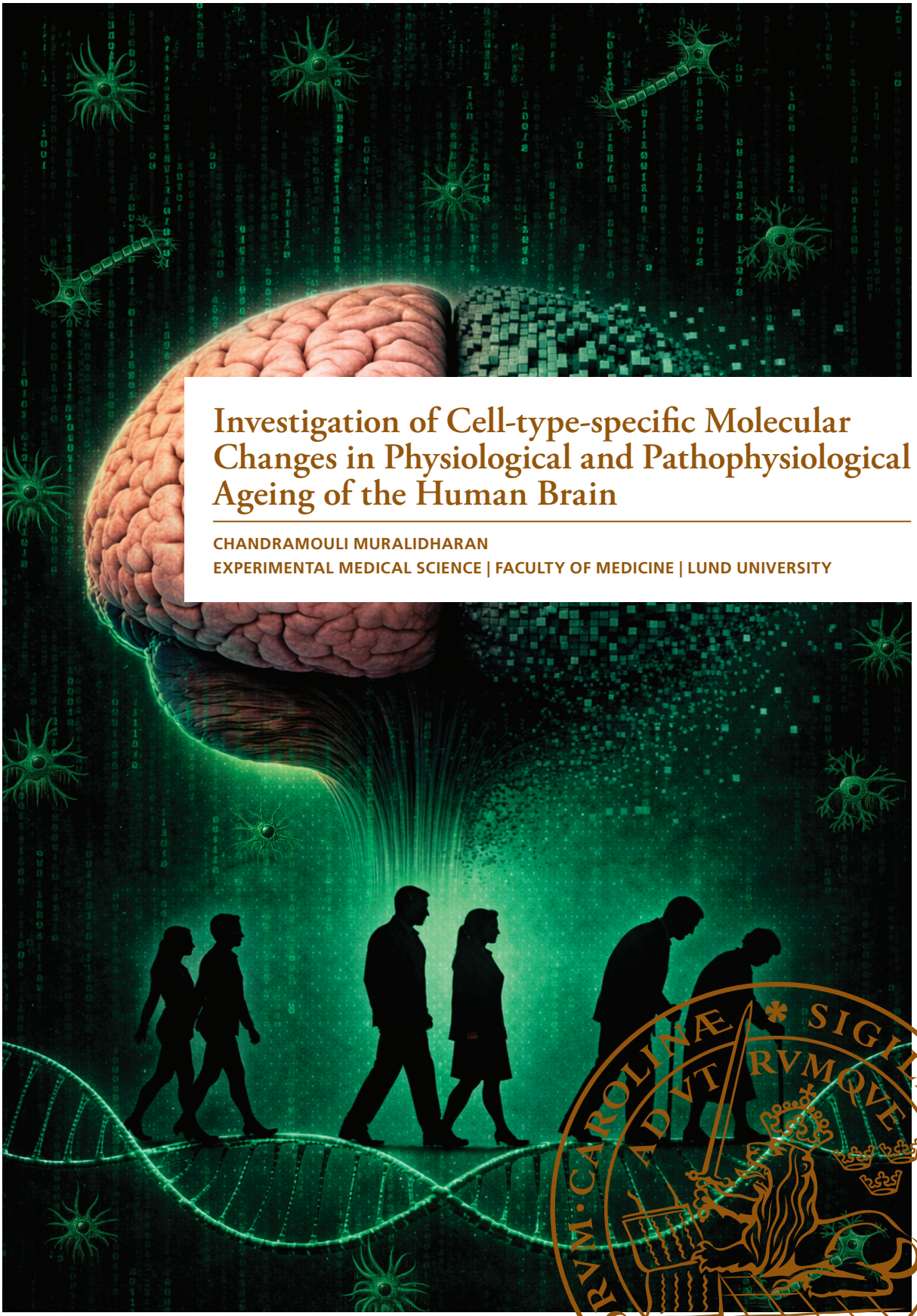
Investigation of Cell-type-specific Molecular Changes in Physiological and Pathophysiological Ageing of the Human Brain

Muralidharan, Chandramouli

2026

Document Version:
Publisher's PDF, also known as Version of record



Investigation of Cell-type-specific Molecular Changes in Physiological and Pathophysiological Ageing of the Human Brain

CHANDRAMOULI MURALIDHARAN
EXPERIMENTAL MEDICAL SCIENCE | FACULTY OF MEDICINE | LUND UNIVERSITY

ted by Media-Tryck, Lund 2026 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Experimental Medical Science

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2026:53
ISBN 978-91-8021-851-1
ISSN 1652-8220

Investigation of Cell-type-specific Molecular Changes in Physiological and Pathophysiological Ageing of the Human Brain

Chandramouli Muralidharan

LUND
UNIVERSITY

DOCTORAL DISSERTATION
for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on 24th of April 2026 at 13.00 in Segerfalksalen, Department of Experimental Medical Science, Sölvegatan 17, 223 62, Lund, Sweden

Faculty opponent
Dr Jerome Mertens
Department of Neurosciences, University of California San Diego, La Jolla, California, USA

Organization: LUND UNIVERSITY

Document name: Doctoral Dissertation **Date of issue** 2026-04-24

Author(s): Chandramouli Muralidharan **Sponsoring organization:**

Title and subtitle: Investigation of Cell-type-specific Molecular Changes in Physiological and Pathophysiological Ageing of the Human Brain

Abstract:

Ageing in the human brain is a major risk factor for most neurodegenerative diseases. Yet the cell-type-specific molecular changes that contribute to this risk remain poorly understood. This thesis investigates cell-type-specific changes across multiple levels of molecular regulation and their roles in physiological and pathophysiological brain ageing.

Using single-nucleus RNA sequencing of postmortem tissue, transcriptomic ageing clocks were developed to measure the progression of ageing at a cell-type-specific level (Paper I). The clocks could additionally predict accelerated ageing in distinct cell types under pathophysiological conditions such as Alzheimer's disease and schizophrenia, highlighting their utility in identifying selective vulnerability. At the post-translational level, the underexplored phosphoproteome of Huntington's disease was profiled in a neuronal context using patient-derived induced neurons (Paper II). The analysis revealed dysregulated components of key homeostatic cell signalling pathways, including autophagy, and identified potential therapeutic targets. To further improve in vitro induced neuronal models, direct neuronal conversion of an alternative, accessible somatic cell source sharing developmental lineage with neurons was established (Paper III). Dental pulp stem cells, isolated from the dental pulp of extracted third molars, were converted into induced neurons (iNs) with superior efficiency compared to dermal fibroblasts, strongly indicating the influence of cell lineage on direct reprogramming.

Individually, each contribution addresses a specific gap in the field. Collectively, they reflect a broader effort to study human brain ageing with greater resolution, across multiple molecular levels, and in more physiologically relevant human cellular contexts.

Key words: Human brain ageing, Neurodegeneration, Ageing Clocks, Transcriptomic Ageing Clocks, Single-cell Ageing clocks, Direct reprogramming, Direct neuronal conversion, Induced Neurons, Huntington's Disease, Dental Pulp Stem Cells, Phosphoproteomics, Multi-omics, Single-nuclei RNA sequencing

Classification system and/or index terms (if any) Supplementary bibliographical information

Language: English **Number of pages**: 78

ISSN: 1652-8220, Lund University, Faculty of Medicine Doctoral Dissertation Series: 2026:53

ISBN: 978-91-8021-851-1

Recipient's notes Price Security classification

Signature Date 2026-03-12

Investigation of Cell-type-specific Molecular Changes in Physiological and Pathophysiological Ageing of the Human Brain

Chandramouli Muralidharan

2026

LUND
UNIVERSITY

Molecular Neurogenetics Laboratory
Department of Experimental Medical Science,
Faculty of Medicine, Sweden

There is a great deal of fun in interpreting art. I would highly recommend reading the thesis first and then revisiting the cover art to form your own perspectives before continuing with this description.

The cover image illustrates the main themes explored in this thesis. The human silhouettes, representing different time points in human adulthood, trace a path formed by DNA, symbolising the molecular progression of human ageing. The brain above represents the alternate fates of brain ageing: the physiological trajectory on the left, and the pathophysiological trajectory on the disintegrating right side. In the background, the raining 1s and 0s, together with the cells, represent the cell-type-specific molecular programs that underlie human brain ageing and its potential trajectories. The overall Matrix-like theme also reflects the computational perspective of my research.

Parts of the cover image were generated using the DALL·E model of ChatGPT. Additional details, including a statement on the use of generative AI, are presented in the Introduction and Methods sections of the thesis.

Published by:
Department of Experimental Medical Science
Faculty of Medicine
Lund University
Lund 2026

ISBN 978-91-8021-851-1
Lund University, Faculty of Medicine Doctoral Dissertation Series: 2026:53
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University, Lund, 2026

Media-Tryck is a Nordic Swan Ecolabel certified provider of printed material. Read more about our environmental work at www.mediatryck.lu.se

MADE IN SWEDEN

To my family

Table of Contents

Abstract

Ageing in the human brain is a major risk factor for most neurodegenerative diseases. Yet the cell-type-specific molecular changes that contribute to this risk remain poorly understood. This thesis investigates cell-type-specific changes across multiple levels of molecular regulation and their roles in physiological and pathophysiological brain ageing.

Using single-nucleus RNA sequencing of postmortem tissue, transcriptomic ageing clocks were developed to measure the progression of ageing at a cell-type-specific level (Paper I). The clocks could additionally predict accelerated ageing in distinct cell types under pathophysiological conditions such as Alzheimer's disease and schizophrenia, highlighting their utility in identifying selective vulnerability. At the post-translational level, the underexplored phosphoproteome of Huntington's disease was profiled in a neuronal context using patient-derived induced neurons (Paper II). The analysis revealed dysregulated components of key homeostatic cell signalling pathways, including autophagy, and identified potential therapeutic targets. To further improve in vitro induced neuronal models, direct neuronal conversion of an alternative, accessible somatic cell source sharing developmental lineage with neurons was established (Paper III). Dental pulp stem cells, isolated from the dental pulp of extracted third molars, were converted into induced neurons (iNs) with superior efficiency compared to dermal fibroblasts, strongly indicating the influence of cell lineage on direct reprogramming.

Individually, each contribution addresses a specific gap in the field. Collectively, they reflect a broader effort to study human brain ageing with greater resolution, across multiple molecular levels, and in more physiologically relevant human cellular contexts.

Popular Science Summary

Have you ever wondered why some get diseases when they grow old and others don't? Because ageing in the human body is beyond just numbers. Your decade milestones mark the number of years you have lived on earth, but not how old you really are. We all seek a good quality of life. To be able to age like a fine wine might be a dream, but to not age like milk is something we sincerely hope for. Growing old, apart from other things, involves many complex changes at a molecular level within the body, especially in the brain. This is why many get brain diseases during old age, primarily those involving rapid death of nerve cells such as in Alzheimer's disease. Research has shown that in such brain diseases, the complex changes progress more rapidly than in healthy brains. So, understanding what changes during ageing, and how it changes differently in diseases, would help us understand these diseases better, and potentially cure or prevent them in the future. In my thesis, I present research that helps address some of these questions in the human brain.

Together with my colleagues, we have developed clocks that measure the pace of ageing in single cells. The brain is a very complex organ all the way down to the cellular and molecular level. There are hundreds of different types of cells, that have very different functions and work together to make the most complex organ in the body. Research has shown that ageing affects the various cell types differently. In fact, in diseases like Alzheimer's, called neurodegenerative diseases, specific types of nerve cells are more prone to death than the others. This makes it important to understand how ageing progresses in individual cell types and help identify which of them age faster in disease conditions. Underlying all the functions in a cell and their identity, are the genes they express – different cell types express different set of genes. And age-related changes reflect in the expression of these genes. So, measuring the changes in the expressed genes in individual cell types, can help identify how far they have aged. Using postmortem brains from people who were 18-95 years old at their time of death, we used advanced single-cell technologies and machine learning to identify age-related patterns in the expression of genes in every cell type, and generated tools that can predict a single cell's age. More importantly, the tools predicted rapid ageing in different cell types in an Alzheimer's disease brain, showcasing its potential.

As odd as the idea sounds, much of research on brain ageing and related diseases are based on dead human brains. This is a major hurdle to research, as postmortem brains serve the role of fossils where you can only guess the course of past events,

rather than observe them in live action. An ingenious solution to this is an advanced technology that can convert skin cells into nerve-like cells. The converted cells have been shown to preserve progression of age-related molecular changes of the donor cells, and show the properties of a nerve cell, making them an excellent live specimen to study ageing. We used this technology to study a fatal genetic neurodegenerative disease, called Huntington's disease, and identified novel targets that have therapeutic potential.

As one may wonder, is it not odd that skin cells can become nerve cells? We tested and succeeded in doing something even more odd. We converted cells from within human teeth, called dental pulp stem cells, into nerve-like cells. In fact, their conversion yielded more nerve-like cells than fibroblasts! Before looking into how it works, it is important to note that the dental pulp stem cells were acquired from extracted wisdom teeth, which is usually discarded unless a tooth fairy needs it. So, they are easier to acquire, making it a good alternate source for nerve-like cells. While in theory any cell can be converted into a nerve cell, how closely they are related to the nerve cells, has been shown to influence the yield. In this case, the dental pulp stem cells have a common origin with the nerve cells, while the fibroblasts have a different origin.

The work presented in this thesis takes us a few steps closer to understanding the complex process of ageing in the human brain, how it unfolds differently across cell types, how it goes awry in diseases like Alzheimer's and Huntington's, and how we can study it better with improved tools and models. Ageing may be inevitable, but understanding it is not out of reach. Every clock developed, every dysregulated pathway uncovered, and every new cellular model established brings us closer to answering why some age like fine wine while others do not, and more importantly, what we can do about it.

Svensk Populärvetenskaplig Sammanfattning

Har du någonsin undrat varför vissa drabbas av sjukdomar när de blir äldre och andra inte? Åldrande i människokroppen handlar om mer än bara siffror. Dina födelsedagar markerar hur många år du har levt på jorden, men inte hur gammal du egentligen är. Vi strävar alla efter en god livskvalitet. Att åldras som ett gott vin kan vara en dröm, men att inte åldras som mjölk är något vi uppriktigt hoppas på. Att åldras innebär bland annat många komplexa förändringar på molekylär nivå i kroppen, särskilt i hjärnan. Därför drabbas många av hjärnsjukdomar på äldre dagar, framför allt sådana som innebär en snabb död av nervceller, såsom Alzheimers sjukdom. Forskning har visat att dessa komplexa förändringar sker snabbare vid sådana hjärnsjukdomar än i friska hjärnor. Att förstå vad som förändras under åldrandet och hur dessa förändringar skiljer sig vid sjukdom kan hjälpa oss att bättre förstå sjukdomarna och i framtiden kanske bota eller förebygga dem. I min avhandling presenterar jag forskning som bidrar till att besvara några av dessa frågor i den mänskliga hjärnan.

Tillsammans med mina kollegor har vi utvecklat så kallade klockor som mäter åldrandets takt i enskilda celler. Hjärnan är ett mycket komplext organ ända ner på cell- och molekylnivå. Det finns hundratals olika celltyper med mycket skilda funktioner som samverkar för att bilda kroppens mest komplexa organ. Forskning har visat att åldrandet påverkar olika celltyper på olika sätt. I sjukdomar som Alzheimers sjukdom, en neurodegenerativ sjukdom, är vissa typer av nervceller mer benägna att dö än andra. Därför är det viktigt att förstå hur åldrandet fortskrider i enskilda celltyper och att identifiera vilka som åldras snabbare vid sjukdom. Grunden för en cells funktion och identitet är de gener den uttrycker – olika celltyper uttrycker olika uppsättningar av gener. Åldersrelaterade förändringar återspeglas i hur dessa gener uttrycks. Genom att mäta förändringar i genuttrycket i enskilda celltyper kan man därför uppskatta hur långt de har åldrats. Med hjälp av postmortala hjärnor från personer som var 18–95 år gamla vid sin död använde vi avancerad enkelcellsteknik och maskininlärning för att identifiera åldersrelaterade mönster i genuttrycket i varje celltyp och skapa verktyg som kan förutsäga en enskild cells ålder. Ännu viktigare är att verktygen visade på snabbare åldrande i olika celltyper i hjärnor från personer med Alzheimers sjukdom, vilket visar deras potential.

Hur märkligt det än kan låta bygger mycket av forskningen om hjärnans åldrande och relaterade sjukdomar på studier av döda mänskliga hjärnor. Detta är en stor utmaning, eftersom postmortala hjärnor fungerar som fossiler där man bara kan försöka återskapa tidigare händelser, snarare än att observera dem i realtid. En elegant lösning på detta är en avancerad teknik som kan omvandla hudceller till nervliknande celler. Dessa omvandlade celler har visat sig bevara åldersrelaterade molekylära förändringar från donatorcellerna och samtidigt uppvisa egenskaper som nervceller, vilket gör dem till utmärkta levande modeller för att studera åldrande. Vi använde denna teknik för att studera en dödlig genetisk neurodegenerativ sjukdom, Huntingtons sjukdom, och identifierade nya mål med terapeutisk potential.

