Response to Review Summary for DISC4-19391
Project Title: Dissecting cell-specific genetic and molecular drivers of amyotrophic lateral sclerosis (ALS) for therapeutic insights
PI team: Michael Snyder, Anshul Kundaje, Melissa Gymrek, Justin Ichida, John Ravits
Key Persons: Jonathan Cooper-Knock, Sai Zhang, David Dulcis, Thomas Karathanos

We thank the reviewers for their thoughtful evaluation and suggestions. We are delighted that the review received a positive appraisal noting, "Overall, this is a fundable, well-developed, ambitious proposal led by leading experts in their respective areas of research. The preliminary data support feasibility, and the project is well-positioned for rapid translation given the stepwise study design and consideration of patient subtypes," and "The project has significant potential for impact. It tackles a major challenge in ALS therapeutics: the unknown genetic drivers of sporadic ALS and incomplete mechanistic understanding of the disease. Successful completion would represent a paradigm shift in understanding ALS etiology."

Reviewers noted weaknesses that we can easily address as follows:

1. Reviewers questioned the feasibility of obtaining diseased donor tissue. **Critique 1:** "This reviewer could not find information on data access routes/agreements for sources from other PI/consortia and sparse information on agreements or routes for accessing biological materials." **Critique 2: "**Feasibility and access to key resources are of key importance. The rationale depends entirely on acquiring 200 high-quality samples from 50 donors from two biobanks. Access and availability are subject to official review."

Response - We appreciate the opportunity to provide more detail on our biospecimen plans. The Ravits lab at UCSD is one of the founding members of the Target ALS CNS biorepository and currently contains 158 CNS (brain and spinal cords) from ALS and non-ALS banked by rapid autopsy and stored in frozen and formalin-fixed formats for correlating structural (in formalin tissues) and functional (from frozen tissues) and will readily meet the demands of the study. As backup, the Target ALS biorepository (targetals.org/research/human-postmortem-tissue-core/) of which the Ravits lab is a member contains 602 CNS from SALS, FALS, and control (about 443 additional cases) which have been collected specifically to support and advance research studies such as the one proposed here. The repostory wasfunded by philanthropy with pre-existing pre-negotiated material transfer agreements which have no encumbrance of intellectual property or discoveries.

2. Reviewers questioned the rationale for including the occipital cortex in our single cell profiling. **Critique 1:** "Despite the high potential impact of this proposal, a more refined strategy focusing on brain regions that will be most informative (are novel results anticipated for the occipital cortex or could that be removed?) would allow for a more thorough, focused investigation while keeping costs similar and enhance likelihood of success." **Critique 2:** "The choice of the occipital cortex as a control region is not justified, and the risk of low neuronal yield from some regions is not addressed."

Response: Use of occipital cortex as a control associated with lower burden of ALS-associated TDP-43 pathology [10.1002/ana.23937] is following a precedence in the literature [10.1186/s13024-025-00820-5] although we note that this study actually identified transcriptome evidence of TDP-43 loss of function in the occipital cortex which is poorly understood. The fact that ALS patients manifest molecular evidence of TDP-43 dysfunction in the occipital cortex without significant clinical symptoms suggests that much can be learned from this region about mechanisms of neuroprotection from the pathology of ALS. Given the large degree of heterogeneity of human samples from biological, anatomical and technical aspects, the occipital cortex (spared in ALS as noted) provides a valuable data set to help interpret all results, including post-hoc analyses and generation of hypotheses. We believe the occipital regions will render significant neuron numbers, but from neuron populations with different disease vulnerabilities.

3. The reviewers suggested long-read sequencing for the whole genome analysis as opposed to the short-read sequencing proposed. **Critique 1**: "For a project emphasizing tandem repeat biology, long-read sequencing is the gold standard for accurate genotyping."

Response: We appreciate this suggestion and agree. We will modify our plan to instead perform long-read WGS.

4. Reviewers requested justification for proposed sample sizes. **Critique 1:** “Power calculations are essentially absent. For example, the n=50 for multi-omics discovery is based on consultation with Target ALS. The statistical methods proposed for tissue validation (Aim 4) appear limited (t-tests, ANOVAs) and do not consider critical covariates.”

Response: We appreciate this question. For aim 1: Pilot data presented for Aim 1 is based upon our own work where we have performed a 10x Multiome study of human motor cortex consisting of 548,323 high-quality nuclei derived from 46 ALS/FTD patients of whom 19 carry a G4C2-repeat expansion of C9ORF72; and 25 age and sex matched controls. In this dataset, which is smaller than the one we propose to create, we have been able to identify cell-type specific differentially expressed genes (DESeq2), differentially accessible regions (Signac) and changes in gene regulatory networks (scDORI, DOI:10.1101/2025.05.13.653733) after stringent FDR multiple testing correction. Based upon this study, and other similar studies in the literature [e.g. DOI:10.1038/s41467-026-69944-6; 10.1016/j.cell.2024.02.031], we propose that we will have sufficient statistical power to identify disease associated changes.

