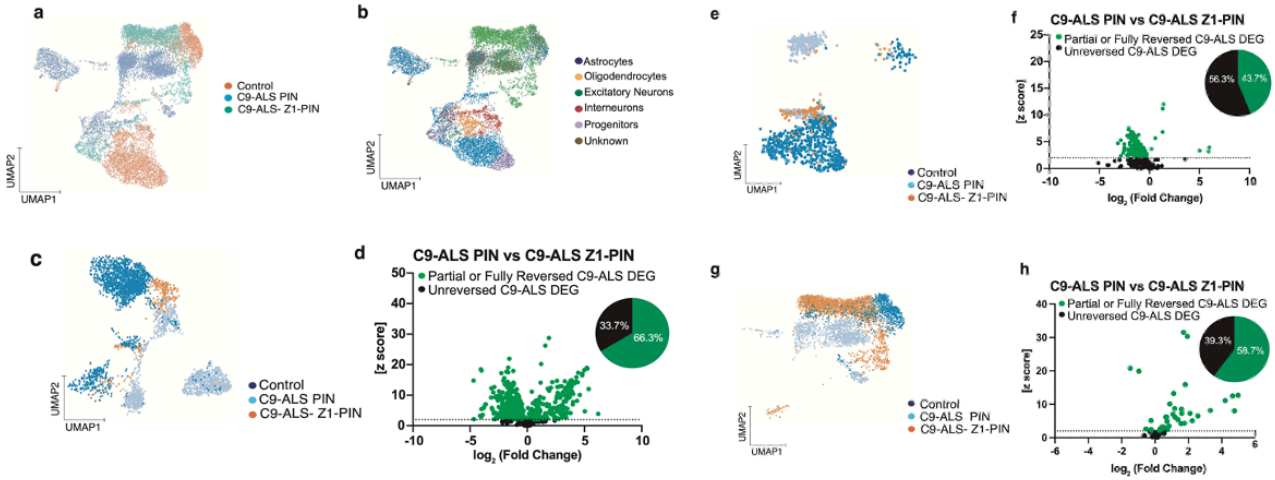

Figure 3. Z1-PIN partially reverses cell-type specific molecular biomarkers of C9-ALS in patient iPSC-derived spinal cord organoids. (A) UMAP visualization of control spinal cord organoids colored by experimental group. **(B)** UMAP visualization of control spinal cord organoids colored by cell-type.**(C,E,G)** Cell type-specific UMAP visualization of control spinal cord organoids colored by experimental group. **(D,F,H)** Volcano plot of cell-type specific C9-ALS DEGs between C9-ALS organoid lines treat with Z1-PIN vs PIN. Large fractions of C9-ALS DEGs correspond to a partial to full reversal of C9-ALS-associated DEGs (green). Fold change is relative to C9-ALS PIN. Significance cutoffs include a standard deviation > +/- 2 and FDR-adjusted p-value < 0.05.