Man kan förstås fråga sig om det inte är märkligt att hudceller kan bli nervceller. Vi testade och lyckades göra något ännu mer oväntat. Vi omvandlade celler från mänskliga tänder, så kallade tandpulpa-stamceller, till nervliknande celler. Faktum är att omvandlingen gav fler nervliknande celler än när vi utgick från fibroblaster. Innan vi undersökte hur detta fungerar är det viktigt att notera att tandpulpa-stamcellerna hämtades från utdragna visdomständer, som vanligtvis kastas bort om inte tandfen behöver dem. De är alltså relativt enkla att få tag på, vilket gör dem till en bra alternativ källa för nervliknande celler. Även om alla celler i teorin kan omvandlas till nervceller har det visat sig att hur nära besläktade de är med nervceller påverkar hur effektiv omvandlingen blir. I detta fall har tandpulpa-stamcellerna ett gemensamt ursprung med nervceller, medan fibroblaster har ett annat ursprung.

Arbetet i denna avhandling tar oss några steg närmare en förståelse av den komplexa åldrandeprocessen i den mänskliga hjärnan — hur den utvecklas olika i olika celltyper, hur den går snett vid sjukdomar som Alzheimers och Huntingtons, och hur vi kan studera den bättre med förbättrade verktyg och modeller. Åldrandet kan vara oundvikligt, men att förstå det är inte omöjligt. Varje klocka vi utvecklar, varje störd signalväg vi kartlägger och varje ny cellmodell vi etablerar för oss närmare svaret på varför vissa åldras som ett gott vin medan andra inte gör det — och viktigare, vad vi kan göra åt saken.

Original research papers

Papers included in this thesis

Paper I

Muralidharan C., Zakar-Polyák E., Adami A., Abbas A.A., Sharma Y., Garza R., Johansson J.G., Atacho D.A.M., Renner E., Palkovits M., Kerepesi C.[#], Jakobsson J.[#], Pircs K.[#] (2025).

Human Brain Cell-Type-Specific Aging Clocks Based on Single-Nuclei Transcriptomics.

Advanced Science, 12(43), e06109.

Paper II

Danics L.[#], **Muralidharan C.**[#], Varga Á., Rezeli M., Gil J., Abbas A.A., Pap Á., Park A., Cserhalmi M., Sőth Á., Jamniczky D., Zsoldos R., Barker R.A., Róna G., Drouin-Ouellet J., Varga G.M., Darula Z., Pircs K. (2025).

Phosphoproteomic profiling reveals post-translational dysregulation in Huntington's disease patient-derived neurons.

BioRxiv.

Paper III

Abbas A.A.[#], **Muralidharan C.**[#], Sramkó B., Gazdik M. E., Vörös K., Varga B., Kis B., Varga Á., Johansson J. G., Kristóf K., Åkerblom M., Storm P., Darula Z., Kerepesi C., Björklund T., Parmar M., Jakobsson J., Lamsa K., Szűcs A., Varga G., Zsembery Á., Anna F., Pircs K.

Direct neuronal reprogramming of human dental pulp stem cells.

Manuscript.

# These authors contributed equally

Original research papers not included in this thesis

Gerdes P. [#], Karlsson O. [#], Garza R. [#], Atacho D. A. M. [#], Hsieh P. H., Prajapati B., **Muralidharan C.**, Adami A., Davis-Hansson C., Johansson E. M., Castilla-Vallmanya L., West W., Vinoud M., Domitrovic L., Johansson P. A., Johansson J., Douse C. H., Kanduri C., Jern P., Eichler E. E., Jakobsson J. (2025).

Retroviral insertions contributed to the divergence of human and chimpanzee brains.

BioRxiv.

# These authors contributed equally

Abbreviations

AD	Alzheimer's Disease
bp	Base Pair
bulk-RNAseq	Bulk RNA sequencing
Co-IP	Co-immunoprecipitation
CNS	Central Nervous System
DNA	Deoxyribonucleic Acid
DPSC	Dental Pulp Stem Cell
FDR	False Discovery Rate
HD	Huntington's Disease
hiPSC	Human Induced Pluripotent Stem Cells
ICC	Immunocytochemistry
IHC	Immunohistochemistry
iN	Induced Neuron
LC-MS/MS	Liquid Chromatography Mass Spectrometry
MAE	Mean Absolute Error
MSC	Mesenchymal Stem Cells
MSN	Medium Spiny Neurons
OPC	Oligodendrocyte Progenitor Cells
PCA	Principal Component Analysis
PD	Parkinson' Disease
PFC	Prefrontal cortex
PMI	Postmortem Interval
PSEA	Phosphosite Enrichment Analysis
PTM	Post-translational Modifications

RNA	Ribonucleic Acid
SCZ	Schizophrenia
shRNA	Short hairpin RNA
snRNAseq	Single-nucleus RNA sequencing
WHO	World Health Organization

Introduction

Ageing is characterized by a progressive functional decline of cells and organs that ultimately limits survival [1]. The process involves several molecular changes associated with a loss of physiological integrity, broadly categorized as the hallmarks of ageing [1,2]. Depending on their progression and, interaction with genetic and environmental factors, ageing in several cases can become pathophysiological [3].

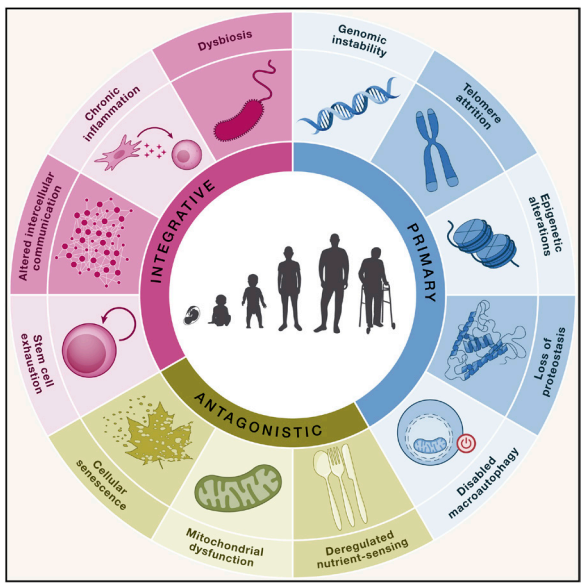


Figure 1: Hallmarks of Ageing. The schematic figure shows the different hallmarks of ageing. Adapted from López-Otín et al., *Cell*, 2023, with permission from Elsevier. © 2023 Elsevier.

While there is a lack of consensus on when ageing begins in humans [4-7], functional and molecular changes accumulate throughout adulthood and increase susceptibility to age-related diseases, such as Alzheimer's and Parkinson's disease in the brain [8,9]. An adult human, based on convention, can be classified into one of three broad age groups based on the age range one falls into, namely, young (18 – 40 years), middle-age (41 – 60 years) and old (>60 years). Those belonging to the old age group, particularly, show the highest functional decline and the greatest vulnerability to age-related diseases [10-15]. Based on current estimates and the increasing life

expectancy[12,16,17], the World Health Organization (WHO) predicts that by 2050, at least 22% of the world population would belong to the old age group, posing an unprecedented burden to the society, economy and health-care [18-21]. Understanding the molecular basis of ageing under physiological and disease conditions is thus important for developing therapeutic strategies that can mitigate the detrimental effects of ageing and help improve quality of life [2,3,11].

Ageing in the human brain

Physiological Ageing

Human brain ageing is characterised by a gradual decline in cognitive function, including learning, memory, decision-making and motor-coordination, together with structural alterations such as reduction in brain volume and blood-brain barrier leakage [13-15,22,23]. A cumulative impact of several molecular processes has been suggested to underlie this decline [24-40]. Notably, age-related increase in inflammation, impairment of proteostasis (the ability of cells to maintain protein homeostasis through synthesis, folding and degradation) and synaptic dysfunction have been identified as prominent features of the ageing brain [3,24,27,31,41,42]. However, how these molecular changes manifest across the diverse cell types of the brain, the specific molecular components affected and how they collectively give rise to functional decline, remains uncharacterised. The brain comprises a rich diversity of cell types with distinct functions [43,44], and age-related molecular changes are highly cell-type- and region- specific [27-40,43]. This makes it essential to investigate age-related molecular changes in the human brain at a cell-type-specific level.

Cell types in the human brain

Broadly, the major cell types found across the human brain include the neuronal populations – excitatory and inhibitory neurons, the major glial populations – oligodendrocytes, astrocytes, microglia and oligodendrocyte progenitor cells (OPCs) – alongside ependymal cells, vascular cells and in some cases infiltrating immune cells [43-45]. Single-nuclei transcriptomic studies of human post-mortem brain tissue have further revealed extensive diversity in subtypes and cellular states within each population, varying across brain regions [43,44]. Notably, many brain cells are either post-mitotic, such as neurons, or exhibit low turnover, limiting the possibility to replenish during cell division [44,46,47]. This lack of cell renewal is thought to increase their susceptibility to age-related molecular alterations and functional decline [3,11].

Cell-type-specific molecular changes

Much of our current understanding of cell-type-specific age-related molecular changes in the brain comes from single-nucleus RNA sequencing (snRNAseq) studies of human postmortem tissue [28-40], with a strong emphasis on the prefrontal cortex (PFC) region due to its high vulnerability to structural and functional alterations in physiological ageing [48-54].

Overall, cell-type-specific markers and genes associated with house-keeping functions, such as macroautophagy and translation, are downregulated during ageing across most cell types, indicating a broad decline in basic cellular functions [28-40]. In neurons, genes associated with oxidative phosphorylation and synapse formation and signalling, are similarly affected, indicating declining energy metabolism and synaptic function [28-40]. Among glial cells, oligodendrocytes, astrocytes and OPCs also show a downregulation of genes associated with postsynaptic modulation and synaptic transmission, alluding to alterations in their neuro-supportive functions [28-40]. Additionally, the glial cells, particularly microglia and astrocytes, show an upregulation of inflammatory and proteostasis-stress response genes, consistent with the inflammatory shift observed at a bulk-tissue level [28-40]. Collectively, transcriptomic observations point to declining house-keeping functions and increasing stress response across brain cell types during ageing [28-40].

Pathophysiological Ageing

Ageing increases the risk of incidence of many diseases in the human brain [3]. Notably, it is a major risk factor for neurodegenerative diseases such as Alzheimer's (AD), Parkinson's (PD) and Huntington's disease (HD) [11,55]. Depending on the disease, ageing may act either as the primary risk factor driving disease onset, as in AD and PD, or as an important modifier that exacerbates disease progression and symptom severity, as in HD [11,55-58]. The pathology of such age-associated neurodegenerative diseases involves selective vulnerability of certain brain regions and cell types, which likely reflects the cell-type-specific nature of molecular ageing trajectories [59,60]. However, the progression of physiological ageing into pathophysiology in some individuals, and its contribution to selective vulnerability in a cell-type-specific manner, remains poorly understood. In all cases of pathophysiological ageing, including neuropsychiatric disorders, such as Schizophrenia (SCZ), molecular changes underlying physiological ageing appear to progress at an accelerated rate [28,31,41,61-72]. The possibility to track this progression in a cell-type-specific manner can aid in discovering vulnerable cell types and better understanding the underlying molecular trajectories [31].

Below is a brief overview of the age-associated neurodegenerative diseases addressed in this thesis:

Alzheimer's Disease

AD is a neurodegenerative disease that leads to memory deficits and cognitive decline [73]. The late-onset sporadic form of AD is the most commonly occurring neurodegenerative disease with ageing as its primary risk factor [56,73]. The pathology of the disease involves extracellular accumulation of amyloid-β plaques (Aβ) plaques and intracellular accumulation of neurofibrillary tangles containing hyperphosphorylated tau aggregates, together with progressive loss of specific neuronal subtypes and aberrant inflammatory response in glial cells [73]. Particularly, excitatory neurons, namely, the layer II entorhinal stellate neurons and the hippocampal CA1 pyramidal neurons show the strongest vulnerability to synaptic loss and death [59,60].

At a molecular level, based on snRNAseq studies comparing AD postmortem brain tissues with that of neurotypical controls, alterations partially mirror but substantially exceeds the changes observed in physiological ageing [74-78]. For example, neurons, mainly excitatory, show a downregulation in synaptic function and signalling genes, while microglia and astrocytes shift towards inflammatory states [74-78].

Huntington's Disease

HD is an autosomal dominant progressive neurodegenerative disorder caused by CAG-repeat expansions in the first exon of the *Huntingtin* (*HTT*) gene [79-82]. The mutation results in polyglutamine expansion in the translated protein, which shows a tendency to misfold and ultimately, aggregate [79,81,82]. The mutant Huntingtin protein (mHTT) is also known to disrupt numerous homeostatic cellular pathways such as those associated with autophagy, energy metabolism, synaptic transmission and transcription, ultimately leading to cellular dysfunction and death [81,83]. Medium spiny neurons (MSN) in the striatum are selectively vulnerable to degeneration [59,60].

HD manifests with cognitive impairment, movement abnormalities, metabolic and psychiatric issues, and eventual death within 20 years of manifestation [79-82]. Age of onset of the disease negatively correlates with the CAG-repeat length of the *HTT* gene, with symptoms typically manifesting 30-40 years of age [79-82]. Although ageing is not the primary risk factor, it is a key factor in the progression of the disease [58]. Molecular ageing has been shown to accelerate and thought to exacerbate the symptoms of the disease [58].

Challenges in studying human brain ageing and related diseases

Study of ageing and related diseases in the human brain, at a cell-type-specific level, has largely depended on postmortem tissue [28-40,74-78]. While animal models have been used to mimic certain aspects of the pathology of different diseases, they are fundamentally different from humans, fail to mimic the complexity of the human

brain and unlike humans they do not naturally develop neurodegenerative diseases, leaving limited insights for translation [84-90]. Postmortem brain tissue in combination with single-nucleus and spatial omic approaches on the other hand has paved way for deciphering the cellular diversity and has enabled snapshot insights on the molecular events in the brain both in physiological and in pathophysiological ageing [28-40,74-78]. However, tissue quality is affected by variables such as postmortem interval (PMI) and cause of death, which are difficult to fully account for [91-94]. Postmortem tissue from young and middle-aged individuals is frequently underrepresented, and when such samples are available, the cause of death is often suicide or accident, further constraining sample availability and skewing the representation of the full age range [95]. Critically, postmortem tissue cannot be experimentally manipulated, limiting mechanistic insight and preventing the assessment of potential therapeutic strategies.

Post-translational changes

While transcriptomic studies have provided valuable insights into cell-type-specific molecular changes during human brain ageing [28-40], gene expression represents only one regulatory layer. Cellular functions are predominantly governed post-transcriptionally by post-translational modifications (PTMs), which dynamically and reversibly regulate protein activity, stability, localization, and interactions [96]. Since PTMs can alter protein function independently of changes in transcript levels, they represent a layer of molecular information that is not captured by transcriptomics, and one that is essential for a comprehensive understanding of molecular ageing.

Among the various PTMs, protein phosphorylation, which is the reversible addition of a phosphate group to serine, threonine or tyrosine residues, is the most common in mammalian cells [97]. It is dynamically regulated by phosphorylating protein kinases and dephosphorylating phosphatases [98]. Phosphorylation plays a pivotal role in several house-keeping cell signalling pathways such as synaptic function, autophagy and transcriptional regulation, that are known to decline in both physiological and pathophysiological ageing [97]. Additionally, dysregulation of phosphorylation plays a critical role in the protein aggregation pathology of AD and HD [99,100]. In HD, the mHTT interacts with and dysregulates multiple kinase and phosphatase pathways, and phosphorylation of mHTT itself has been shown to influence its aggregation propensity and neuronal toxicity, implicating phosphorylation dysregulation as both a consequence and a driver of disease progression [100]. Despite this, a characterisation of the entire phosphoproteomic landscape in HD and many other neurodegenerative diseases in humans, particularly in a neuronal context, has been lacking. Such a characterisation could help uncover potential therapeutic targets [97].

In vitro models of ageing

Cells of the human brain have been modelled *in vitro* using various methods [101,102]. This has enabled the possibility to study live cells closely resembling those in the human brain, in morphology, function and physiology [103-111]. They provide several key advantages over postmortem tissue in enabling experimental manipulation in a controlled environment to draw mechanistic insights, scalability, and early access to disease processes [101]. Further, due to their human-specific biology they overcome several disadvantages of animal models, such as by avoiding cross-species extrapolation for translational applications, modelling human genetics and minimising ethical concerns. These factors make *in vitro* models an excellent alternative to postmortem tissue and animal models for studying human brain ageing and related diseases.

