

# Establishing iPSC Lines From PBMCs of African Centenarians for Neuroscience and Biomedical Research

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A. I. Alkhamis<sup>1,2,5</sup>, I. G. Bako<sup>2</sup>, Umar Adam<sup>2</sup>, Larema Baba Zau<sup>1,4</sup>, Muhammad Mustapha<sup>1</sup>, Muktar Lawan<sup>1</sup>, H. S. Kwairanga<sup>1</sup>, Mubarak Isamil<sup>1</sup>, Dawoud Usman<sup>1</sup>, Zaid Muhammad<sup>1,3</sup> Mahmoud Bukar Maina<sup>1,3</sup>

1-Biomedical Science Research and Training Center, Yobe State University, Damaturu

2-Ahmadu Bello University, Zaria

3-Sussex Neuroscience, University of Sussex, UK

4-Yobe State University Teaching Hospital, Damaturu

5-Human Physiology Department, Yobe State University, Damaturu

## Introduction

Induced pluripotent stem cell (iPSC) technology has revolutionized disease modeling by enabling the generation of patient-specific cell lines for studying neurodegenerative disorders and developing precision therapeutics (Takahashi et al., 2007; Shi et al., 2017). However, identifying appropriate control iPSC lines for late-onset neurodegenerative diseases remains a major challenge because healthy control individuals may still harbor genetic variants that predispose them to future disease. Consequently, iPSC lines derived from exceptionally healthy individuals, such as centenarians ( $\geq 100$  years), may provide more suitable reference models with a lower likelihood of developing age-related neurodegenerative conditions (López-Otín et al., 2023).

Centenarians exhibit exceptional longevity and resilience against many age-related diseases, making them an invaluable population for investigating protective genetic, molecular, and environmental mechanisms associated with healthy aging (Sebastiani & Perls, 2012; Franceschi et al., 2018). Generating iPSC lines from the peripheral blood mononuclear cells (PBMCs) of African centenarians offers a unique opportunity to establish biologically relevant control models while addressing the significant underrepresentation of African populations in stem cell resources and genomic research. These models will facilitate investigations into mechanisms of aging, neuroprotection, and neurodegenerative disease within diverse genetic backgrounds (Bentley et al., 2020).

By expanding iPSC biobanks to include African genetic diversity, this study aims to improve our understanding of genetic variation influencing cellular function, disease susceptibility, and therapeutic responses, thereby contributing to more inclusive and globally representative biomedical research (Bentley et al., 2020; Sirugo et al., 2019).

