**Significance of the work**

The ongoing work is crucial for the advancement of antiviral drug development against a backdrop where rapid responses to emerging viral threats are increasingly necessary. By perfecting iPSC-derived models for high-throughput screening, we're paving the way for the discovery and optimization of antiviral compounds with greater efficiency and cost-effectiveness. This work directly addresses the urgent need for innovative approaches to antiviral therapy, particularly in the face of pandemics.

Significant project-generated resources

1. Enhanced iPSC-derived cell models optimized for antiviral drug screening.
2. Streamlined pneumocyte differentiation protocols that reduce cost and time.
3. RNA-seq and single-cell RNA-seq data sets characterizing cellular responses post-viral infection.
4. Adapted protocols for medium-throughput antiviral assays in 96-well formats.

**A. Specific Aims for the MP/DRP** (1 page max)

If the Specific Aims have not been modified, state “The Specific Aims have not been modified from the original, competing application.”. If they have been modified, give the revised aims and the reason for the modification.

The Specific Aims have not been modified from the original, competing application.

**B. Studies and Results** (5 pages max)

Describe the studies directed toward specific aims during the current budget year and the positive and negative results obtained. If applicable, address any changes to the innovative potential of the project. If technical problems were encountered in carrying out this project, describe how the approach was modified.

During the current budget year, we focused on five specific areas to support the Specific Aims:

* Optimization of Cell Culture Protocols: We refined the differentiation protocol for type II pneumocytes, enhanced culture efficiency with various substrates, and reduced costs by adjusting cytokine application.
* Inclusion of Additional iPSC Lines: We integrated a new iPSC line (tz14), which demonstrated our protocol’s robustness and potential for widespread application.
* Comprehensive Cell Characterization: Through RNA-seq and single-cell RNA-seq, we established a cellular signature that indicates susceptibility to SARS-CoV-2 infection and analyzed the expression profiles post-infection.
* Expansion of Differentiable Cell Types: We successfully broadened our scope by optimizing differentiation protocols for various cells, including neurons and macrophages, and initiated their infection with multiple viruses.
* **Readiness for Antiviral Drug Screening: We have prepared our iPSC-derived cell models for automated, medium-throughput antiviral drug screening.**

**Challenges and Innovations**:

We faced challenges in ensuring model fidelity and assay scalability. These were addressed by validating our models against primary tissues and conducting rigorous optimization and standardization for high-throughput formats.

**C. Significance** (1 page max)

The accomplishments to date have significant implications for both the scientific community and public health. The optimized protocols for iPSC-derived cells and their readiness for drug screening have the potential to significantly shorten the drug discovery pipeline. Moreover, the comprehensive cell characterization has provided valuable insights into the cell-specific mechanisms of viral infection and replication, which could lead to targeted therapies and vaccines.

**D. Plans** (1 page max)

Summarize plans to address the Specific Aims during the next year of support. Include any important modifications to the original plans.

In the forthcoming year, we plan to:

* **Expand Viral Infection Studies**: We will assess infectivity and viral replication in additional iPSC-derived cell lines to encompass a broader range of viruses.
* **Characterize New Cell Lines**: We will perform detailed analyses of the new iPSC lines, focusing on expression patterns and viral replication capabilities.
* **Optimize Protocols for High-Throughput Screening**: We will refine our protocols for both 96- and 384-well formats, ensuring our cell models are prepared for extensive antiviral drug testing.

**Training and Professional Development (For Mentored Projects only)**

Describe training and professional development activities during the previous funded year of the award.

Not applicable as this is not a mentored project.