Approaches

Two approaches have been widely used for *in vitro* modelling of various human brain cell types, human induced pluripotent stem cell (hiPSC)-derived reprogramming and direct reprogramming [102]. The first approach takes advantage of hiPSCs' pluripotency, wherein under the influence of developmental morphogens, small molecules and/or transcription factors, the cells are differentiated into one of the human brain cell types, such as neurons [112,113]. Given that the hiPSCs are typically derived from donor's dermal fibroblasts through induction of pluripotency, the models recapitulate the genetics of the human donor and enables patient-specific modelling of disease genetics [102]. In this regard, hiPSC-derived neuronal cells have been used to successfully model the genetics of diseases such as HD, and non-idiopathic versions of AD and PD [102]. However, the reprogramming in induced pluripotency has been suggested to erase age-related epigenetic alterations in the donor cells leading to a reset of donor age [114]. For this reason, hiPSC-based reprogramming fails to model ageing, late-onset neurodegenerative diseases and essential age-influenced pathology in disease like HD [102,114].

Direct reprogramming overcomes the main disadvantage of hiPSC-based reprogramming in modelling ageing by bypassing the pluripotency stage during conversion [101,115]. Donor-derived cells, typically dermal fibroblasts, are transdifferentiated into brain cell types using cocktails of small molecules, microRNAs or forced expression of lineage-specific transcription factors [101,115]. Further, the induced cells recapitulate age-related molecular features of the donor cells, making them excellent *in vitro* models for studying ageing [116,117]. For example, induced neurons (iNs) show chronological DNA methylation patterns and age-related decline in RanBP17 expression, features observed in the corresponding source fibroblasts [116,117]. Several protocols exist, where donor-derived dermal

fibroblasts have been converted into neuron-like (or iNs), astrocyte-like or oligodendrocyte-like cells [101,103,104,118]. Different approaches also yield specific neuronal subtypes such as motor, striatal medium spiny, dopaminergic, GABAergic and cholinergic neurons [105-111], which highlights the potential of the method to model diverse cell types in the brain. From a disease context, direct neuronal conversion has been used to successfully model HD, late-onset AD and PD, where important neuron-specific molecular features of the respective diseases were recapitulated [119-121].

Alternate somatic cell sources

Human dermal fibroblasts have been widely used as a source for direct reprogramming due to their accessibility from skin biopsies, high proliferative rate in standard media and viability post cryopreservation. However, their mesodermal origin differs from the neuroectodermal lineage of brain cell types such as neurons, astrocytes, and oligodendrocytes, which is suggested to reduce their conversion efficiency [122,123]. Furthermore, sources sharing neuroectodermal origin, such as astrocytes and neural progenitors, have shown greater conversion efficiency, i.e., higher yield and faster conversion rates, supporting the importance of shared lineage [124-126]. Nevertheless, despite their superior conversion efficiency, such sources are difficult to access from living human donors.

Human dental pulp stem cells (hDPSCs) offer a compelling alternative to dermal fibroblasts, particularly for direct neuronal reprogramming [127]. They are multipotent, mesenchymal-like stem cells that reside in the dental pulp of human teeth, with neurogenic potential, that they readily differentiate into neurons in the presence relevant growth factors [127]. Owing to their cranial neural crest origin, which is closely aligned developmentally with the neuroectoderm, they have the potential to convert into neurons with superior efficiency over dermal fibroblasts [128]. Importantly, hDPSCs are harvested non-invasively from discarded exfoliated deciduous or extracted third molars, providing an ethically favourable and clinically accessible source [129].

Ageing Clocks

Although chronological age provides a measure of time since birth, it does not reflect the progression of molecular changes underlying ageing, particularly in complex organs such as the human brain [22]. As a result, chronological age fails to address the inter-individual difference in susceptibility to pathophysiological ageing [61,62,66,67,130,131]. Ageing clocks, which are machine learning models trained to measure biological age based on age-associated molecular features, help address this gap [132].

The models are typically trained on omics data, such as methylomic, proteomic or transcriptomic data, and capture age-correlating molecular patterns, based on which they predict the biological age [133]. Further, ageing clocks have been used to successfully predict age acceleration in individuals with pathophysiological ageing, such as in AD or HD [61,62,66,67,130,131], highlighting their potential to predict alterations in pathophysiological conditions.

Majority of ageing clocks developed have been based on bulk omic data and consequently lack cell-type-specific resolution [132-135]. Given the cell-type-specificity of molecular changes in human brain ageing, most ageing clocks have failed to provide the required insights. Development of clocks that can predict cell-type-specific biological age for the human brain would aid in understanding how ageing progresses in different cell types and leads to selective vulnerability in neurodegenerative diseases. Single-cell/nucleus RNA sequencing enables the development of such cell-type-specific clocks due to their high-throughput nature and excellent cell type resolution [136]. In fact, cell-type-specific transcriptomic clocks, have been successfully developed in mice, where they could measure biological age in different cell types, apart from predicting deceleration post rejuvenation events [137]. This highlights the potential and the need of such clocks for the human brain.

Use of generative AI in thesis

This thesis has in part been produced with the assistance of the generative AI tools Claude, ChatGPT and DeepL. I have processed the generated text and image and take full responsibility for the content. A more detailed account of how and when the AI models were used can be found in the methodology section.

Rationale

Despite decades of research, our understanding of the molecular changes underlying human brain ageing and their contribution to neurodegenerative diseases remains limited. Two challenges have been particularly persistent: the cell-type-specificity of age-related molecular changes [27-40,43], which bulk tissue approaches cannot adequately resolve, and the lack of suitable human cellular models in which to study them. Furthermore, ageing is a systemic process that manifests across multiple levels of gene regulation, from transcription through to PTM, and a comprehensive understanding therefore requires investigation at each of these levels. High-throughput omic approaches are well-positioned to address this, offering the scale and resolution needed to uncover age-associated molecular changes across the full complexity of the human brain [43,136,138].

A vital step toward understanding cell-type-specific brain ageing is the ability to quantify its progression in individual cell types, enabling the study of both cell-type-specific dynamics and selective vulnerability under physiological and pathophysiological conditions. Ageing clocks, machine learning models trained on omics data to predict biological age, offer a promising framework for this purpose [61,62,66,67,130,131]. However, existing clocks have not been well-suited to the human brain, either lacking cell-type resolution or being derived from non-human sources [132-135,137]. Developing transcriptomic ageing clocks trained on human brain cell-type-specific data therefore represents an important and unmet need.

Beyond the transcriptome, age-related decline in housekeeping functions, including proteostasis, autophagy and cell signalling, is a defining feature of both physiological and pathophysiological ageing, and is further accelerated in neurodegenerative diseases [11,55]. Protein phosphorylation, as the most abundant PTM in mammalian cells, is central to the regulation of these pathways [97]. Further, in HD, where protein phosphorylation plays an important role in the pathology of the mutant HTT protein, a comprehensive insight on the phosphoproteomic landscape has been lacking [100]. Characterising phosphoproteomic dysregulation in a neuron-specific context holds considerable potential for uncovering disrupted molecular mechanisms and identifying therapeutic targets [97].

Progress in studying cell-type-specific molecular changes in brain ageing has been significantly advanced by the development of in vitro neuronal models through direct reprogramming of donor-derived somatic cells [101,115]. Unlike hiPSC-based

approaches, direct reprogramming preserves the age-associated molecular signatures of the donor, making it uniquely suited for modelling ageing and late-onset neurodegeneration [102,114,116,117]. However, the field has remained largely depended on dermal fibroblasts as the source cell, which due to its lack of shared developmental origin with neurons has reduced conversion efficiency [122,123]. Identifying and validating an easily accessible alternative somatic cell source, that has closer developmental relationship with neurons, such as DPSCs, could help improve the conversion efficiency, and help accelerate discoveries [124-129].

Aims of the thesis

The overall aim of this thesis was to investigate cell-type-specific molecular changes associated with physiological and pathophysiological ageing in the human brain.

The specific aims of the thesis were to:

1. develop ageing clocks that measure cell-type-specific progression of ageing in the human prefrontal cortex (Paper I)
2. investigate neuronal phosphoproteomic changes in Huntington's disease (Paper II)
3. develop an *in vitro* neuronal model of ageing using direct reprogramming of dental pulp stem cells (Paper III)

Summary of Key Methods

Direct Neuronal Conversion

Dermal fibroblasts (in Paper II) or DPSCs (in Paper III) were directly converted into iNs using a previously described third-generation lentiviral vector expressing short hairpin RNAs (shRNAs) against REST and the transcription factors ASCL1 and BRN2 [139,140]. Lentiviral particles were produced and titrated as reported previously, and fibroblasts or DPSCs were transduced under standard conditions. Neuronal conversion was carried out using NDiff-based media, and iNs were harvested at day 17 (DPSC-iNs) or 28 (fb-iNs) for subsequent experiments.

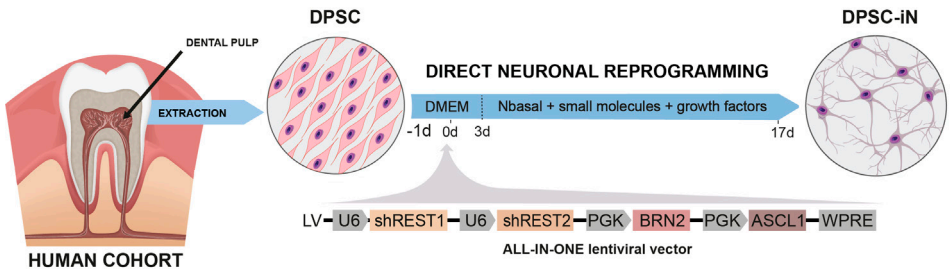


Figure 2: Schematic of direct neuronal conversion of DPSCs. The schematic describes the direct neuronal conversion of DPSCs into DPSC-iNs using a previously established protocol.

Table 1: Summary of demographic information on HD patient and control fibroblast donors.

ID	Age	Sex	CAG repeats (WT/MUT)	Age at onset of HD
HD1	28	Male	15/39	premanifest
HD2	31	Male	20/45	33
HD3	43	Male	17/42	38
HD4	43	Male	19/44	36
HD5	47	Male	NA/40	premanifest
HD6	53	Male	19/42	premanifest
HD7	59	Male	16/39	33
C1	27	Male	17/17	-
C2	30	Male	19/24	-
C3	52	Female	19/23	-
C4	54	Female	15/20	-
C5	61	Male	17/23	-
C6	61	Female	17/17	-
C7	66	Male	24/24	-

Table 2: Summary of demographic information of dental pulp donors.

Donor ID	Age (years)	Sex
DPSC1	18	Female
DPSC2	24	Male
DPSC3	28	Female
DPSC4	29	Female

Transcriptomics

Single-nucleus RNA Sequencing

Sequencing

Single-nuclei isolated from frozen post-mortem tissue were processed for snRNAseq sequencing using the Chromium Next GEM Single Cell 3′ platform (10x Genomics) according to the manufacturer's protocol. Single-nucleus gel bead–in-emulsion (GEM) generation, reverse transcription, and cDNA amplification were performed using Chromium Next GEM reagents, followed by library construction with unique dual indices. Libraries were pooled and sequenced on an Illumina NovaSeq 6000 platform.

Table 3: Summary of demographic and pre-clinical information on the human postmortem tissue used for generating training dataset in Paper I.

Sample ID	Age (years)	Sex	PMI (h)	Brain Region	Cause of death
y1	22	Male	8	Middle frontal gyrus	suicide
y2	36	Female	2	Middle frontal gyrus	suicide
y3	31	Male	8	Middle frontal gyrus	suicide
y4	31	Male	7	Middle frontal gyrus	suicide
y5	36	Male	6	Ventrolateral prefrontal cortex	suicide
y6	35	Male	6	Middle frontal gyrus	suicide
y7	18	Male	8	Middle frontal gyrus	suicide
y8	25	Male	8	Middle frontal gyrus	suicide
y9	39	Female	12	Middle frontal gyrus	suicide (hanging)
m1	42	Male	3.5	Ventrolateral prefrontal cortex	cardiac insufficiency
m2	55	Male	1	Ventrolateral prefrontal cortex	acute myocardial infarction
m3	47	Male	1	Ventrolateral prefrontal cortex	cardiac insufficiency
m4	50	Male	2	Ventrolateral prefrontal cortex	myocardial infarction
m5	53	Male	5	Ventrolateral prefrontal cortex	pulmonary embolism
m6	56	Female	6	Ventrolateral prefrontal cortex	cardiorespiratory insufficiency
m7	49	Female	6	Ventrolateral prefrontal cortex	suicide (drug overdose)
m8	44	Female	5	Dorsolateral prefrontal cortex	acute cardiac insufficiency
o1	86	Male	3	Ventrolateral prefrontal cortex	acute cardiac insufficiency
o2	73	Male	6	Ventrolateral prefrontal cortex	acute cardiac failure; general arteriosclerosis
o3	81	Male	5	Ventrolateral prefrontal cortex	acute ischaemic heart failure;cardiogen shock
o4	80	Male	4.5	Ventrolateral prefrontal cortex	cardiorespiratory insufficiency
o5	76	Male	5.5	Ventrolateral prefrontal cortex	stroke*
o6	94	Female	6	Ventrolateral prefrontal cortex	cardiac failure
o7	74	Female	1.5	Ventrolateral prefrontal cortex	cardiac insufficiency; pulmonary embolism
o8	70	Female	7	Ventrolateral prefrontal cortex	stroke*
o9	75	Male	8	Ventrolateral prefrontal cortex	stroke*
o10	74	Male	1	Ventrolateral prefrontal cortex	acute cardiac insufficiency
o11	80	Female	1	Ventrolateral prefrontal cortex	acute respiratory insufficiency
o12	85	Female	4	Ventrolateral prefrontal cortex	acute cardiorespiratory; renal insufficiency
o13	72	Female	1	Ventrolateral prefrontal cortex	pulmonary embolism
o14	85	Female	8	Ventrolateral prefrontal cortex	stroke*; hypertension

**microdissected region not same as the stroke affected region.*

Pre-processing

Raw reads in fastq format were pre-processed, for extraction of cell ranger barcodes and UMI, mapping to the GRCh38 genome assembly and counting, using the Cell Ranger (v7.0.0) count pipeline [136].

All downstream processing steps were done using R and the standard Seurat workflow [141].

Quality control

High quality nuclei were filtered based on read counts thresholds of 1,200 – 100,000, that of gene counts of 800 – 12,000, and percentage mitochondrial genes less 5.

Normalisation, Dimensional reduction and Clustering

The gene expression data was log-normalized and scaled, followed by data integration over sequencing batches and individual samples using the Harmony package [142]. Further, the data was clustered using 39 corrected principle components and a resolution of 0.2. The clusters were annotated based on the expression of cell-type-specific canonical markers of the human PFC. Potential doublets or multiplets in the annotated clusters were predicted using scDblFinder and were removed [143].

Differential expression analysis

The differential gene expression analysis was performed using the FindMarkers function of Seurat package with Wilcoxon Rank Sum Test, between the different age groups followed by Benjamini–Hochberg correction to determine false-discovery rate. The specific comparisons made were namely, old versus young, old versus middle-aged, and middle-aged versus young. Significance of results were determined based on an adjusted p value threshold of 0.05 and an absolute log2 fold-change threshold of 0.5.

Gene ontology analysis

A gene over-representation test was performed using the ClusterProfiler package, where the gene ontology terms enriched for the genes significantly differentially expressed for each group-wise comparison were determined. Additionally, a Benjamini–Hochberg correction was included to the determine false-discovery rate. Significance of results were determined based on an adjusted p value threshold of 0.05.

Bulk RNA Sequencing

Sequencing

Total mRNA was extracted from native DPSC and DPSC-iN cell lines using the RNeasy Micro Kit (Qiagen). In the case of DPSC-iNs, $NCAM^{+}/PI^{-}$ cells were first FACS sorted prior to mRNA extraction for neuronal purity. Full-length cDNA libraries were prepared using the SMART-Seq mRNA Kit (Takara/Clontech) and sequenced on an Illumina NovaSeq 6000 or NovaSeq X Plus platform with paired-end 150 bp reads.

Pre-processing

Raw base call (.bcl) files generated from sequencing were converted to FASTQ format and demultiplexed using bcl2fastq package. The raw reads from the .fastq files were mapped to GRCh38.p14 human genome with Gencode hg38 annotations (v47) using the STAR aligner package with default settings. The mapped reads, which were paired-end and unstranded, were quantified at the gene level using featureCounts function of the Subread package with corresponding settings.

All downstream analysis on the gene counts were performed using R. Additionally, only counts of protein-coding genes that were non-zero across all the samples were included.

Principal component analysis

First, a regularized log transformation was performed on the gene counts using rlog function from the DESeq2 package. Then, the prcomp function from the stats package was applied on the top 500 most variable genes with "centre" option.

Differential expression analysis

The DESeq2 package was used for differential expression analysis, with a paired design for samples from each donor. The log-fold change values were shrunk using the lfcshrink function with the ashr algorithm. Significance of results were determined based on an adjusted p value threshold of 0.05 and an absolute log2 fold-change threshold of 0.5.