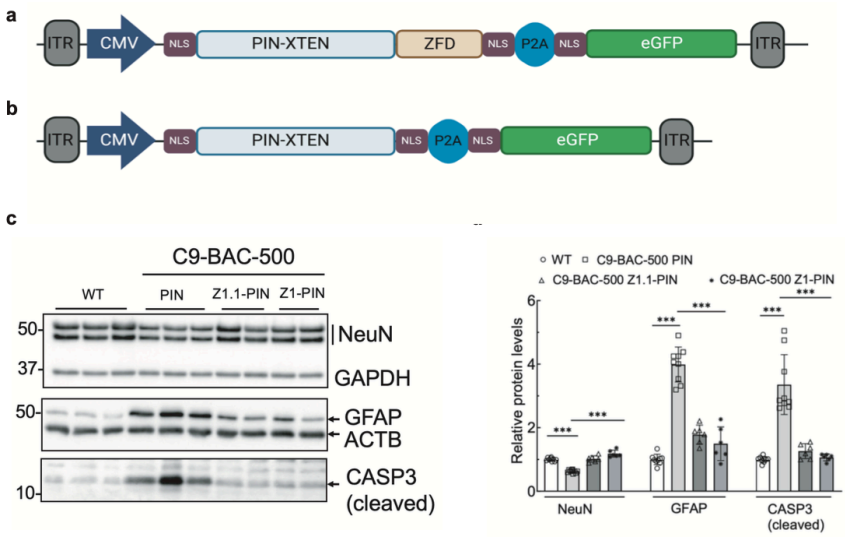

Figure 4. *In vivo* testing of Z1-PIN in BAC-*C9ORF72* mice. Mice were injected ICV at 24 wks of age with PIN (control), Z1.1-PIN, or Z1-PIN and brain extracts were evaluated by western blotting 4 wks later. **(A,B)** Diagram of AAV vector containing Z1, Z1.1 (A) or PIN-only (B) ORFs. **(C)** Each lane represents an extract from an individual mouse. ACTB/GAPDH was used as a loading control, and the blots shown are representative of three replicate blots. (**D)** Quantitative analysis of Western blots in A**.** One-way ANOVA with Bonferroni multiple comparisons test. Data are mean ± SD, ns = p > 0.05, **p < 0.01, ***p < 0.001.

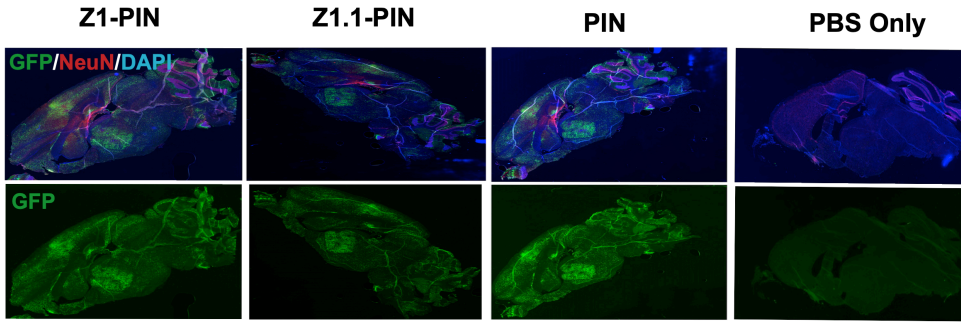

Figure 5. Expression of Z1-PIN *in vivo*. Transverse sections of the cerebral cortex from wildtype and BAC-C9-500 showing distribution of GFP-tagged ZFD con-structs. Scale bar = 1mm

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

Dr. Yeo presented this and other research in numerous public outreach talks, including the CIRM Bridges Trainee Meeting, July 26, 2022, aimed at promoting the participation of individuals representing the diversity of California's population.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Our data provides proof of principle that a member of a new class of zinc-finger RNA-targeting effectors can potentially treat C9-ALS, with implications for other neurodegenerative diseases such as frontotemporal dementia.

What was the impact on other disciplines?

We have shown proof-of-principle for an alternative and new strategy using human RNA-targeting zinc fingers that is in theory minimally immunogenic, biologically stable, can degrade both sense and antisense toxic transcripts, and can be encoded in adeno-associated viral (AAV) vectors allowing long-term continuous in-organism production of therapeutic materials. This design strategy of RNA-targeting zinc fingers has broad implications for the treatment of other neurodegenerative disorders as RNA repeat expansions in the C9ORF72 locus are also the most known cause of frontotemporal dementia, and has also been associated with Alzheimer's and Parkinson's disease pathogenesis.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

Publications, conference papers, and presentations

Nothing to report.

- **Journal publications.** The results of this study are currently under review at *Science*.

  Books or other non-periodical, one-time publications.

  Nothing to report.

Other publications, conference papers and presentations.

Dr. Yeo presented the results of this study at numerous symposia, conferences, and departmental seminars.

- **Website(s) or other Internet site(s)**

  Nothing to report.