## Methods

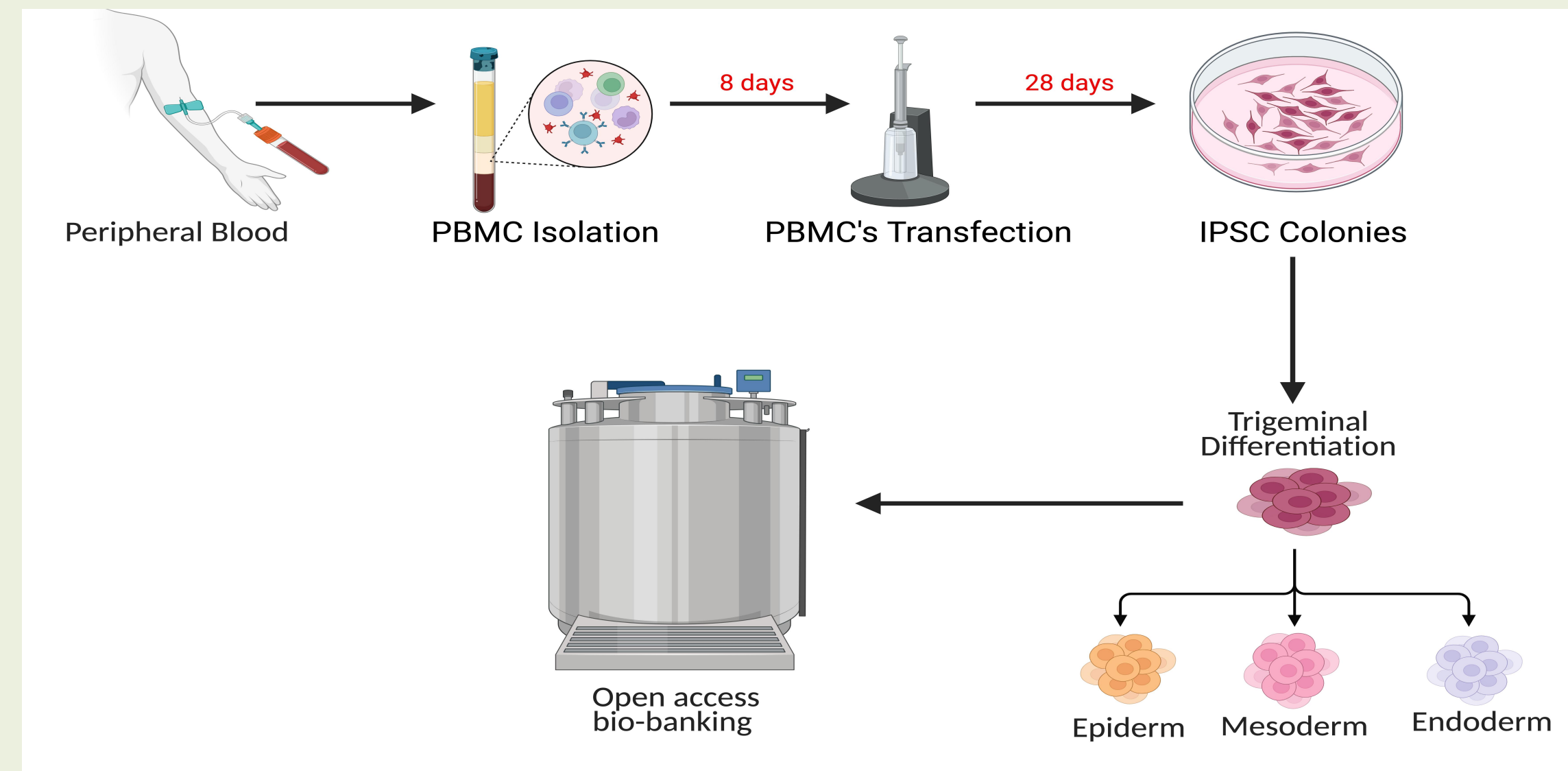


Figure 1: Protocols Showing Isolation, Pre-enrichment, Expansion of PBMCs, Mycoplasma Detection, Reprogramming and Differentiation of iPSCs