Gene ontology analysis

A gene over-representation test was performed using the ClusterProfiler package, where the gene ontology terms enriched for the genes significantly upregulated or downregulated were determined separately. Additionally, a Benjamini–Hochberg correction was included to the determine false-discovery rate. Significance of results were determined based on an adjusted p value threshold of 0.05.

Cell-type-specific Ageing Clocks

Ageing clock models were trained on gene expression data of each cell type, individually, with donor-level five-fold cross validation using ElasticNet regression with the Glmnet python package. Models were trained on 80% of the donors and tested on the remaining 20% over five rounds (/folds). The final clock models were based on the average coefficients across the five folds. In all cases, mitochondrial and sex chromosome genes were excluded to avoid noise and bias respectively. Different approaches with respect to the gene expression data were tested.

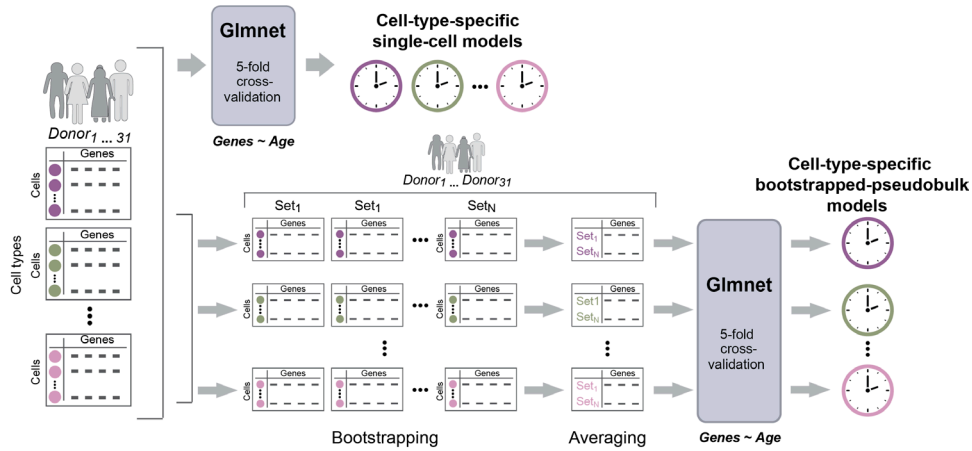


Figure 3:Schematic of cell-type-specific transcriptomic ageing clock development. The schematic figure describes the different approaches used for the development of the cell-type-specific transcriptomic ageing clocks. Adapted from Muralidharan et al., *Adv. Sci.*, 2025.

Approaches

Single-cell

The models were trained on the log-normalised gene expression values of each single cell (represented by nuclei) and every single cell was considered an observation.

Simple Pseudobulk

Log-normalised gene expression data was averaged across cells of a given cell type in each donor, resulting in pseudobulk gene expression data. The models were trained on the pseudobulk expression data, and each pseudobulk cell, one per cell type per donor, was considered an observation.

Bootstrapped-pseudobulk

From the cells of a given cell type, of each donor, a fixed number of cells were randomly sampled (bootstrapping) and the log-normalised gene expression data was averaged across the sampled cells. Clocks were trained on resulting bootstrapped-pseudobulk expression data, and every boostrapped-pseudobulk cell, several per cell type per donor, was considered an observation.

Validation of Clock Predictions

The correlation between the chronological age of the donors, and predicted age from the clock models from each approach in each cell type, were compared using a

Pearson's correlation test. Additionally, the average difference between the predicted and chronological age, called mean absolute error (MAE) of the predictions, was calculated. Both the Pearson's correlation coefficient (r) and the MAE values were used for assessing the predictions.

In validating predictions for the training dataset, the results from the testing rounds were assessed. While, in external independent and published snRNAseq datasets, the clocks were first applied on the log-normalised gene expression data, and the resulting predictions were assessed. Necessary transformations on the data were made as required for the different approaches. Additionally, expression values for missing genes in the external datasets were imputed based on the average expression value in the original training dataset.

Age Acceleration

Clock models were applied on published snRNAseq datasets of cohorts representing physiological ageing and age-related neurological conditions. Least-squares linear regression models were fit to the chronological age of the donors and the predicted age separately for each condition, and age acceleration was calculated as the difference between the predicted age and the fitted line. When comparing age acceleration between conditions, mixed linear models in the case of single-cell approach and generalised linear models for the remaining approaches were used.

LC-MS/MS Proteomics

Global Proteomics

Sample preparation and LC-MS/MS

DPSC and corresponding converted DPSC-iN cell lines were processed for quantitative proteomics using SDS-based lysis, S-Trap digestion, and TMTpro 16-plex labelling. Fractionated peptide samples were analysed by LC–MS/MS on an Orbitrap Fusion Lumos instrument.

Pre-processing

Protein identification, TMT-based quantification and normalization were performed in Proteome Discoverer using Sequest HT, with reporter-ion–based normalization applied prior to downstream analysis. Protein abundances were normalized to the total peptide amount.

All downstream data analysis was performed using R. Proteins were first filtered to only include those identified with a confidence level marked as High.

Principal component analysis

A log2 transformation was applied on the normalized counts of filtered proteins. Then, the prcomp function from the stats package was applied on the top 500 most variable proteins with "scale" and "centre" options.

Differential abundance analysis

The limma package was used for differential abundance analysis, with a paired design for samples from each donor. Significance of results were determined based on an adjusted p value threshold of 0.05 and an absolute log2 fold-change threshold of 1.

Pathway enrichment analysis

A gene over-representation test was performed using the ReactomePA and ClusterProfiler packages, where the reactome pathways enriched for the proteins significantly upregulated or downregulated were determined separately. Additionally, a Benjamini–Hochberg correction was included to the determine false-discovery rate. Significance of results were determined based on an adjusted p value threshold of 0.05.

Phosphoproteomics

Sample preparation and Phospho-enriched LC-MS/MS

The iN cell lines (7 control and 7 HD) were processed for quantitative phosphoproteomics using SDS-based lysis and S-Trap digestion. A triplicate design was used overall. Peptides were enriched for phosphopeptides by Fe(III)-IMAC and analyzed by nanoLC–MS/MS in data-dependent acquisition mode on a Q-Exactive HF-X mass spectrometer.

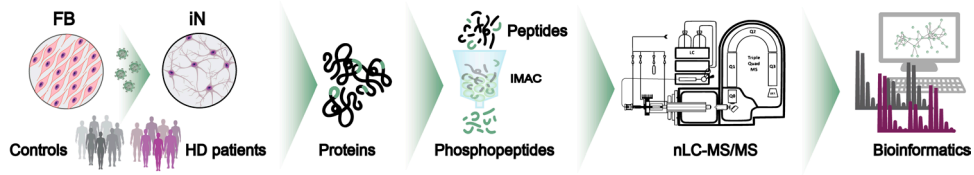


Figure 4: Schematic of phosphopeptide-enriched LC-MS/MS workflow. The schematic describes the workflow of phosphopeptide-enriched LC-MS/MS from iNs derived from the dermal fibroblasts of 7 control and 7 HD donors.

Pre-processing

Proteome Discoverer was used for processing the raw data, with the SEQUEST HT search engine, the human UniProt database as reference and ptmRS for locating phosphosites. Peptide and protein identifications were filtered at a 1% false discovery rate (FDR) prior to further steps.

Normalization and Intensity Correction

The intensities of the filtered phosphopeptides were log2-transformed and median-centered per sample to correct for differences in sample loading. Further, the intensities of technical replicates were averaged. Only peptides quantified in at least four samples in either one of the groups were retained for further processing and data analysis. Finally, to infer phosphopeptide abundance independent of the mapped protein's abundance, the log2-transformed intensities of the phosphopeptides were subtracted by that of the corresponding protein in the global proteomic data.

Principal component analysis

PCA was performed using Perseus software.

All downstream data analyses after above steps were performed using R.

Differential abundance analysis

Using Shapiro-Wilk test and unpaired t-test the phosphopeptide abundances were compared between the HD-iNs and Ctrl-iNs. Significance of results were determined based on a Shapiro-Wilk p-value threashold of 0.05 (>) and a t-test p-value of 0.05 (<).

Pathway enrichment analysis

Pathway enrichment analysis was performed using String-db v12.0, where the reactome pathways enriched for the proteins corresponding to the phosphopeptides significantly upregulated or downregulated were determined separately. In all cases, the background for the analysis included all the proteins identified across the phosphoproteomic and global proteomic data. Significance of results were determined based on a FDR threshold of 0.05.

Phosphosite Enrichment Analysis

The Phosphosite Enrichment Analysis (PSEA) tool as part of Kinase Library webtool on the PhosphositePlus portal was used for predicting the kinases phosphorylating the differentially abundant phosphopeptides [144-146]. Significance of results were determined based on the detection of the kinases in the global proteomic dataset, and a p-value threshold of 0.05

Co-Immunoprecipitation

Sample preparation and LC-MS/MS

Cells from iN cell lines (4 control and 4 HD) were lysed under non-denaturing conditions, and soluble protein fractions were subjected to immunoprecipitation using an anti-MXRA8 antibody coupled to Protein A/G magnetic beads. Additionally, as background, a control fibroblast and a control iN cell line were subjected to immunoprecipitation using an anti-IgG antibody. Immunoprecipitated proteins were eluted and digested with trypsin using an S-Trap–based workflow. Peptides were analyzed by nanoLC–MS/MS in data-dependent acquisition mode using an Evosep One system coupled to an Orbitrap Fusion Lumos mass spectrometer with FAIMS.

Pre-processing

Proteome Discoverer was used for processing the raw data, with the SEQUEST HT search engine and the human SwissProt database as reference. Protein identifications were filtered at a 1% FDR. Peptide abundances were normalized to the total peptide amount.

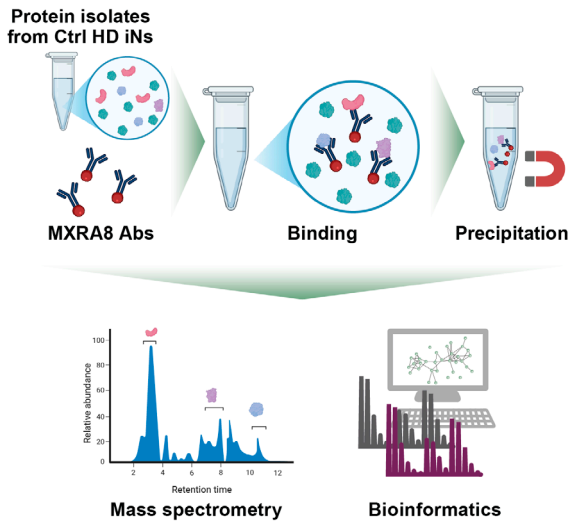


Figure 5: Schematic of anti-MXRA8 Co-IP workflow. The schematic describes the anti-MXRA8 co-immunprecipitation experiment of control and HD iNs, which was followed by LC-MS/MS.

Differential abundance analysis

Relative protein abundances were quantified in Proteome Discoverer using pairwise peptide ratio–based quantification, in which protein ratios were calculated from the ratios of associated peptide abundances. The protein-level abundance changes were

assessed using Proteome Discoverer's background-based t-test and Benjamini-Hochberg's FDR. The significance of protein-level abundance changes were determined based on an absolute log2 fold-change threshold of 1 and FDR thershold of 0.05.

The results were processed further using R. Only proteins identified with "High" confidence were included. Further, all proteins identified as differentially abundant in the anti-IgG immunoprecipitation of control fibroblasts when compared to control iNs, were further excluded as background. Finally, only proteins quantified in at least 3 samples belonging to one group were considered significant.

DNA Methylation Array

DNA methylation profiling

Genomic DNA from parental DPSCs and DPSC-derived iNs was extracted and bisulphite converted prior to genome-wide DNA methylation profiling using the Illumina Infinium MethylationEPIC v2.0 BeadChip array, following the manufacturers' protocols.

Epigenetic ageing clocks

The epigenetic clocks were applied on the DNA methylation data using the DNA Methylation Age Calculator of the Clock Foundation portal (https://dnamage.clockfoundation.org). The raw data, available as IDAT files, was uploaded to the portal, where standard data pre-processing and quality control was done prior to the application of the clocks. The results were downloaded from the website.

Generative AI

AI tools, namely Claude, ChatGPT and DeepL, were used in writing for translation of lay summary from English to Swedish, rephrasing text in several parts to reduce redundancy, improve clarity and readability, and for generating parts of the cover image of this thesis. Any AI-generated content was thoroughly reviewed by the author, who retained full responsibility for all critical analysis, interpretation, and engagement with primary sources. The AI tools served a strictly supportive function and were not involved in any analytical or decision-making processes.

Summary of Key Results

Transcriptomic ageing clocks uncover cell-type-specific progression of ageing in the human PFC (Paper I)

To measure the progression of ageing in the human prefrontal at a cell-type-specific level, and potentially predict its acceleration in pathophysiological conditions, we developed transcriptomic ageing clocks for each cell type based on snRNAseq data of human postmortem PFC tissue. We used snRNAseq, given that previous studies had highlighted the potential of single-cell transcriptomics for predicting age based on age-related transcriptomic patterns [137,147]. Additionally, it had been successfully used in characterising transcriptomic ageing in the human brain [28-30,36,37], apart from having higher throughput and scalability when compared to other single-cell/nuclei omic technologies [136].

The training dataset

First, we generated a snRNAseq dataset from human postmortem PFC tissue samples of adults, 18 – 94 years of age at the time of death, with short PMIs, spanning all three age groups of adulthood and representing both the sexes (Table 3, Fig. 6a and b). From across ~74,000 high-quality nuclei, based on the expression of known major transcriptomic markers, we identified all the major cell types of the human PFC, namely oligodendrocytes, excitatory neurons, inhibitory neurons, astrocytes, microglia and OPCs (Fig. 6c, d).

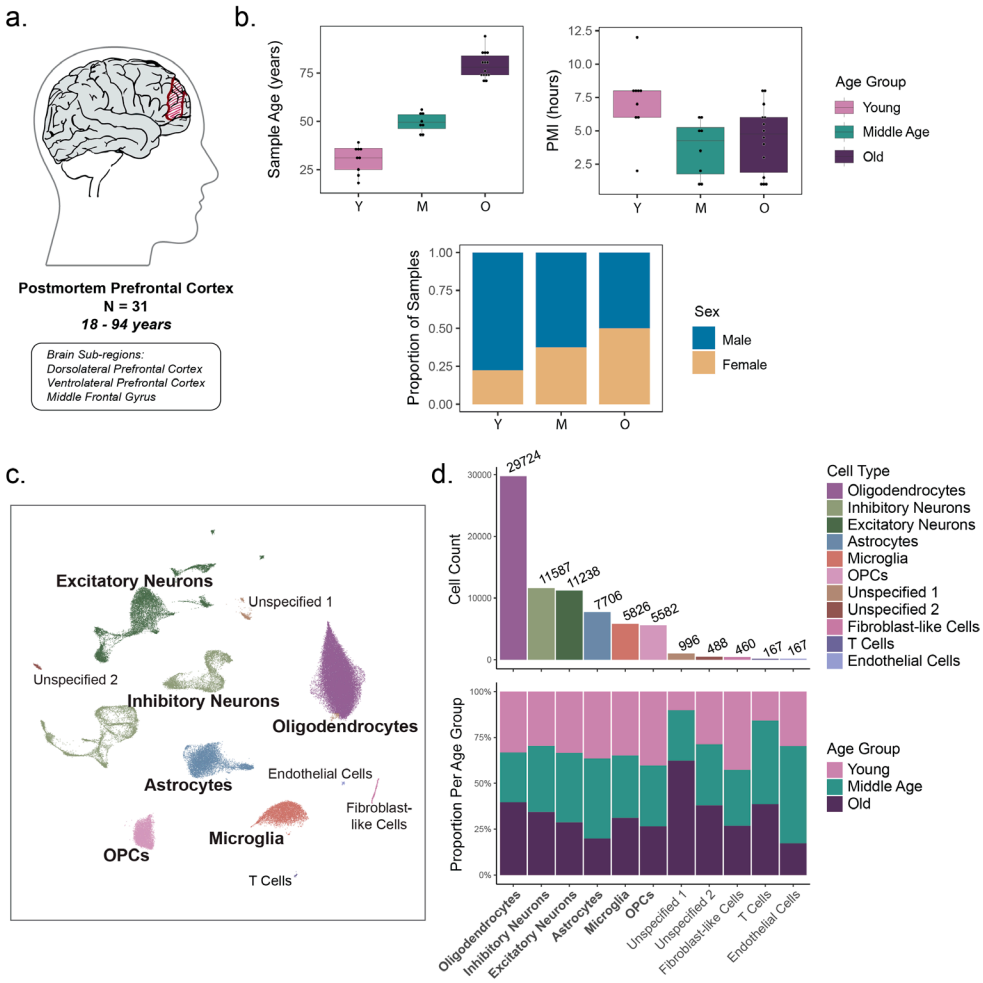


Figure 6: Characterising the training dataset. a. Schematic illustrating the PFC of the adult human brain, the region from where the postmortem tissue was collected for the training dataset. Additional details describe the number of samples, age range and specific brain sub-regions corresponding to the samples. b. The graphs show the distribution of different sample parameters of the training dataset by age groups, namely, young, middle-age and old. The parameters described, left-right then top-bottom, are donor age at the time of death (years), PMI (years) and sex ratio. c. UMAP showing the clusters identified in the training dataset. All clusters are annotated by the cell types of the adult human PFC. The major cell types that were the focus of the study are highlighted in bold. d. The top graph shows the number of cells per cell type that were identified post quality control in the training dataset. The bottom graph shows the proportion of cell found across the samples in the different age groups in each cell type.