- **Technologies or techniques**

  Zinc-finger targeting of RNAs

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Stefan Aigner
Project Role:	Project Scientist
Researcher Identifier (e.g. ORCID ID):	0000-0002-9511-3328
Nearest person month worked:	0
Contribution to Project:	Project coordination
Funding Support:	

Name	Mariana Bravo-Hernandez
Project Role	Postdoc
Researcher identifier	0000-0001-8762-0357
Nearest person month worked	0
Contribution to project	Animal husbandry, *in vivo* characterization
Funding support	

Name:	Esau Estrada
Project Role:	Technician
Researcher Identifier (e.g. ORCID ID):	0000-0001-7949-4151
Nearest person month worked:	2
Contribution to Project:	Stem cell culture
Funding Support:	

Name:	Megan Huang
Project Role:	Technician
Researcher Identifier (e.g. ORCID ID):	n/a
Nearest person month worked:	12
Contribution to Project:	Cell culture, molecular experiements
Funding Support:	

Name:	Nicole Lopez
Project Role:	Graduate student
Researcher Identifier (e.g. ORCID ID):	0009-0000-1226-2022
Nearest person month worked:	1
Contribution to Project:	Stem cell culture
Funding Support:	

Name:	Martin Marsala
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0001-5048-6422
Nearest person month worked:	0
Contribution to Project:	Lead of in vivo experiments
Funding Support:	

Name:	Kathryn Morelli
Project Role:	Postdoc
Researcher Identifier (e.g. ORCID ID):	0000-0002-3123-5369
Nearest person month worked:	4
Contribution to Project:	*In vivo* characterization
Funding Support:	

Name:	Hugo Medina Munoz
Project Role:	Postdoc

Researcher Identifier (e.g. ORCID ID): 0000-0003-0941-4106
Nearest person month worked: 0
Contribution to Project: Molecular cloning
Funding Support:

Name: Joshua Schwartz
Project Role: Postdoc
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 3
Contribution to Project: Stem cell differentiation
Funding Support:

Name: Anthony Vu
Project Role: Graduate student
Researcher Identifier (e.g. ORCID ID): 0000-0001-8922-6409
Nearest person month worked: 1
Contribution to Project: Stem cell differentiation
Funding Support:

Name: William West
Project Role: Bioinformatics Programmer
Researcher Identifier (e.g. ORCID ID): n/a
Nearest person month worked: 5
Contribution to Project: Bioinformatics analysis
Funding Support:

Name: Eugene Yeo
Project Role: PI
Researcher Identifier (e.g. ORCID ID): 0000-0002-0799-6037
Nearest person month worked: 0
Contribution to Project: Overall project lead
Funding Support:

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Changes are indicated relative to the 1-year annual report.

YEO

Closed:

Subcellular RNA dynamics in HD
Major Goals: We aim to define global mRNA localization landscapes in normal and Huntington's disease iPSC-motor and striatal neurons to evaluate mRNA localization in nuclear, cytoplasmic, and insoluble fractions.
Project Number: 2021-235103 (5022)
Name of PD/PI: Thompson
Source of Support: Chan Zuckerberg Initiative
Primary Place of Performance: University of California, San Diego
Project/Proposal Start and End Date: (MM/YYYY) (if available): 07/2021-06/2022
Total Award Amount (including Indirect Costs):
Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	00.12

Identification of HTT interaction partners
Major Goals: The goal of this project is to use state-of-the art mass spectrometry and cross-linking immunoprecipitation assays in pluripotent stem cell-derived striatal neurons to identify protein and RNA interaction partners of mutant and normal huntingtin, and to validate these in postmortem brain tissue.
Project Number: UCSD Proposal ID: 29557
Name of PD/PI: Yeo
Source of Support: CHDI Foundation
Primary Place of Performance: University of California, San Diego
Project/Proposal Start and End Date: (MM/YYYY) (if available): 11/2020-10/2022
Total Award Amount (including Indirect Costs):
Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2022	00.12

Effect of TDP-43 targeting biologics on stress granule formation
Major Goals: The Yeo lab will (1) generate lentiviral vectors from 4 proprietary plasmid constructs provided by ProMIS, and (2) perform stress granule resolution assays in iPSC-derived ALS and control motor neurons treated with the lentiviral vectors.