## Results

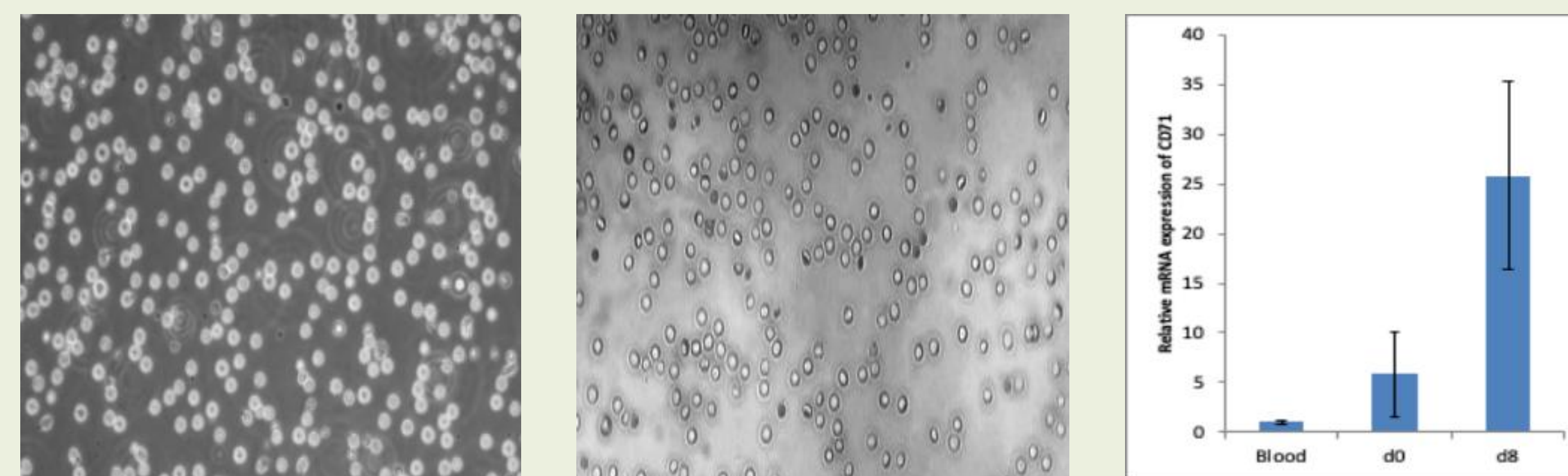


Figure 2: Showing Phase contrast photomicrographs of Peripheral Blood Mononuclear Cells and qPCR results showing the relative mRNA expression of CD71 after 8 days of expansion of PBMCs to Erythroid Progenitor Cells

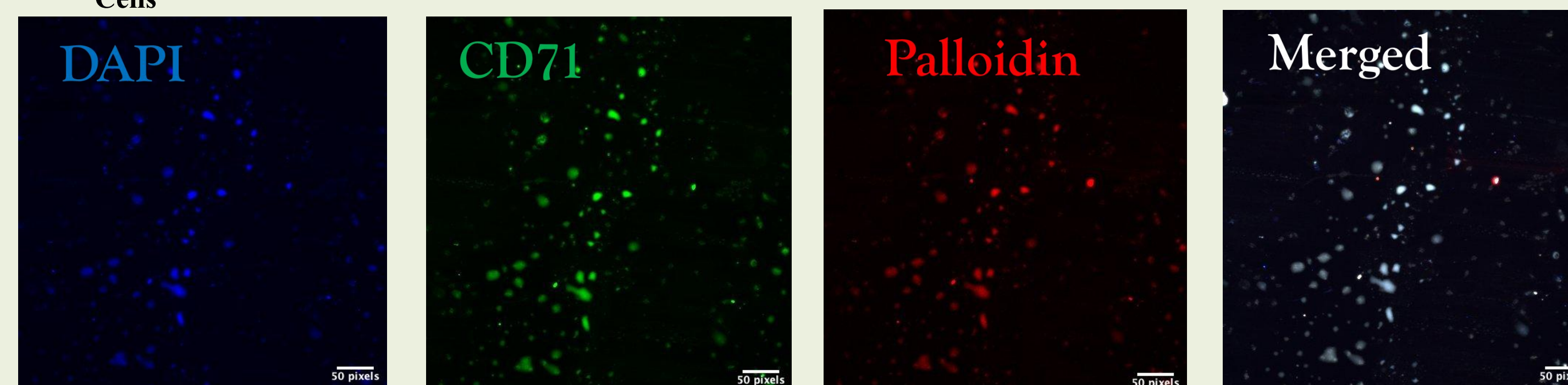


Figure 3: Confocal images showing DAPI (nuclei), CD71 (proliferation), and phalloidin (cytoskeleton) in erythroid progenitor cells derived from PBMCs on days 0 and 8.