Further, to check the suitability of the dataset for training ageing clocks, we checked for the presence of cell-type-specific age-related changes using group-wise differential gene expression comparison. We observed distinct cell-type-specific changes, with the most prominent being in microglia and astrocytes. In microglia, in line with previous findings in the aged human PFC[28-35], we found an upregulation of inflammatory response genes in the old samples (*FOXP1*, *TLR2* and *CD163*)

suggesting increased inflammatory response, while a downregulation of homeostatic microglia markers (*CX3CR1* and *P2RY12*) suggesting functional decline (Fig. 7a). Similarly, in astrocytes, we found an upregulation of genes associated with reactive astrogliosis (*TPST1*, *SAMD4A*, *CNN3*, *STAT3*, and *SORBS1*) in the old samples (Fig. 7b). With gene ontology analysis, we identified further support for our observations (Fig. 7c, d). Overall, we showed that the training dataset captured cell-type-specific age-related trends, emphasising its suitability for training cell-type-specific transcriptomic ageing clocks.

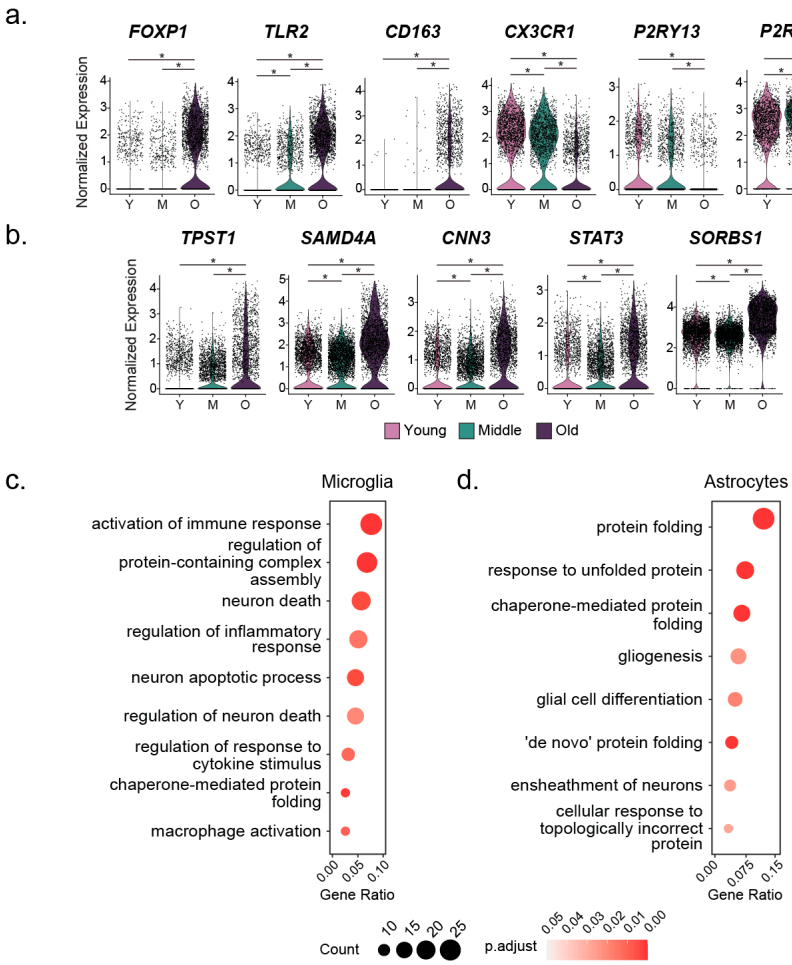


Figure 7: Prominent age-related differences in the training dataset. a-b. The violin plots show the distribution of normalized expression of selected genes significantly differentially expressed in the old samples when compared to the young or middle-age samples in the training dataset, in microglia (a) and astrocytes (b). Wilcoxon-rank sum test and Benjamini-Hochberg correction were perfomed to test the differences. c-d. The plots show the top gene ontology terms that were significanlty over-represented for genes significantly differentially expressed in the old when compared to the young samples, in microglia (a) and astrocytes (b). Significance was measured based on ClusterProfiler's over-representation test and Benjamini-Hochberg correction, with adjusted p-value < 0.05. * adjusted p-value < 0.05 and absolute log2 fold-change > 0.5.

Cell-type-specific clock predictions show strong correlation to chronological age

We developed the transcriptomic ageing clocks for each cell type, based on three approaches, namely, single-cell, simple pseudobulk and bootstrapped-pseudobulk (Refer Methods, Fig. 3), and validated them. From the testing rounds in the training dataset, the predictions from single-cell and bootstrapped-pseudobulk approaches showed the best performance, with strong positive correlation with the chronological age in all the cell types, indicating that the clocks could capture transcriptomic patterns that correlate with age (Fig. 8a, b). To test the applicability of the clocks in external datasets, we applied them on independent datasets of human adult post-mortem brain samples with a broad and continuous age range. In the dataset from Fröhlich et al 2024 [28], we observed similar trends in the predictions in individual cell types, with the single-cell and the bootstrapped-pseudobulk clocks showing positive correlations between the predicted age and the chronological age (Fig. 8c). Based on these observations, we showed that the clocks could predict age from transcriptomic patterns at a cell-type-specific level not only in the training but also in an independent dataset.

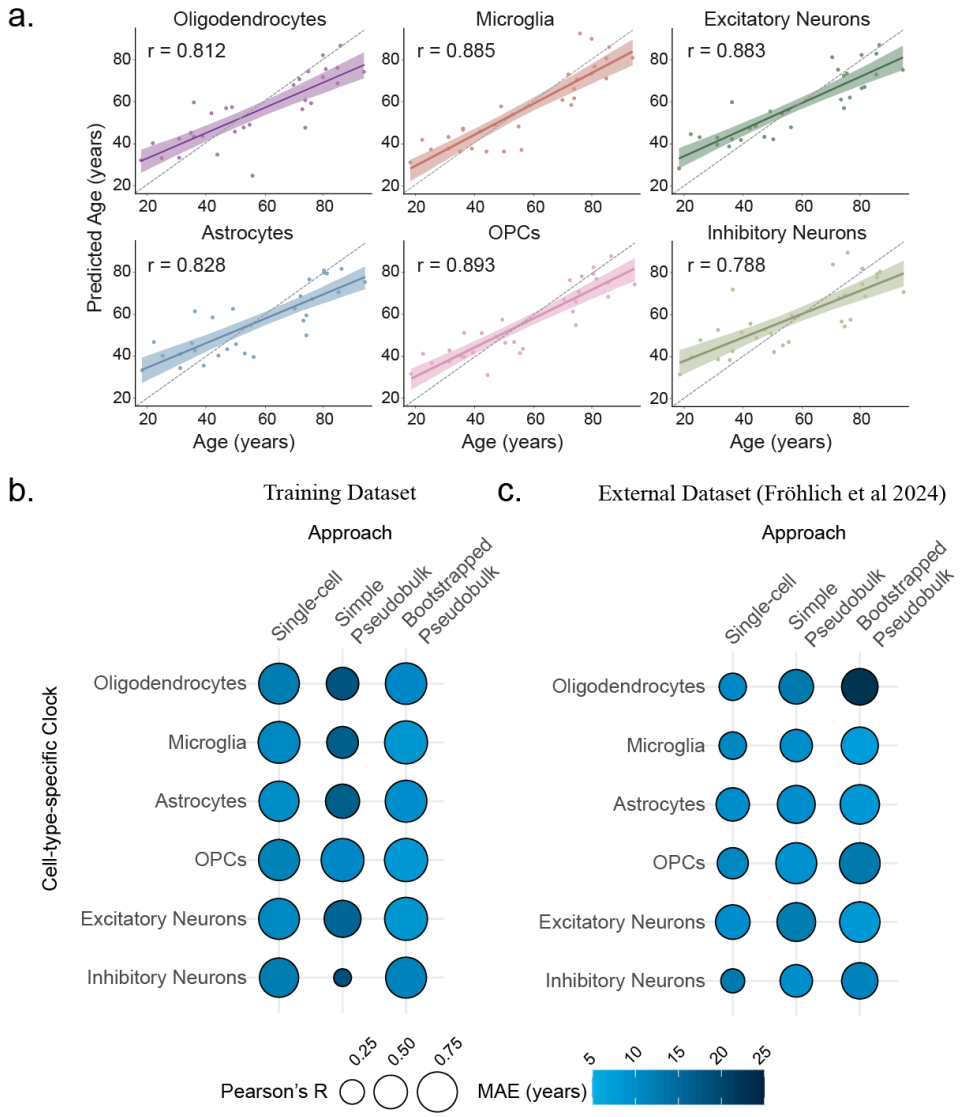


Figure 8: Validation of cell-type-specific ageing clock predictions. a. The plots describe the relationship between the age predicted by the respective cell-type-specific bootstrapped-pseudobulk transcriptomic ageing clock and the corresponding chronological age of the donors. The r value shown is the Pearson's correlation coefficient calculated for the relationship. b-c. The plot summarises the Pearson's correlation coefficient (size of circles) and the MAE (darkness of blue) that were computed in the assessment of the cell-type-specific clocks' predictions from different approaches with respect to the chronological age of the donors in the training dataset (b) and an external independent dataset from Fröhlich et al 2024 [28] (c).

Clock feature genes capture cell-type-specific ageing patterns

To check the biological relevance of the transcriptomic patterns captured by the clock models, we studied the feature gene sets associated with each cell-type-specific clock model. We found that most feature genes in every clock model were unique to the respective cell type, indicating the cell-type-specificity of the transcriptomic patterns captured (Fig. 9a). The only gene shared across all the clock models, was *FKBP5*, which had a strong positive correlation with age (Fig. 9b). This was in line with previous findings that indicate an upregulation of *FKBP5* with age and its association with altered cognitive function in the human brain [148-150]. Furthermore, with gene ontology analysis on the clock feature genes, particularly in the single-cell clock models, we found an enrichment of biological processes related to inflammatory response in the microglia clock for genes with a positive average coefficient, suggesting a positive association between the corresponding genes' expression with increasing age (Fig. 9c). On the other hand, in the neuronal and other glial cell-type-specific clocks, genes with negative average coefficient were associated with trans-synaptic signalling and synapse formation (Fig. 9c). Previous studies have shown a similar trend in gene expression in the corresponding cell types during ageing in the human PFC [28-35]. Overall, our observations indicated that the clock models captured biologically relevant cell-type-specific ageing pathways, thereby strongly suggesting the physiological relevance of their predictions.

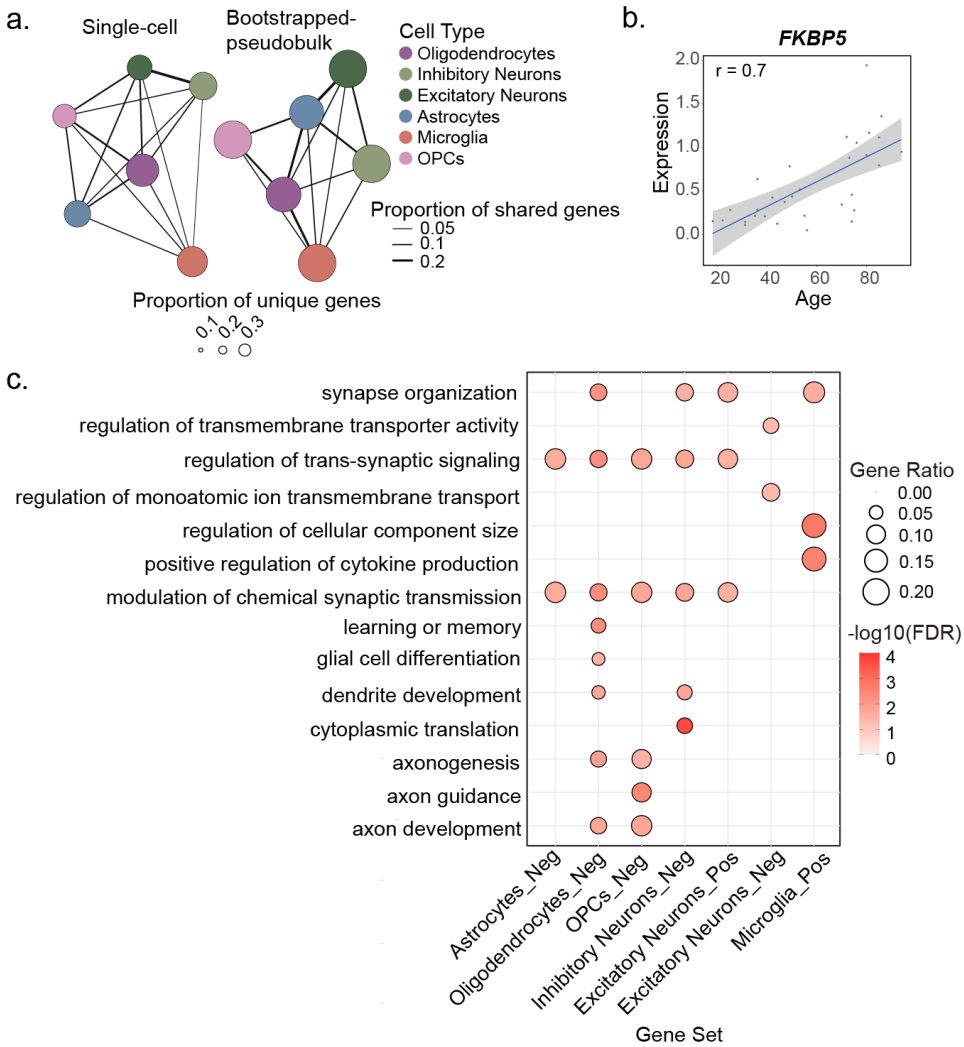


Figure 9: Biological relevance of clock models. a. The plot describes the proportion of unique (size of circle) and shared (width of the connecting lines) clock feature gene sets in the cell-type-specific ageing clock models of single-cell (left) and bootstrapped-pseudobulk (right) approaches. b. The plots show the correlation between age and donor-wise average expression of the gene *FKBP5*. A Pearson's correlation test was performed to compute the Pearson's correlation coefficient. Significance was measured at p-value < 0.05. c. The plot summarises the results of top gene ontology terms enriched from across the gene over-representation tests of single-cell cell-type-specific clock feature gene sets, separately on those with positive and negative average coefficient. The tests were performed using ClusterProfiler with Benjamini-Hochberg correction. Signficance was measured at adjusted p-value < 0.05.

Cell-type-specific clocks predict age acceleration in neurological disorders

To determine the relevance of the clock predictions in pathophysiological conditions, where in many cases ageing is known to be accelerated [28,41,61-72], we applied the clock models on previously published snRNAseq datasets of cohorts diagnosed with AD [78] or SCZ [28]. In all cases, we calculated age acceleration in the predictions (Refer Methods) and compared the same between the pathological conditions and their respective controls. Overall, in AD samples, the clock predictions showed a significant acceleration of age in oligodendrocytes and microglia, while in the remaining cell types, the predictions had similar but not significant trends (Fig. 10a). In SCZ samples on the other hand, all cell types, except the excitatory neurons showed a significant acceleration of age (Fig. 10b). Our results highlighted that the clock models could predict cell-type-specific alterations in pathophysiological ageing.

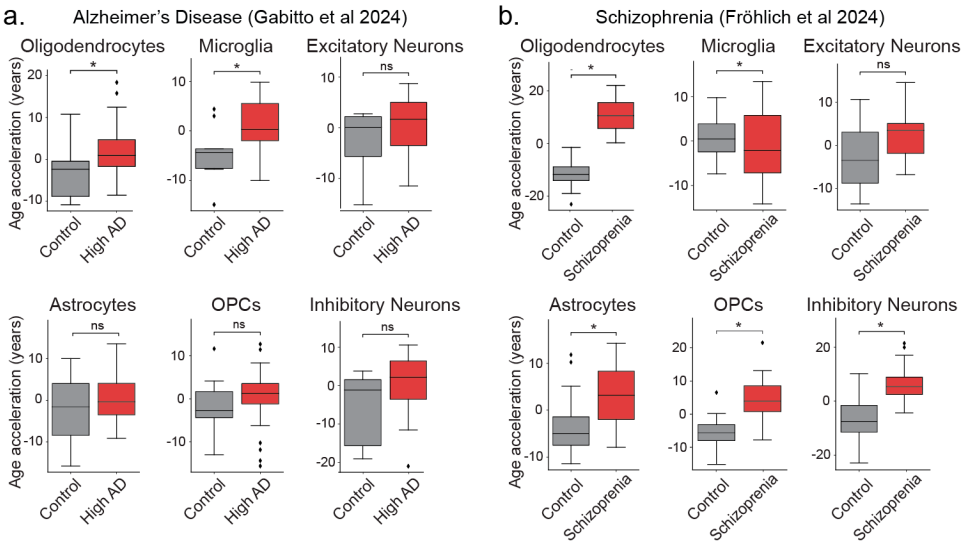


Figure 10: Application of ageing clocks in neurological disorders. a-b. The box plots show the distribution of age acceleration in (a) the control and High-AD samples from the dataset published in Gabitto et al 2024 [78], and (b) the control and SCZ samples from Fröhlich et al 2024 [28]. *p < 0.05, generalised linear model was used to compute the difference between the control and disease samples.