Project Number: UCSD #2020111 (Lab service agreement)
Name of PD/PI: Yeo
Source of Support: ProMIS Neurosciences, Inc, Canada (**FOREIGN ENTITY**)
Primary Place of Performance: University of California, San Diego
Project/Proposal Start and End Date: (MM/YYYY) (if available): 12/2021-12/2022
Total Award Amount (including Indirect Costs):
Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2022	00.01

Modulation of RNA subcellular localization for the treatment of neurological diseases

Major Goals: The goal of this project is to generate comprehensive maps of RNA localization in cultured neurons and brain tissue at subcellular resolution and transcript isoform specificity, relevant to Parkinson's disease.
Project Number: Roche ROADS Innovation Grant
Name of PD/PI: Yeo
Source of Support: F. Hoffmann – La Roche Switzerland (**FOREIGN ENTITY**)
Primary Place of Performance: University of California, San Diego
Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/2021-12/2022
Total Award Amount (including Indirect Costs):
Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2022	00.01

New:

Title: ***Recruitable and inducible tools to map protein-RNA interactions at scale***
Major Goals: In this NHGRI Genome Technology Program Opportunity Fund grant application, we propose to develop novel base editor technologies not addressed in the parent grant R01 HG011864.
Project Number: N/A
Name of PD/PI: Yeo, Kohli
Source of Support: NIH/NHGRI via the JAX TDCC
Primary Place of Performance: University of California, San Diego
Project/Proposal Start and End Date: (MM/YYYY) (if available): 03/2023-02/2024
Total Award Amount (including Indirect Costs):
Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2023	00.06

Title: ***"Mirror" cloaking of therapeutic payloads***
Major Goals: The goal of this grant is to develop "mirror" capsids for cloaking internal non-mirrored macromolecular payloads as therapeutic agents.
Project Number: N/A (Ion-ARPA Initiative grant)
Name of PD/PI: Devaraj
Source of Support: Ionis Pharmaceuticals
Primary Place of Performance: University of California, San Diego
Project/Proposal Start and End Date: (MM/YYYY) (if available): 04/202-03/2024
Total Award Amount (including Indirect Costs):
Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2023	00.12
3. 2024	00.12

Title: ***Total microbial knowledge of wastewater on a college campus: lessons for NWSS***
Major Goals: The goal of this contract is to perform a COVID-19 genomic sequencing project from wastewater sources to understand viral transmission dynamics. Dr. Yeo will be responsible for ensuring data quality of the test and sequence data.
Project Number: Proposal in response to BAA 75D301-22-R-72097
Name of PD/PI: Knight
Source of Support: U.S. Centers for Disease Control

Primary Place of Performance: University of California, San Diego
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2022-08/2024
Total Award Amount (including Indirect Costs):
Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2023	00.24
3. 2024	00.24

Title***: Mechanisms of A-I RNA editing-dependent mislocalization of TDP-43***
Major Goals: Dr. Yeo will act as a consultant and contribute his experience towards the data analysis and interpretation of the sequencing data generated from Drosophila CLIP-seq and RIP-seq experiments.
Project Number: R21 NS130492
Name of PD/PI: Sattler
Source of Support: NIH/NINDS
Primary Place of Performance: University of California, San Diego
Project/Proposal Start and End Date: (MM/YYYY) (if available): 12/2022-11/2024
Total Award Amount (including Indirect Costs):
Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2023	00.01
2. 2024	00.01

Title: ***Adopting genome-scale chromatin tracing in human tissues***
Major Goals: The aim of this grant is to validate and disseminate Genome-Scale Chromatin Tracing within our local genomic community. To enable this in a scalable manner with the prospect of a broad range of applications, the effort will be physically concentrated within the imaging core of the Center for Epigenomics. Dr. Yeo will be contributing computational efforts to standardize the tools used for chromatin imaging data visualization and exploration.
Project Number: N/A (CZI Advancing Imaging Through Collaborative Projects grant)
Name of PD/PI: Ren, Bintu
Source of Support: Chan Zuckerberg Initiative
Primary Place of Performance: University of California, San Diego
Project/Proposal Start and End Date: (MM/YYYY) (if available): 03/2023-02/2025
Total Award Amount (including Indirect Costs):
Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2023	00.12
2. 2024	00.12