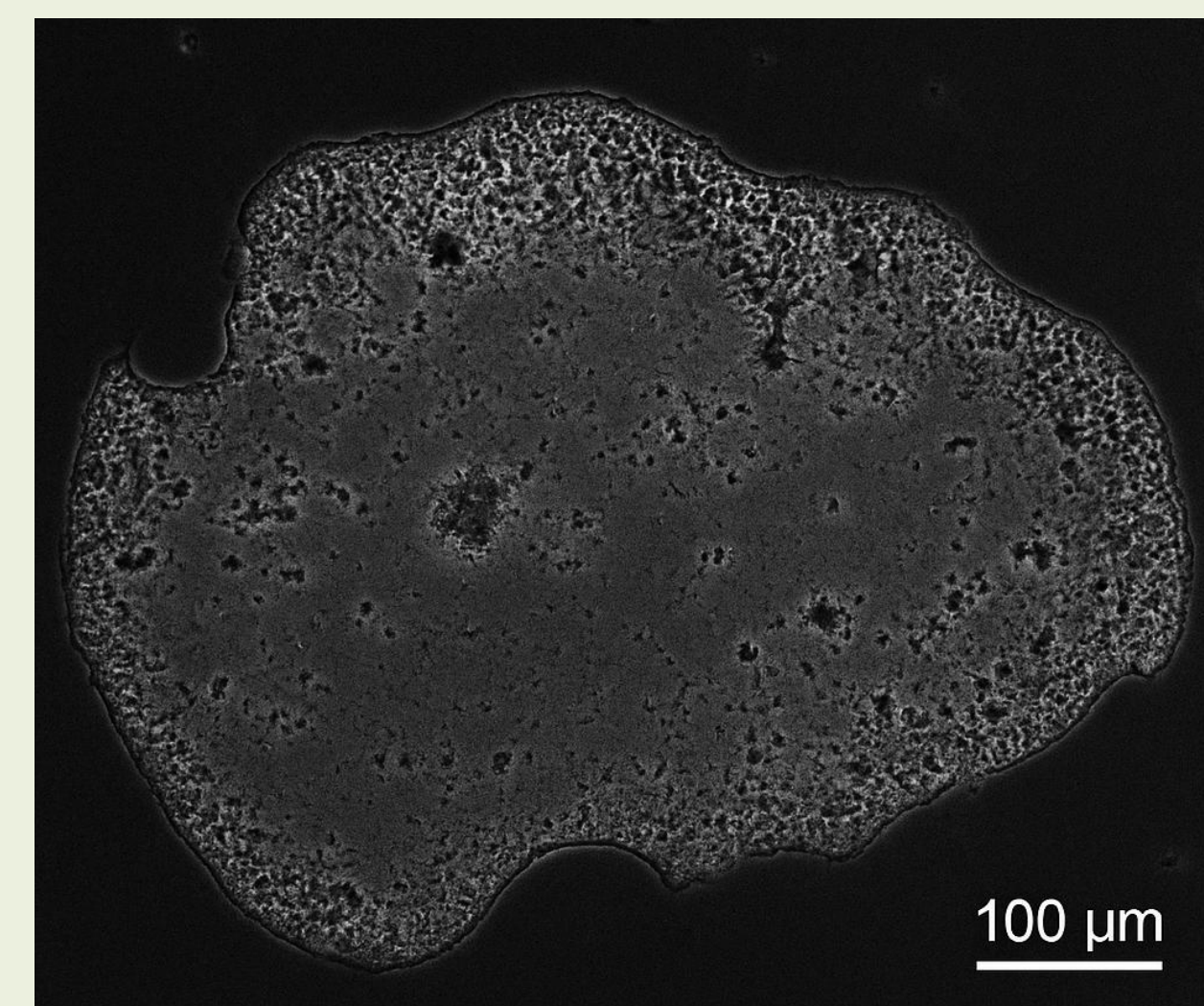


Plate I: Phase-contrast image (10 $\times$ ) of iPSC colonies derived from centenarian PBMCs after 28 days of reprogramming, showing compact colonies with smooth borders (red arrows) and typical pluripotent morphology.