In summary, we developed transcriptomic ageing clocks for the human PFC that measure biological age at a cell-type-specific level. Further, the clocks capture biologically relevant cell-type-specific transcriptomic patterns and predict age acceleration in pathophysiological ageing. The clocks overall represent important tools to measure the progression of ageing at a cell-type-specific level in both physiological and pathophysiological ageing.

Phosphoproteomic profiling of HD patient-derived iNs (Paper II)

To investigate neuronal phosphoproteomic changes in HD, we performed phosphopeptide-enriched LC-MS/MS on an established iN model of HD (Refer methods, Fig. 4) [119]. LC-MS/MS-based phosphoproteomics was chosen for its ability to provide large-scale, unbiased and quantitative profiling of phosphorylation events across the proteome, a resolution that antibody-based approaches cannot achieve at scale [138]. The model, derived from dermal fibroblasts of HD-patient donors, was previously shown to have several pathological features of HD, with a clear dysfunction in autophagy, strong proteomic differences showing several affected kinases and phosphatases, and an accelerated epigenetic age based on DNA methylation patterns [119]. We used iNs derived from seven HD-patient donors and equal number of age-matched controls for the study and combined it with the previously published global proteomic (LC-MS/MS) dataset from the same samples to draw parallel insights (Table 1) [119].

HD iNs show a distinct phosphoproteomic profile

We identified a total of 4,183 phosphopeptides corresponding to 1,409 unique phosphoproteins in our dataset. Upon performing PCA, we observed a grouping of samples based on the disease condition, with a distinction stronger than that previously observed in the global proteomic dataset (Fig. 11a). This indicated the presence of more characteristic differences at the phosphoproteomic level in HD. To identify the potentially dysregulated candidates, we performed a differential abundance analysis comparing the HD and control iN samples and identified 71 phosphopeptides (corresponding to 55 proteins) significantly upregulated in HD, while 106 phosphopeptides were downregulated (corresponding to 86 proteins) (Fig. 11b). Upon checking overlap with phosphoproteins dysregulated at the proteomic level, we identified only 8 in total. All the overlapping proteins were downregulated at the phosphopeptide level while upregulated at the proteomic level (Fig. 11c). The small size of the overlap further highlighted the distinct profile of HD-iNs at a post-translational level and indicated that protein regulation transcends beyond transcriptomics and global proteomics.

Phosphoproteomic disruption of house-keeping pathways in HD-iNs

To inspect the functional consequences of the phosphoproteomic differences, we performed a pathway analysis on the proteins corresponding to the differentially abundant phosphopeptides (refer methods), and found an over-representation of autophagy-related pathways, such as PTEN regulation or PIP3-AKT signalling among the upregulated phosphopeptides (Fig. 12a), while mRNA splicing pathways were associated with the downregulated phosphopeptides (Fig. 12b). This suggested a post-translational dysregulation of autophagy and mRNA splicing pathways in the

HD-iNs. The former was further supported when we identified a complete overlap with our dataset, the phosphoproteins that were previously identified to be dysregulated in autophagy disruption of hiPSC-derived neuronal models (CRISPRi-*ATG7* and *ATG14*) (Fig. 12c) [151]. Our observations were in line with the known disruption of autophagy and splicing pathways in HD and strongly suggested the involvement of post-translational dysregulation in the same.

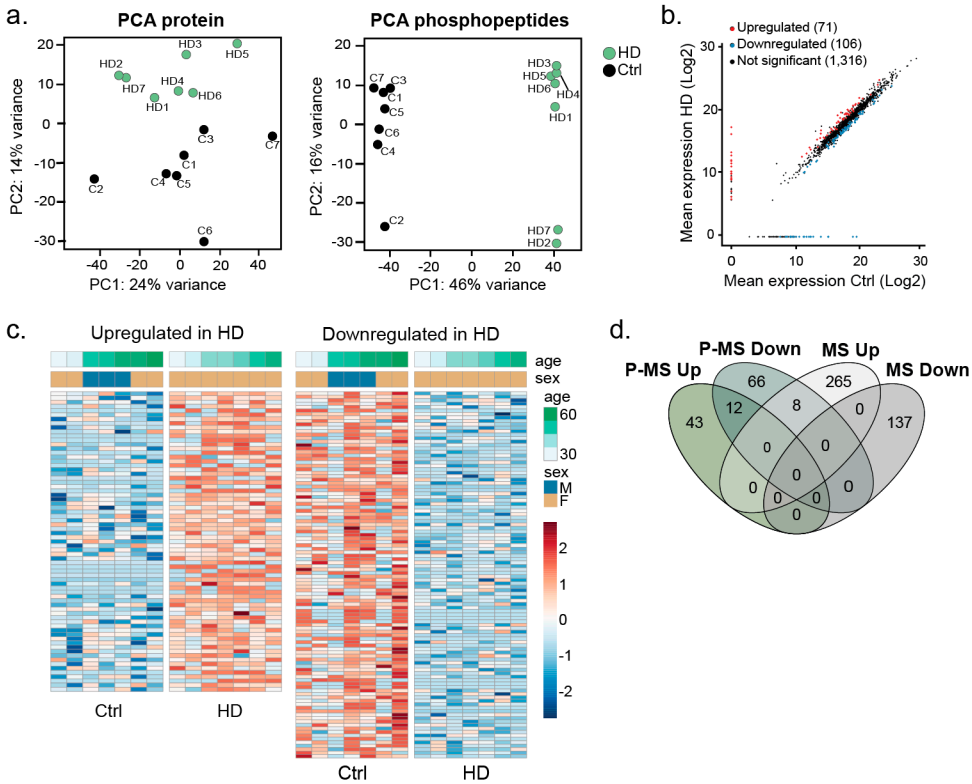


Figure 11: Phosphoproteomic landscape of HD-iNs. a. PCA plots based on global LC-MS/MS (left) and phospho LC-MS/MS (right) datasets of 7 HD and 7 control iN samples. b. Scatter plot showing the distribution of log2 mean abundance of phosphopeptides in iNs from Ctrl (x-axis) and HD (y-axis) samples. The phosphopeptides significantly upregulated in HD-iNs when compared to Ctrl-iNs are represented by red dots, while those that are significantly downregulated are represented by blue dots, and the non-significant ones by black dots (Shapiro-wilk test $p > 0.05$; unpaired t-test $p < 0.05$). c. Heatmaps showing the abundance of upregulated phosphopeptides (left) and downregulated phosphopeptides (right) in each sample. d. Venn diagram showing the number of overlapping genes predicted to be dysregulated in the global proteomic and phosphoproteomic datasets of control and HD iNs.

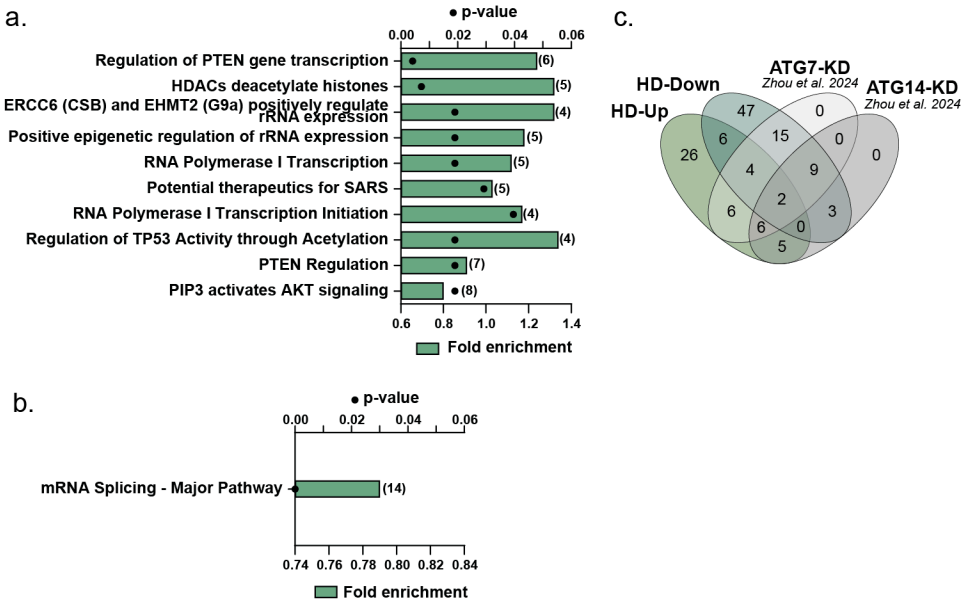


Figure 12: Functional characterisation of phosphoproteomic changes in HD-iNs. a-b. Top reactome pathways associated with genes corresponding to phosphopeptides significantly (a) upregulated or (b) downregulated. Based on pathway enrichment analysis using String-db v12.0. Significance is based on p-value < 0.05. c. Venn diagram showing the number of overlapping phosphoproteins predicted to be dysregulated in HD-iNs and in autophagy disruption experiments of hiPSC-iNs.

Dysregulated kinase activity in HD-iNs

We checked the kinases potentially involved in the dysregulation of the differentially abundant phosphopeptides. To do this, we performed a PSEA on the phosphosites associated with the candidate phosphopeptides (refer methods) and identified 48 kinases with potentially increased activity in the HD-iNs, while 20 with decreased activity (Fig. 13a). Among these kinases, majority of those with increased activity belonged to the CAMK and AGC families, while those with decreased activity belonged to the STE family (Fig. 13b). Further, when we checked the proteomic abundance of the kinases with dysregulated activity, only a few kinases were significantly differentially abundant at the proteomic level, and we found 4 of these, namely, CAMK2G, CAMKK2, STK10 and EEF2K to have kinase activity and differential abundance in the same direction (Fig. 13c). Our findings further supported the alterations in phosphorylation and identified potential drivers of the dysregulation in the HD-iNs. This additionally attributes the identified kinases as potential therapeutic targets to alleviate the dysregulation.

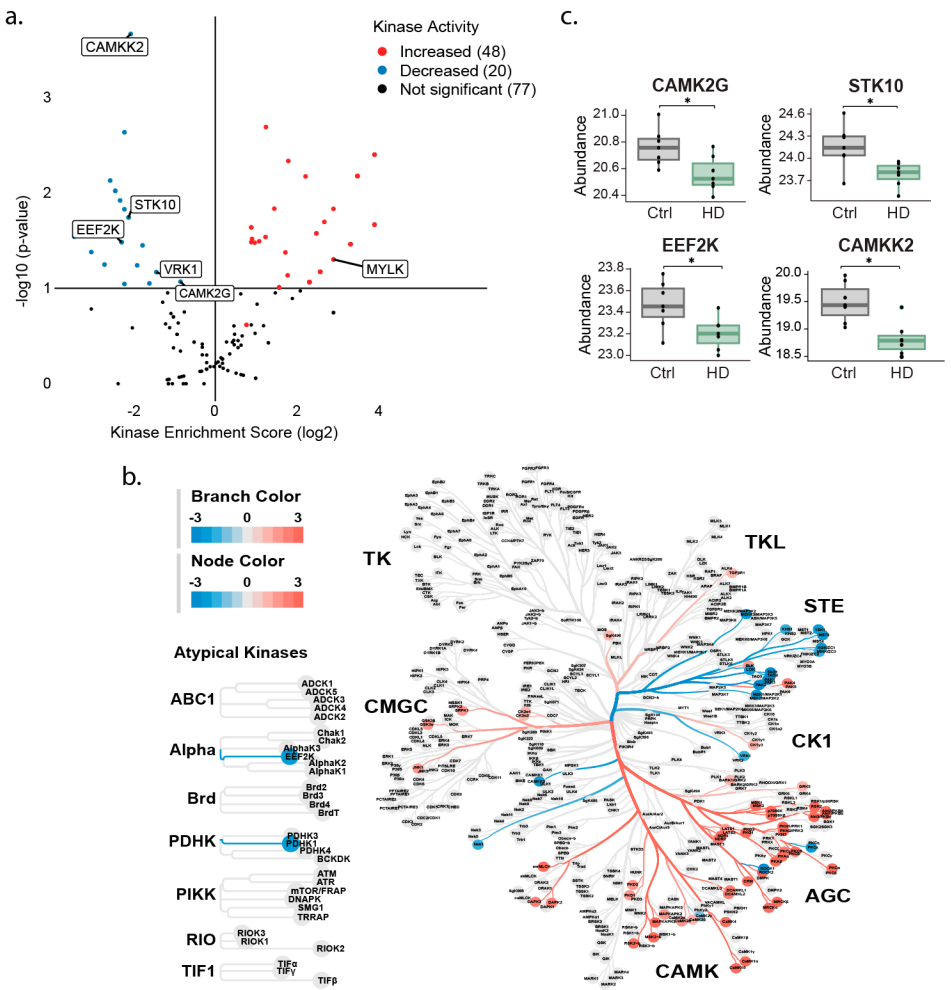


Figure 13: Kinase dysregulation in HD-iNs. a. The scatter plots shows the distribution of predicted kinases from PSEA of the differentially abundant phosphopeptides in the HD-iNs dataset. Significance was measured based on p-value < 0.05. b. The kinase tree shows the highlights the kinases predicted based on increased or decreased log2 fold-change of activity (predicted) in HD-iNs. c. The box plots show the distribution of proteomic abundance of shortlisted kinases. Shapiro-Wilk and unpaired t-test were used to test the difference between HD and control iNs. *Shapiro-Wilk p-value > 0.05 and t-test p-value < 0.05.

MXRA8 – a phosphoswitch with therapeutic potential

Among the various phosphopeptides we identified to be potentially dysregulated in the HD-iNs, we found subsets of phosphoswitches, that were completely absent in either the controls or the HD-iNs (Fig. 14a, b). 27 of these, corresponding to 26 phosphoproteins had no abundance in the HD-iNs (ON-OFF) (Fig. 14a), while 19 phosphopeptides corresponding to 17 phosphoproteins, had no abundance in the controls (OFF-ON) (Fig. 14b). With the general transitionary state of protein

phosphorylation, the phosphoswitches overall, with their condition-specific phosphorylation, represent potentially robust candidates for discovering pathological mechanisms tightly coupled to phosphorylation and can serve as therapeutic targets.

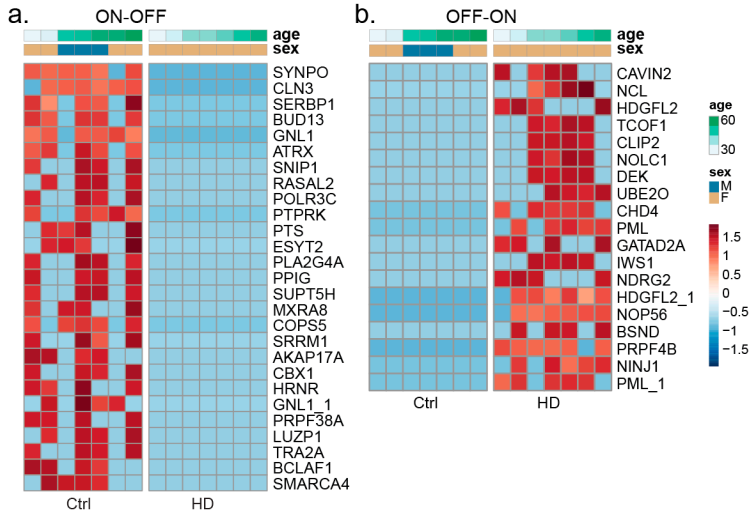


Figure 14: Phosphoswitches in HD-iNs. The heatmaps show abundances of (a) ON-OFF and (b) OFF-ON phosphopeptides.

When we inspected the global proteomic abundance of the phosphoswitches, among the ON-OFF phosphopeptides, Matrix-remodelling protein 8 (MXRA8) with phosphorylation detected on a serine at position 377 of the protein, was the only phosphoswitch that was differentially abundant at the proteomic level (Fig. 15a). Interestingly, the protein had an increased abundance in HD-iNs, while the phosphorylation was absent (Fig. 15a), suggesting a potentially compensatory mechanism at a proteomic level for the lack of phosphorylation. Further, given that MXRA8-pS377 was mono-phosphorylated and unique in the dataset, it showed potential as a suitable target for follow-up studies.