For Aim 4: While our preliminary power analysis (based on large effect sizes in focused studies) suggests N=8-15 per group is sufficient for high-contrast proteinopathy markers, our study is uniquely positioned to leverage a much larger cohort of N=140 for the final validation of the 10 candidate genes. This larger sample size allows us to detect subtle (small-to-medium) effect sizes ($d<0.5$) with >80% power, which is critical for identifying 'upstream' molecular changes in relatively preserved anatomical regions where pathology may be less overt. We agree that the complexity of human tissue requires rigorous control of confounding variables. Our revised statistical framework will utilize Multivariate Regression and Linear Mixed-Effects Models (LMMs). This allows us to treat 'Donor ID' as a random effect—accounting for multiple measurements per patient—while including Age, Sex, PMI, and Genotype as fixed-effect covariates. Furthermore, we will use 'Anatomical Region' and 'TDP-43 Pathological Grade' (0-4 scale) as interaction terms to formally test our hypothesis that candidate gene expression correlates with the 'upstream' or 'downstream' status of the tissue.

5. Reviewers highlighted the predominance of donors of European ancestry in the Project MinE resource. **Critique 1:** “The proposal fails to address the demographic composition of the crucial 50-donor post-mortem cohort or the existing datasets (e.g. Project MinE). This omission significantly limits the generalizability of the findings.”

Response: Project MinE data freeze 3 consists of ~12,000 WGS samples and the forthcoming WGS data release from ALS Compute includes 40,494 WGS (19,704 ALS and 7,657 healthy controls). Both datasets are enriched for Europeans but include samples from diverse populations; Project MinE now includes an entire working group dedicated to recruiting ALS researchers and patients from under-represented populations. We will continue to update our analyses in Aim 2 to incorporate these datasets.

6. Reviewers sought greater explanation for usage of clinical data in the analysis. **Critique 1:** “Given that the outcome is to identify genetic drivers associated with ALS risk and severity, how will clinical features be integrated and how are they expected to affect findings? What is the anticipated impact of onset segment (note: proposal indicates “favor limb onset over limb onset” so effect of onset segment is not clear and relevance to design is not discussed). Are differences expected given that postmortem tissues likely all represent late-stage disease?” **Critique 2:** “Is FTD status known for participants selected for Aims 1 & 2, and how is this expected to affect the analysis results for the different tissues (e.g., frontal cortex)?”

Response - One of the key clinical aspects of ALS is the heterogeneity of clinical phenotypes. Three main determinants of this are: (1) site of onset (arm, leg, trunk or bulbar), (2) variable degrees of degeneration of upper motor neurons in cortex and lower motor neurons in brainstem and spinal cord, and (3) rates of progression. Knowing these phenotypes (along with standard histological/neuropathological phenotyping of the tissues) will allow important ways to stratify the -omics results and uncover more direct and meaningful clinical-neurobiologic correlation. The co-occurrence of ALS and FTD is primarily ascertained by clinical judgement by the neurologist (most ALS patients do not undergo formal neuropsychological testing) and these are recorded in the phenotype, albeit inexactly. A secondary level of identification is neuropathological (“FTLD”) (admitting that the neuropathological distinctions between ALS, ALS-FTLD-TDP-43, and FTLD-TDP-43 are not

clear.) This will add further ability to stratify and explore the data (post-hoc analyses) and generation of hypotheses.

7. Reviewers sought greater explanation for development of the models in Aim 2b. **Critique 1: "**There is little information on how the VMIS score is going to be developed, lacking specifics on model integration, benchmarking, and validation. Developing a useful variant score is a very challenging undertaking so the lack of detail and dedicated time and resource for this questions the feasibility of this objective."

Response - We appreciate this concern. In Aim 2b, we generate modality-specific predictions for each prioritized SNV, indel, or TR across ALS-relevant cell types and regulatory contexts, including Δ-accessibility from variant-aware ChromBPNet, Δ-expression from variant-aware Borzoi, and Δ-splicing/Δ-APA from splice- and APA-aware Borzoi heads. These component models are benchmarked independently using held-out chromosomes and concordance with observed genetic effects, including caQTL and eQTL effect size and direction. More broadly, our groups have already developed and extensively benchmarked related model classes for QTL detection and ranking, prioritization of fine-mapped GWAS variants, rare disease variant prioritization across diverse cellular contexts, and cross-species generalization of sequence perturbation effects. These benchmarking frameworks are already in active use by the community.
Importantly, VMIS is intended here as a practical prioritization framework for experimental follow-up in this specific ALS setting, not as a universal variant scoring system. The reviewers are correct that building a fully generalizable variant impact score would require substantially broader resource generation and validation than is in scope for this project. Our objective is instead to construct a transparent and well-calibrated score that is fit for purpose in the context of this study.