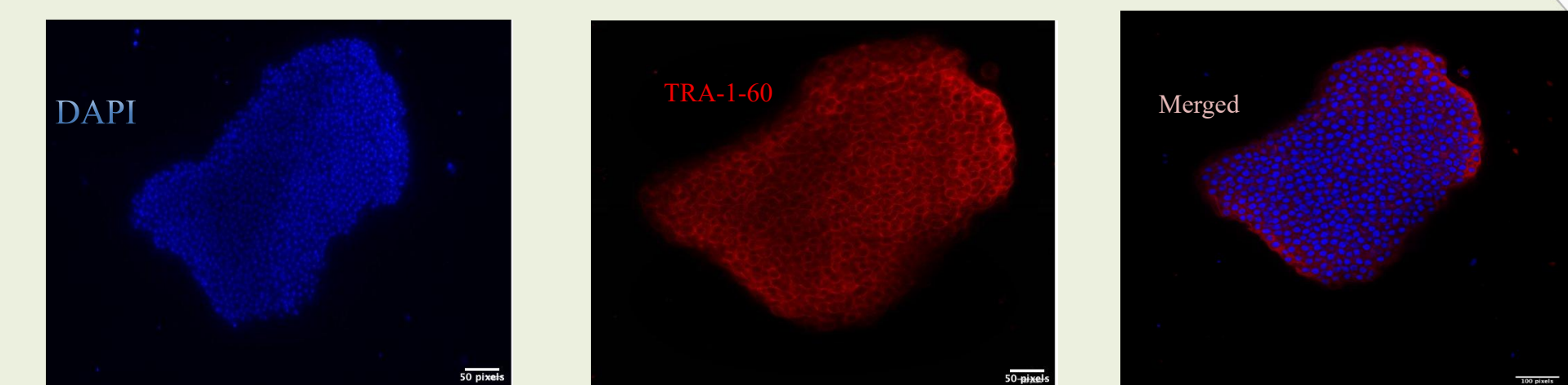


Plate II: Confocal images of iPSCs stained with DAPI and TRA-1-60 showing high nuclear-to-cytoplasmic ratio, strong TRA-1-60 expression, and merged images confirming typical pluripotent colony morphology.

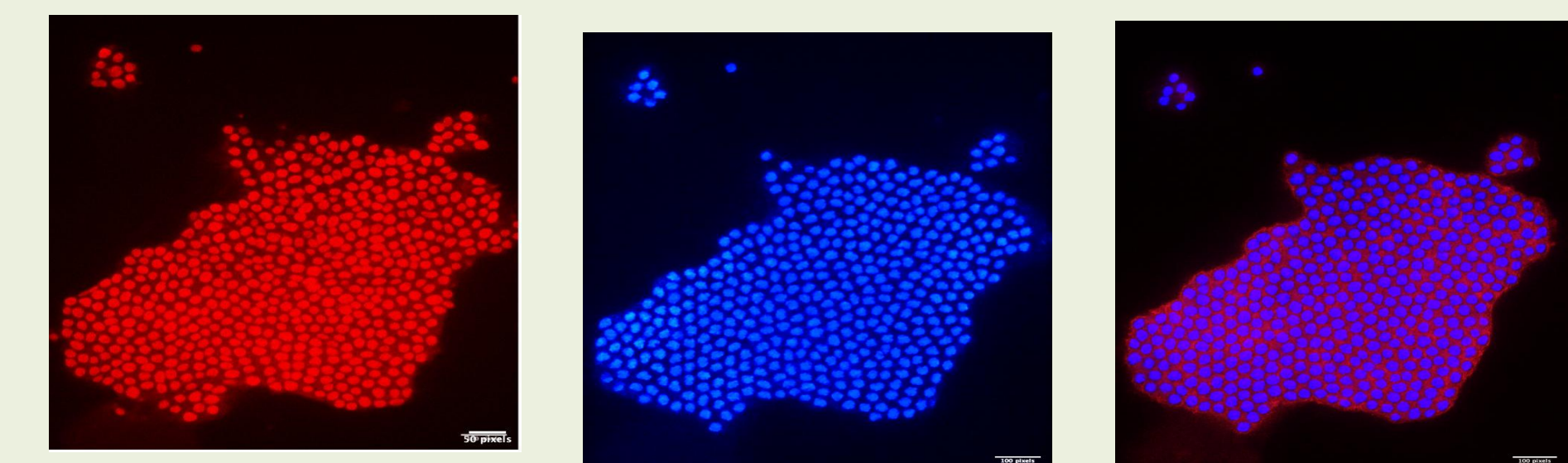


Plate I: Immunofluorescence of centenarian-derived iPSCs showing nuclear OCT4 (red) and DAPI (blue), confirming pluripotency and typical iPSC morphology.