Title: ***RNA granules as novel therapeutic targets in ALS/FTD***
Major Goals: The goal of this grant is to generate safety and efficacy data in mice for candidate therapeutics targeting stress granule components, relevant to ALS
Project Number: N/A (Target ALS Industry-Led Consortium grant)
Name of PD/PI: Lewcock
Source of Support: Target ALS Foundation
Primary Place of Performance: University of California, San Diego
Project/Proposal Start and End Date: (MM/YYYY) (if available): 04/2023-03/2025
Total Award Amount (including Indirect Costs):
Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2023	00.12
2. 2024	00.12

Title: ***Ribo-STAMPEDE: novel tools for molecular profiling of brain cell types***
Major Goals: In this project, we develop new modalities of our STAMP method (Surveying Targets by APOBEC-Mediated Profiling) to enable cell-type and temporal resolution, with a particular focus on applications relevant to neuroscience.
Project Number: RF1 MH126719-01A1
Name of PD/PI: Lippi, Yeo
Source of Support: NIH/NIMH
Primary Place of Performance: University of California, San Diego
Project/Proposal Start and End Date: (MM/YYYY) (if available): 08/2022-07/2025
Total Award Amount (including Indirect Costs):
Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2024	00.91
3. 2025	00.91

Title: ***The Diversity and Science Lecture (DASL) Series and Platform***
Major Goals: Dr. Yeo received funds to support work on project 'Diversity and Science Lecture (DASL) Series and Platform' to support the Diversity and Science Lecture Series project (DASL), a trainee-led effort to elevate the science of student and postdoctoral life science researchers from underrepresented backgrounds. Funding supports administrative costs, speaker honoraria and similar efforts led at other institutions.
Project Number: 2021-238695
Name of PD/PI: Yeo
Source of Support: Chan Zuckerberg Initiative via the Silicon Valley Community Foundation
Primary Place of Performance: University of California, San Diego
Project/Proposal Start and End Date: (MM/YYYY) (if available): 11/2021-10/2023
Total Award Amount (including Indirect Costs):
Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2023	00.01

MARSALA

Closed:

Cervical SCI cell-grafting efficacy study
Major Goals: Characterization of treatment effect after spinal human oligodendrocyte cell grafting in rats with spinal cervical traumatic injury.
Project Number: 306395-00001
Name of PD/PI: Marsala, Martin
Source of Support: Lineage
Primary Place of Performance: UCSD
Project/Proposal Start and End Date: 03/2021 – 03/2022
Total Award Amount (including Indirect Costs):
Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	variable

Disease mechanism in ALS and frontotemporal dementia
Major Goals: In this project, we uncover how mutation in these genes triggers either disease. Key questions to be tackled will be determining how mutation in more than one gene drives either disease, how partial inactivation of one specific gene results in ALS/FTD, and whether (and if so, how) there is disease- causing damage that spreads from cell to cell.
Project Number: R01 NS027036
Name of PD/PI: Cleveland
Source of Support: NIH/NINDS
Primary Place of Performance: UCSD
Project/Proposal Start and End Date: 04/1989 – 08/2022
Total Award Amount (including Indirect Costs): (total for FY2022)
Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	variable

New:

Spinal Subpial Gene Delivery for Treatment of Amyotrophic Lateral Sclerosis
Major Goals: In this project, we test the treatment potency of a novel spinal cord gene delivery in the treatment of ALS. Effect of mutated gene (mSOD1) silencing will be tested in rat G93A model of ALS and the treatment potency defined by the degree of neurological function protection and long-term animal survival.
Project Number: R01 NS130104
Name of PD/PI: Marsala, Martin
Source of Support: NIH/NINDS
Primary Place of Performance: UCSD
Project/Proposal Start and End Date: 09/2022 – 07/2027
Total Award Amount (including Indirect Costs): (total for FY2022)
Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	variable

What other organizations were involved as partners?

Organization Name: University of Utah
Location of Organization: (if foreign location list country): Salt Lake City, UT
Partner's contribution to the project: Collaboration

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

N/A

QUAD CHARTS:

N/A (final

report)

9. APPENDICES:

N/A