MXRA8 had previously been characterised for its role in glial cells, particularly in mediating blood-brain barrier integrity and astrocyte-associated neuroinflammation [152,153]. However, its role in neurons have been unknown. In this regard, we first validated its expression in neurons using immunostaining in iNs, where we counterstained anti-MXRA8 with the mature neuronal marker, TAU. We observed its expression largely in the cell bodies of the TAU+ cells, with a significant increase in the spot area and spot count in the HD-iNs, suggesting an increase in its expression (Fig. 15b), in line with our findings in the global proteomic data. We found further support for its neuronal expression in human postmortem cortical samples from HD patients and corresponding controls, where anti-MXRA8

immunostainings were consistently visible in the cell bodies of MAP2+ cells across all the samples (Fig. 15c). Taken together, our findings established that MXRA8 is expressed in human neurons, revealing a previously unrecognized neuronal expression of this protein.

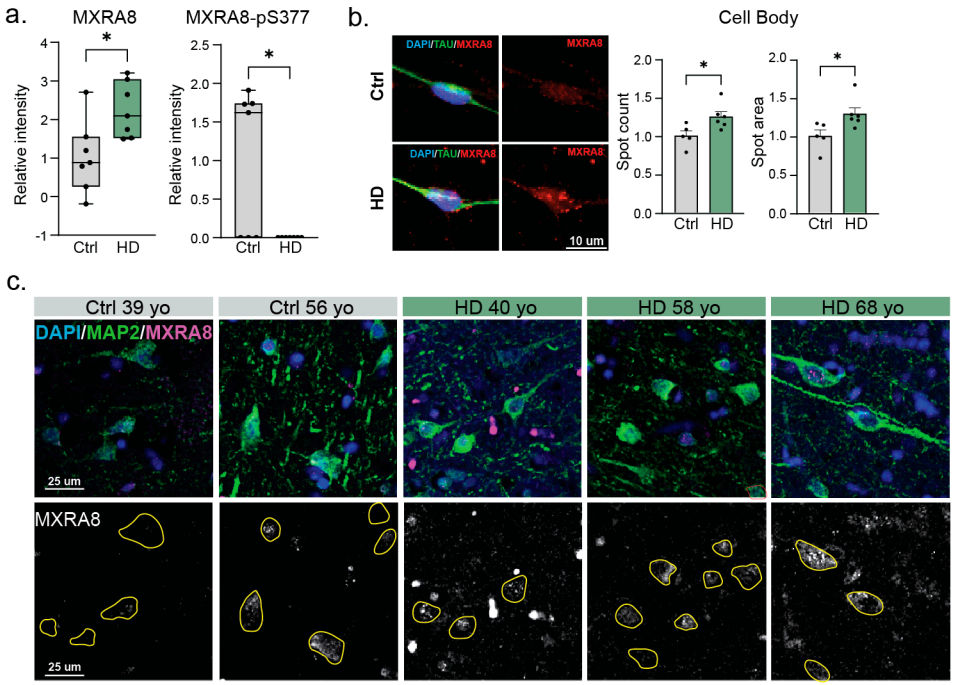


Figure 15: Neuronal expression of MXRA8. a. The box plots show the abundance of MXRA8 protein in the global proteomic dataset (left) and the MXRA8-pS377 phosphopeptide in the phosphoproteomic dataset, in 7 control and 7 HD-iNs. b. MXRA8 localisation and abundance were validated by high content analysis in TAU counterstained samples of 7 HD-iNs and 7 controls. The bar graphs on the left show spot count, while that on the right shows spot area, both within the cell body. The representative images show one control and one HD-iN sample immunostained with DAPI, TAU and MXRA8. c. The images represent IHC validation of MXRA8 protein expression in post mortem human cortical brain samples from 2 Ctrl and 3 HD individuals counterstained with MAP2 and DAPI. The yellow outlines mark neuronal cell bodies based on MAP2 staining. Scale bar 25 µm. (b-c) Each dot represents one one HD or one control adult human iN cell line, the columns of the bars show the mean value of the individual values, the error bar indicates SEM. For statistical analysis two tailed t-tests were used, * $p<0.05$.

To speculate the potential neuronal function of MXRA8 and the molecular pathways it is involved in in HD-iNs, we performed Co-IP using anti-MXRA8 antibodies across 4 HD-iNs and 4 matching controls, followed by LC-MS/MS to identify the potential binding partners differentially abundant in HD-iNs (refer methods) (Fig. 5). In total, among the top 20 such candidates, we found 6 of them to be robustly identified in at least half the samples in either one of the conditions (Fig. 16). Of those, based on the abundance ratio, we predicted TGM2 and LAMP1, components of autophagy [154,155], respectively, to have increased binding to MXRA8 in HD-iNs (Fig. 16). On the other hand, we predicted CYP1B1, involved in the maintenance of

blood-brain barrier integrity [156,157], PFKP, a vital enzyme in the glycolysis pathway [158], ESYT1, regulator of membrane calcium transport [159], to have reduced binding (Fig. 16). The binding partners we predicted overall reflected a potential role of MXRA8 in neurovascular integrity and stress-response pathways that may be involved in neurodegeneration.

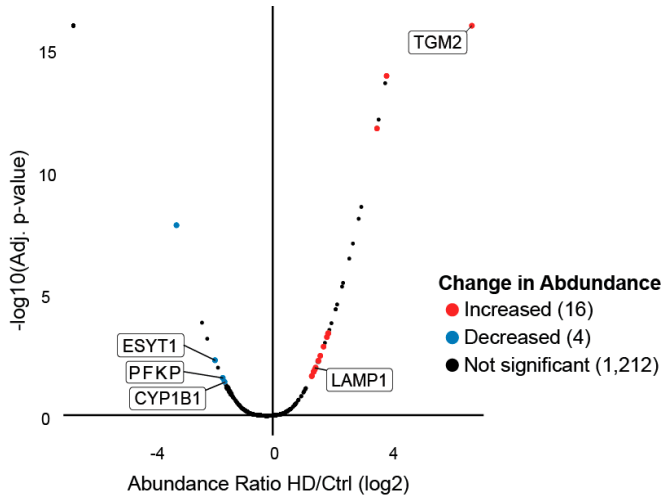


Figure 16: Binding partners of MXRA8. The volcano plot shows binding partners of MXRA8 of 4 control and 4 HD-iN samples. On the volcano plot binding partners with increased abundance in HD-iNs are shown in red, while those with decreased abundance are shown in blue and the non-significant ones by black dots. Significance was measured based on an adjusted p-value < 0.05 among other parameters.

In summary, our analysis revealed distinct phosphoproteomic dysregulation in HD patient–derived iNs, particularly affecting autophagy and mRNA processing pathways. This enabled the identification of potential therapeutic targets, including kinases, ON–OFF phosphopeptides and a novel phosphoprotein with a previously uncharacterised role in neurons or neurodegeneration. Together, the findings highlighted the importance of studying PTMs and the value of iNs as an *in vitro* model for investigating neuron-specific mechanisms in neurodegenerative disease.

Direct neuronal reprogramming of hDPSCs (Paper III)

DPSCs represent an excellent alternative somatic source for direct neuronal conversion, as they are neural crest–derived and therefore share developmental lineage with the central nervous system (CNS) [128]. In addition, DPSCs are readily obtainable from exfoliated deciduous or extracted wisdom teeth, where dental pulp is typically discarded as biological waste, making them an accessible and minimally invasive cell source [127,129]. Based on these advantages, we evaluated the generation, and characteristics of iNs derived from DPSCs. We used our previously established protocol for direct neuronal conversion of dermal fibroblasts, and optimized it for DPSCs (refer methods, Fig. 2). The DPSCs were derived from the extracted third molars of 4 adult human donors (Table 1).

DPSC-iNs reveal neuronal phenotype at an earlier time point

At day 17 post initiating the conversion, we observed a clear neuronal morphology, a timeline about twice as fast as that for dermal fibroblasts (Fig. 17a). When we checked the immunostaining of known mature neuronal markers, the converted cell lines (henceforth referred to as DPSC-iNs) showed a significantly higher percentage of NeuN+, TUJ1+, MAP2+ and TAU+ cells when compared to the corresponding DPSC lines (Fig. 17b, c). On the other hand, we observed a significantly reduced proportion of SSEA3+ cells, a marker for hDPSCs, in the DPSC-iNs. To assess differences in conversion yield, we compared the proportion of TAU+ cells across four fb-iN and four DPSC-iN samples, revealing a significantly higher proportion in DPSC-iNs than in fb-iNs (Fig. 17d). Taken together, these findings demonstrate that DPSCs can convert into neurons expressing mature neuronal markers at approximately twice the rate of dermal fibroblasts and with a superior yield.

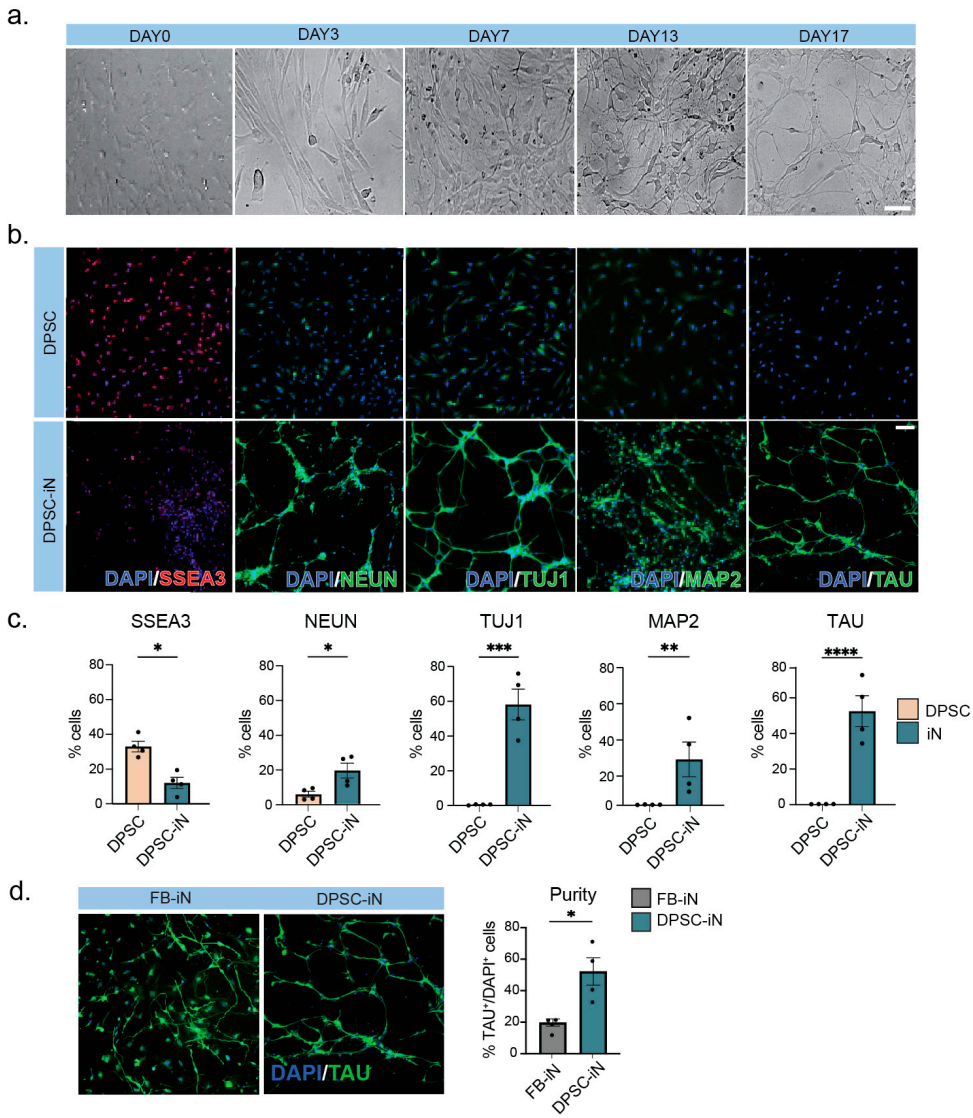


Figure 17: DPSC-iNs show neuronal phenotype. a. The brightfield images show morphological progression from spindle-shaped DPSCs (day 0) to neuron-like cells with neurites (day 7 - 17). Scale bar: 200 µm. b. The ICC images represent DPSCs and DPSC-iNs stained for SSEA3, NeuN, TUJ1, MAP2 and TAU. c. HCA quantification of marker expression (% of $DAPI^+$ cells, n = 4 donors). d. The ICC images are a representative comparison of neuronal purity ($\%TAU^+/DAPI^+$) between fibroblast-derived iNs and DPSC-derived iNs (n = 4 donors each). The bar plot shows significantly higher purity in DPSC-iNs. All results are shown as mean ± SEM. Paired two-tailed t-tests were used and significance was measured at *p < 0.05, **p < 0.01, ***p < 0.001.

Multi-omic profile of DPSC-iNs show mature neuronal characteristics

To further define the molecular profiles of the DPSC-iNs, we performed bulk RNA sequencing (bulk-RNAseq) and LC-MS/MS-based proteomics on the native and converted cell lines. For bulk-RNAseq, we sorted NCAM1+ cells in the DPSC-iNs, to only include cells expressing the neuronal marker. With PCA, we observed a clear separation between the native DPSC and DPSC-iN cell lines in both the transcriptomic and proteomic dataset, indicating a strong distinction in their molecular profiles at both transcriptional and translational levels (Fig. 18a). To further identify the specific molecular changes, we performed a differential expression comparison between the DPSC-iN and the native DPSC cell lines. Overall, we observed a high number significantly differentially expressed genes and proteins at the transcriptomic and proteomic levels respectively, indicating profound shifts in the molecular profiles during the conversion (Fig. 18b).

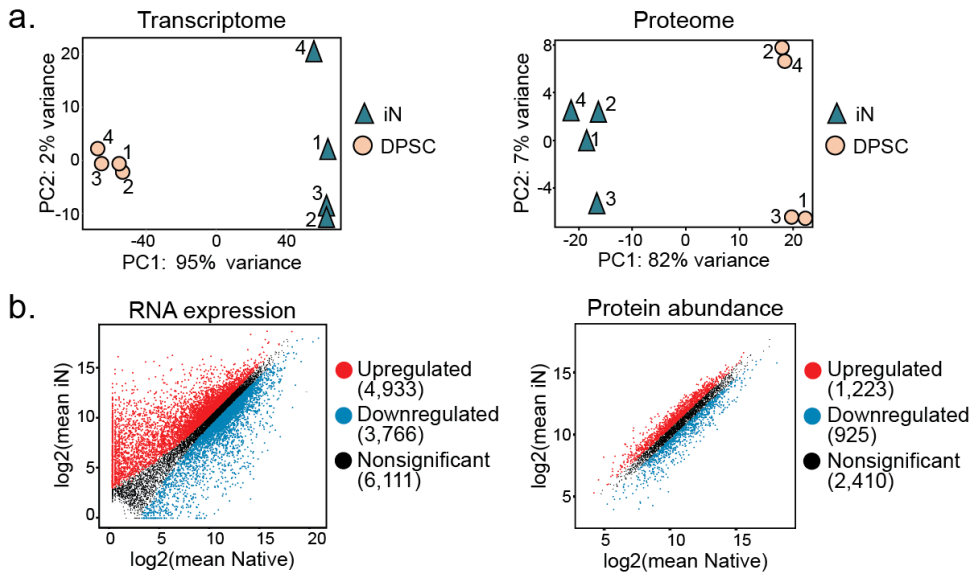


Figure 18: DPSC-iNs show a distinct transcriptomic and proteomic profile. a. PCA plots based on the top 500 most variable protein-coding genes in the bulk RNA-seq dataset (left) and the top 500 most variable proteins in the LC-MS/MS dataset (right) showing clear separation between DPSCs and DPSC-iNs across four donors (n = 4 donors). b. Scatter plot showing the distribution of log2 mean abundance of genes in the bulk RNA-seq data (left) and LC-MS/MS proteomic data (right) in native DPSC (x-axis) and DPSC-iN (y-axis) samples (n = 4 donors). Significance was measured based on adjusted p-value < 0.05, and absolute log2 fold-change > 0.5 (bulk RNA-seq) or > 1 (LC-MS/MS).

Using functional enrichment analysis, we found the genes upregulated at the transcriptomic level in DPSC-iNs were associated with neuron-related biological processes such as axonogenesis and synapse signalling, while the downregulated genes were associated with DNA replication and mitosis (Fig. 19a). At the proteomic level, we found the upregulated proteins in DPSC-iNs to be associated

with reactome pathways related to oxidative phosphorylation and lipid metabolism, while those related to mitotic cell cycle were downregulated (Fig. 19b). Differentiated post-mitotic neurons have been known to depend on oxidative phosphorylation and lipid metabolism to support their energy demands and synaptic transmission [160]. Above observations indicated a molecular shift in DPSC-iNs towards a mature post-mitotic neuronal profile across transcriptomic and proteomic levels of gene expression.