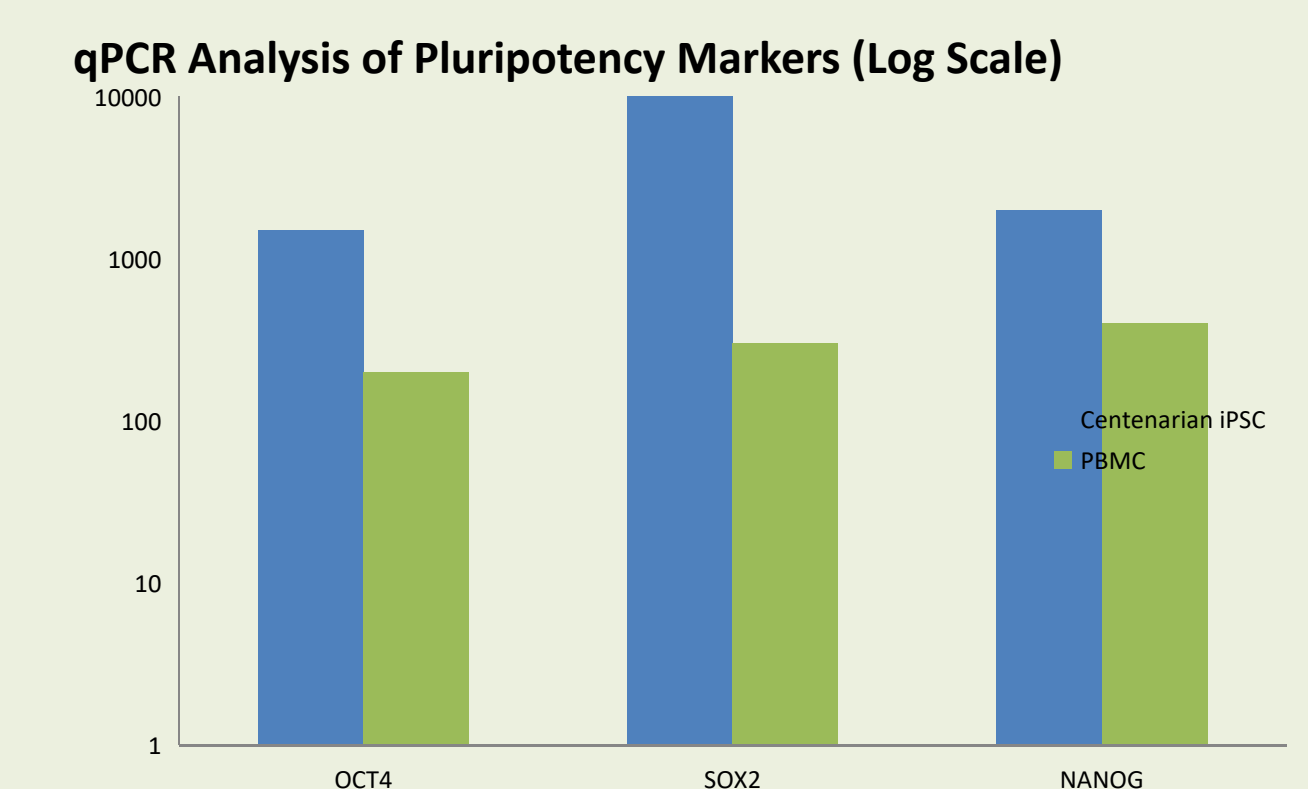
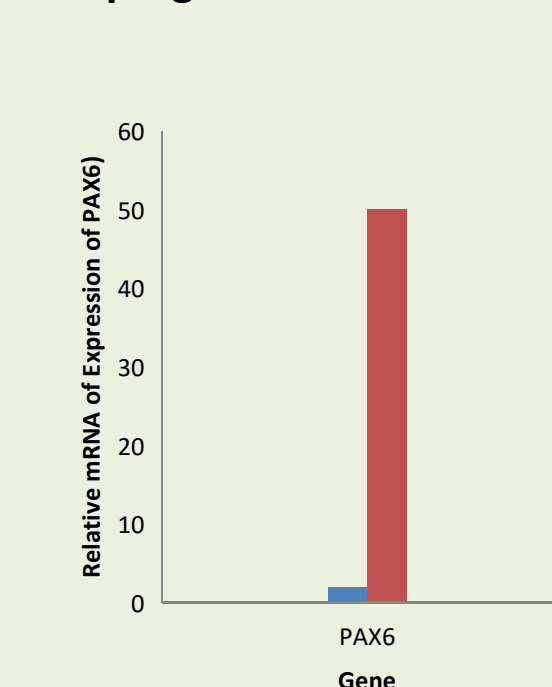
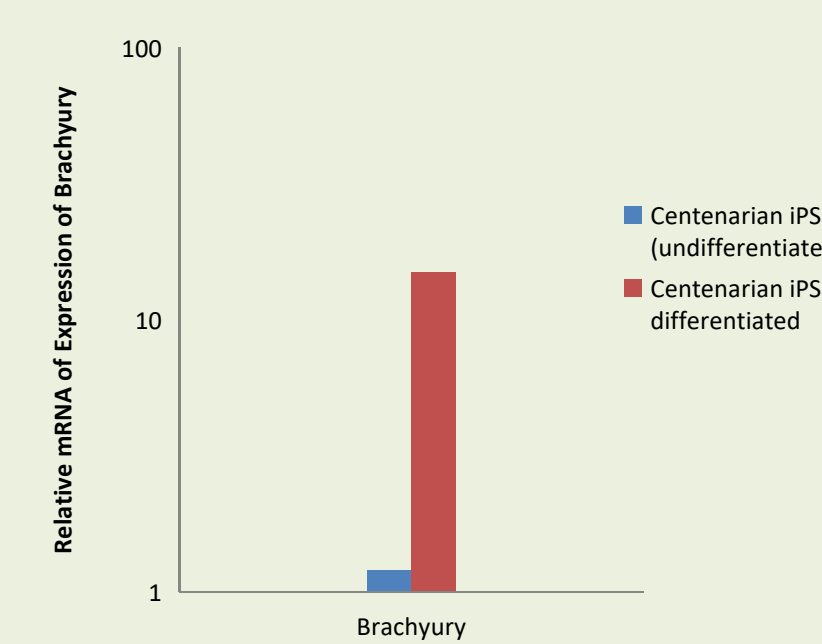