The implementation is straightforward. For each variant×context, we convert modality-specific predictions into standardized evidence components, including signed effect size, calibrated uncertainty, agreement across modalities, fine-mapping support, QTL colocalization, and convergence of implicated target genes on known or plausible ALS-relevant genes and pathways. We then combine these normalized components using a transparent weighted linear score, such that stronger and more concordant evidence yields a higher VMIS. In this formulation, VMIS is primarily a ranking tool for downstream experimental validation. We will enrich validation experiments for high-VMIS variants to maximize the probability of success, while also sampling variants and genes across the broader score distribution. This design will allow us to empirically assess the contribution and calibration of each score component and, if supported by the validation data, subsequently fit a regularized meta-model that further improves ranking based on observed experimental outcomes.

8. Reviewers noted limited detail on our plans for outreach. **Critique 1:** "Outreach and partnership components are minimal"

Response: We appreciate the opportunity to clarify our plans for outreach. As one of the reviewers noted, "Translational aspects of the proposal and letters of support reflect partnerships that will support future translation." In addition to structuring Aims 3-5 around iterative refinement in the evaluation of therapeutic targets and antisense oligonucleotides to target them, we also value insights from industry. Dr. Ichida, a Co-I on this proposal, is a co-founder of two biotech companies. He regularly attends conferences with industry leader participants, such as the NEALS Workshop on Advancing ALS Clinical Trials, the Drug Information Association (DIA) annual meeting, the annual Muscular Dystrophy Association meeting, the annual Target ALS meeting, the annual Packard ALS meeting, and the annual California ALS Research Summit, and will present on the findings of this project and learn from advancements in bio-tech.

We also value outreach to the ALS patient community. Dr. Ravits, Co-I on this proposal, is the director of the ALS clinic at the University of California, San Diego, overseeing a team of clinicians and implementing clinical trials, who will bring perspective on the unmet needs in clinical management and treatment for ALS. Both Dr Ravits and Dr. Cooper-Knock (key person) regularly attend the annual California ALS Research Summit (Jan), annual Packard Center ALS Research Symposium (March), annual Target ALS Meeting (May), and annual International ALS MND symposium (December). We regularly participate in community-led initiatives such as sharing research insights and receiving feedback from patients and family members via ALS Untangled (https://www.alsuntangled.com/), and 'I AM ALS' (https://www.iamals.org/). They will continue to participate in these meetings to seek feedback on the project.

Please see as well a letter of support from ALS Network.

March 18, 2026

CIRM Application Review Subcommittee (ARS)
Re: Application# DISC4-19391

"Dissecting cell-specific genetic and molecular drivers of amyotrophic lateral sclerosis (ALS) for therapeutic insights"

Dear CIRM Application Review Subcommittee,

I am writing to offer my strongest support for the DISC4 application entitled *"Dissecting cell-specific genetic and molecular drivers of amyotrophic lateral sclerosis (ALS) for therapeutic insights"* (DISC4-19391).

I serve as President and CEO of the ALS Network, a California-based nonprofit organization dedicated to improving the lives of people affected by ALS through comprehensive care services, advocacy, and the acceleration of research and therapeutic development. California is home to the largest population of people living with ALS in the United States, and the ALS Network supports thousands of individuals and families across the state each year. In parallel with our care programs, we invest in and catalyze research collaborations nationally and globally, giving us a deeply informed perspective on the most urgent scientific gaps that must be addressed to advance effective therapies.

One of the most critical barriers in ALS therapeutic development is our limited understanding of the genetic and molecular drivers of sporadic ALS, which accounts for approximately 85-90% of all ALS cases. While research has revealed important insights into familial forms of the disease, the genetic mechanisms underlying the vast majority of ALS cases remain poorly understood. Closing this knowledge gap is essential to identifying actionable therapeutic targets that could significantly benefit the broader ALS population.

The DISC4-19391 proposal addresses this challenge through a highly innovative and multidisciplinary strategy that integrates machine learning, large-scale genetic analysis, neuropathology, and stem cell-based functional screening to identify genetic drivers of ALS. The distinguished investigative team, including Michael Snyder (Stanford, Lead PI), Justin Ichida (USC), John Ravits (UCSD), Melissa Gymrek (UCSD), and their collaborators, brings together exceptional expertise spanning computational biology, human genetics, neuropathology, and stem cell biology. Importantly, this collaboration connects leading institutions across California and leverages the state's extraordinary biomedical

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The significance of this work is strongly supported by the ALS Network's Scientific Advisory Committee, which includes internationally recognized experts in ALS research and clinical care. In our collective view, initiatives that combine advanced computational methods with stem cell-based functional discovery represent one of the most promising paths toward identifying new therapeutic targets for sporadic ALS, the form responsible for the overwhelming share of cases and representing one of the most urgent unmet challenges in ALS research.

By illuminating the genetic and molecular drivers of sporadic ALS, this project has the potential to generate a pipeline of novel targets for therapeutic development that could ultimately benefit people living with ALS in California and around the world. In addition, the proposed work will advance the broader field of neurodegeneration research and further strengthen California's leadership in stem cell science and biomedical innovation.

For these reasons, I strongly encourage the committee to support funding for this outstanding and timely proposal. The discoveries enabled by this work could play an important role in transforming our understanding of ALS and accelerating the development of meaningful treatments for patients who urgently and desperately need them.

Sincerely,

Sheri Strahl, MPH, MBA
President & CEO
ALS Network