Plate I: qPCR confirmed significant upregulation of OCT4, SOX2, and NANOG in centenarian-derived iPSCs relative to PBMCs, indicating successful reprogramming.

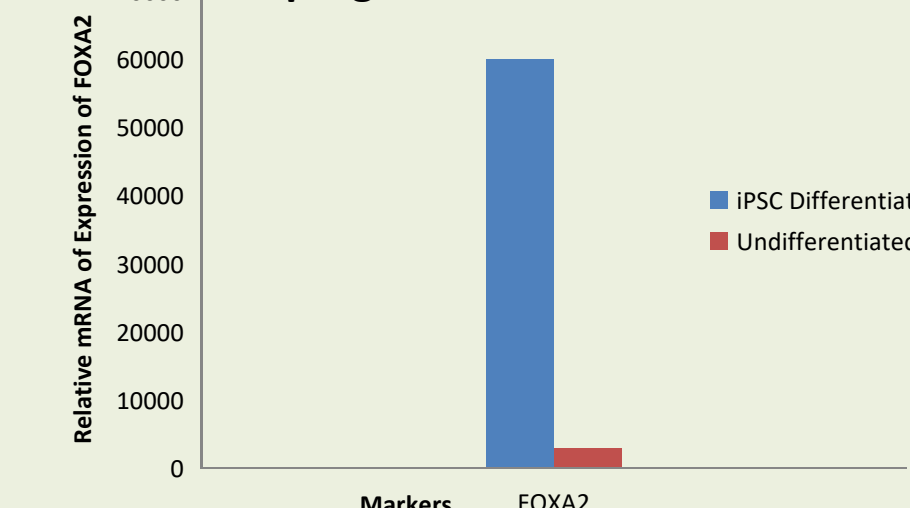
### Upregulation of ectodermal markers



### Upregulation of Mesodermal Markers



### Upregulation of endodermal markers



Figures 9–11: Directed differentiation of centenarian-derived iPSCs resulted in significant upregulation of the ectodermal (PAX6), mesodermal (Brachyury/T), and endodermal (FOXA2) lineage markers compared with undifferentiated controls (all  $p \leq 0.05$ ), confirming successful trilineage differentiation.

## Future Directions

STR profiling and karyotyping will be performed to fully characterize the centenarian-derived iPSC lines, which will then be banked and differentiated into neural lineages for disease modeling and shared through the African iPSC initiative.

## Acknowledgements



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