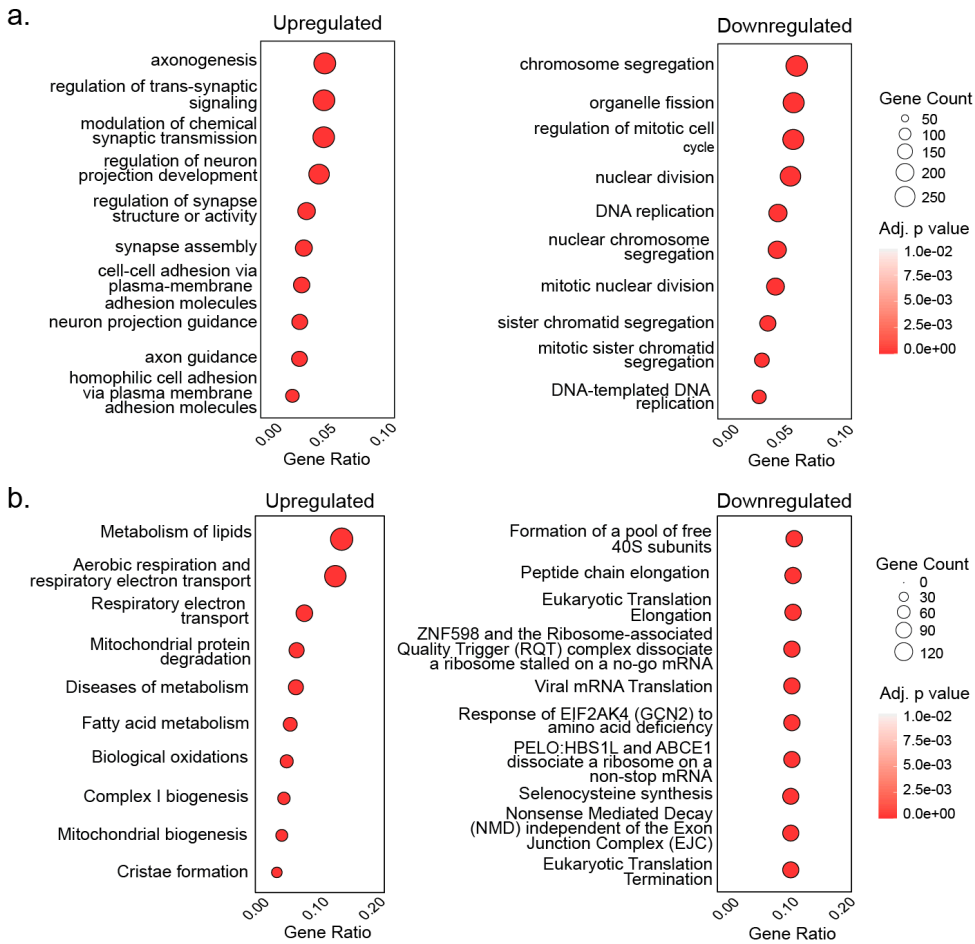


Figure 19: Functional relevance of transcriptomic and proteomic profiles DPSC-iNs. a. Plots show the top gene ontology terms (biological processes) enriched for the up- (left) and downregulated genes in DPSC-iNs when compared to the DPSCs in the bulk RNA-seq data. b. Plots show the top reactoem pathways enriched for the up- (left) and downregulated genes in DPSC-iNs when compared to the DPSCs in the LC-MS/MS data. In all cases, an over-representation test was used using either ClusterProfiler or ReactomePA, and Benjamini-Hochberg correction was used for determining FDR. Signficance was measured at adjusted p-value < 0.05.

Our observations were further supported by the differential expression of several well-known markers, used previously to characterise fibroblast-neuron conversions, across transcriptomic and proteomic levels (Fig. 20a, b). We found DPSC or mesenchymal-stem cell markers, such as CD44 (/*CD44*) and ENG (/*ENG*), to have lower expression in DPSC-iNs, indicating a loss of the corresponding identity (Fig. 20a, b). This was further supported by the downregulation of epigenetic modifiers PRC1 (/*PRC1*) and DNMT1 (/*DNMT1*) in DPSC-iNs, that are known to help maintain an undifferentiated state in cells. Further, several mitotic cell cycle genes, such as MCM2 (/*MCM2*), MKI67 (/*MKI67*), TOP2A (/*TOP2A*) and PCNA (/*PCNA*) were downregulated in DPSC-iNs strongly indicating the exit from the mitotic cell cycle (Fig. 20a, b). Neuronal profile of DPSC-iNs was indicated by the upregulation of pan-neuronal markers such as TUBB3 (/*TUBB3*), NCAM1 (/*NCAM1*) and NEFM (/*NEFM*). At the transcriptomic level, several other post-mitotic mature neuronal markers such as *MAPT*, *MAP2*, *SYN1*, *CDKN1A* and *CDKN2A* were also found to be upregulated in DPSC-iNs.

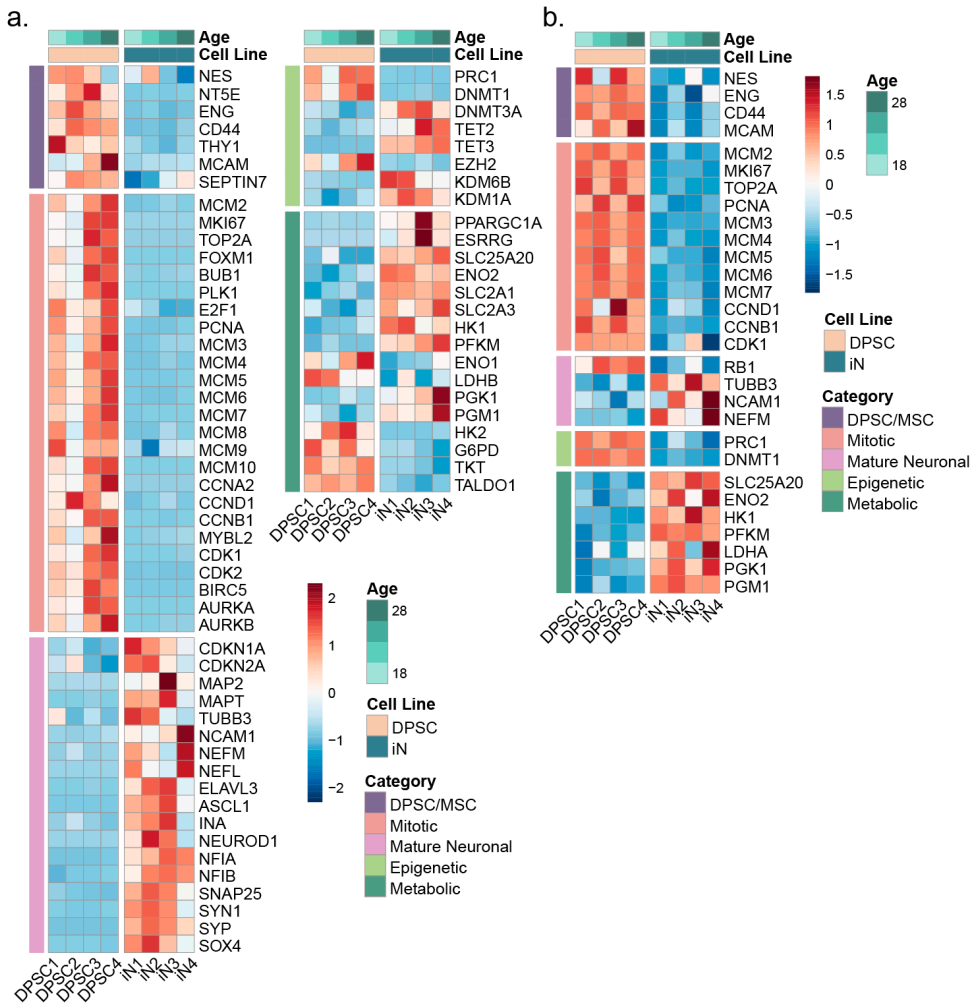


Figure 20: Expression of neuronal markers at transcriptomic and proteomic levels in DPSC-iNs. The heatmaps show expression of Mesenchymal Stem Cells (MSC)/DPSC, cell cycle and neuronal markers in (a) bulk RNA-seq data and (b) LC-MS/MS data across the DPSC and DPSC-iN samples (n = 4 donors).

Indications of epigenetic age-recapitulation in iNs

To check whether the DPSC-iNs capture age-associated molecular changes, an essential feature for an *in vitro* model of ageing, we computed the epigenetic age of the cells. We profiled the methylome of the native and DPSC-iN cell lines using DNA methylation array (refer methods) and applied DNA methylation-based cortical ageing clock on the dataset [134]. While the correlation between the predicted age and chronological age in the DPSC-iNs was not statistically significant, we observed a clear trend with a Pearson's coefficient of 0.88. We reasoned that a larger sample size and wider age range, would help necessary for statistical significance.

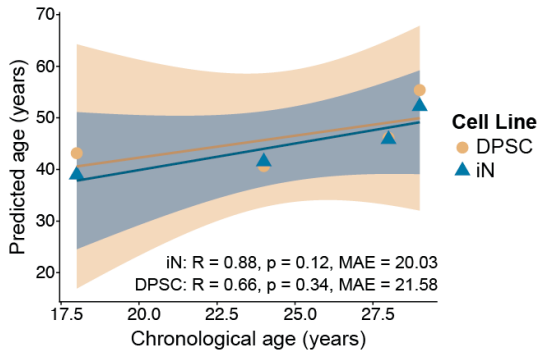


Figure 21: Epigenetic age prediction in iNs. The scatter plot shows age prediction in the DPSC-iNs and the corresponding DPSCs by the DNAm Cortical clock. A person's correlation test was performed, each in DPSCs and DPSC-iNs, between the chronological age and predicted age to measure significance. Significance was calculated at p-value < 0.05.

In summary, DPSCs converted to iNs at a faster rate and with higher yield compared to dermal fibroblasts, demonstrating superior conversion efficiency. The resulting cell lines exhibited distinct transcriptomic and proteomic profiles, reflecting a clear shift in cellular identity toward a pan-neuronal, post-mitotic state. Furthermore, DPSC-derived iNs showed indications of epigenetic age retention, suggesting their potential as *in vitro* neuronal models for studying ageing.

Discussion, Conclusions and Future Perspectives

Ageing in the human brain is a major risk factor for most neurodegenerative diseases [2,11]. The role of age-related molecular changes, particularly those at a cell-type-specific level contributing to this risk remain poorly understood. This thesis makes a collective contribution toward addressing this gap by investigating molecular changes associated with physiological and pathophysiological brain ageing at multiple levels of gene regulation – from transcriptomic dynamics in individual cell types, through post-translational phosphoproteomic dysregulation, to the development of improved cellular models for studying these changes in a human neuronal context.

The cell-type-specific transcriptomic ageing clocks presented in Paper I represent a vital step forward in understanding the progression of ageing in the major cell types of the human PFC. That the majority of genes in each clock's feature set were unique to the corresponding cell type underscores the cell-type-specificity of molecular ageing at the transcriptomic level. Further, the ability of the clocks to predict accelerated age in different cell types in AD and SCZ, highlights the cell-type-specificity of the age-related molecular mechanisms exacerbated in pathophysiological ageing. In AD, age acceleration was significant in microglia and oligodendrocytes but not in the remaining cell types, which may reflect the heterogeneity within broader cell type classifications and acceleration may be more pronounced in specific subtypes that are diluted when considered at the cell-type level. Training the clocks at a sub-cell-type resolution would therefore represent a major improvement, potentially enabling more precise prediction of selective vulnerability in neurodegeneration. Additionally, given the strong regional specificity of molecular ageing in the human brain, extending this framework beyond the PFC to other regions could provide region-specific insights into vulnerability. Training on larger datasets with uniform age group representation and greater demographic and ethnic diversity would further enhance the generalisability of the clocks across external datasets.

Paper II extends the molecular investigation of pathophysiological ageing beyond the transcriptome, addressing the underexplored layer of post-translational regulation. The high-throughput phosphoproteomic characterisation of HD patient-derived iNs revealed widespread dysregulation in key homeostatic signalling

pathways, including autophagy, and identified potential therapeutic targets, demonstrating that PTM-level profiling can uncover disease-relevant molecular changes that transcriptomic approaches would miss. This reinforces the broader argument that a comprehensive understanding of molecular ageing requires integrating multiple levels of gene regulation. Future work could extend this approach to other PTMs of relevance to ageing and neurodegeneration. Ubiquitination, which is central to proteostasis through its role in tagging proteins for degradation, is a particularly promising avenue, as dysregulation of the ubiquitin-proteasome system is a well-established feature of both ageing and proteinopathies such as AD and HD [100,161].

Several challenges inherent to PTM research will need to be addressed in this pursuit. The transient nature of many modifications makes them difficult to capture reliably, and many of the phosphorylation events identified by LC-MS/MS-based approaches, including in our own dataset, have not previously been functionally characterised, making experimental validation challenging [162,163]. This gap between discovery and functional validation is an important bottleneck in translating phosphoproteomic findings into therapeutic leads [163]. Furthermore, substrate-enzyme relationships for many PTMs, particularly for phosphatases in the context of protein phosphorylation, remain poorly defined, complicating the identification of upstream regulators and downstream effectors of dysregulated pathways [164]. Targeted follow-up experiments, including kinase activity assays, genetic perturbation studies and interaction proteomics, will be essential to bridge this gap.

The phosphoproteomic study in Paper II was performed on fibroblast-derived iNs, which carry a pan-neuronal molecular profile. While this does not recapitulate the selective vulnerability of MSNs in HD [59,60], it provides a valuable characterisation of phosphoproteomic dysregulation common to neurons carrying the HD genotype. However, it is important to note that the overall proportion of successfully converted iNs within the cell population represents a limitation, as the presence of unconverted cells in the bulk preparation may dilute or obscure cell-type-specific phosphoproteomic signals. This underscores the importance of improving conversion yield, as discussed in the context of Paper III, and of cross-validating the phosphoproteomic findings identified here against postmortem brain tissue from HD patients, where the disease-relevant cellular context can be independently confirmed. An important and tractable next step would be to extend this analysis to HD patient-derived MSN-like cells [108], which could uncover post-translational dysregulation specific to the selectively vulnerable population and contribute to more targeted therapeutic strategies.

The use of iNs in Paper II also highlights the broader importance of direct reprogramming as a strategy for studying cell-type-specific molecular changes in ageing and neurodegeneration, particularly when using bulk omic technologies that lack the single-cell resolution of snRNA-seq. Unlike hiPSC-based reprogramming, direct conversion preserves the age-associated molecular signatures of the donor,

making it uniquely suited for modelling late-onset disease and ageing-related pathology [101,116,117]. However, for direct reprogramming to fulfil its potential in high-content drug screening and disease subtyping applications, improvements in conversion efficiency and purity are essential. Paper III addresses this need by establishing, for the first time, direct neuronal conversion of DPSCs – an accessible somatic cell source with a shared cranial neural crest developmental origin with neurons [128]. The faster conversion rate and distinct neuronal characteristics of DPSC-derived iNs, together with a higher proportion of NCAM1+ and TAU+ cells compared to fibroblast-derived conversions, support the hypothesis that developmental proximity to the target cell type facilitates more efficient conversion. This is consistent with findings from conversions using astrocytes and neural progenitor cells, and suggests that lineage is an important determinant of conversion outcome [124-126].

Nevertheless, important questions remain. The composition and molecular profile of the converted cell population has not yet been fully characterised, and further studies using snRNA-seq will be necessary to determine the transcriptomic identity of the converted cells and the degree of heterogeneity within the population. Lineage tracing in combination with snRNA-seq would be particularly informative, enabling the tracking of successfully converted cells back to specific subpopulations within the source DPSC pool and potentially identifying a subpopulation with superior conversion competence [165]. Such findings could ultimately guide the derivation of purer, more defined neuronal cell lines. Additionally, the present study was limited by a narrow age range and small sample size, which precluded meaningful conclusions about the influence of donor age on conversion outcome. This is an important question, as studies of aged dental pulp have shown that DPSCs exhibit senescence and reduced proliferative and differentiation potential with age [166-168]. Rather than being solely a limitation, this observation raises an interesting possibility – if DPSCs accumulate age-associated molecular changes, the neurons derived from aged donors may themselves carry ageing signatures, making them potentially useful for modelling neuronal ageing across the lifespan, provided that conversion efficiency is maintained. Addressing this will require studies with broader age representation and larger cohorts.

Ageing and age-related diseases of the human brain continue to pose an enormous burden on individuals, healthcare systems and society, and the absence of disease-modifying treatments for most neurodegenerative conditions makes a deeper understanding of the underlying molecular processes both urgent and essential. This thesis contributes new tools and models toward that understanding – cell-type-specific clocks that can track the progression of molecular ageing, a phosphoproteomic map of pathophysiological dysregulation in a neuronal model of HD, and a more accessible and efficient cellular platform for future ageing research. Individually, each contribution addresses a specific gap; collectively, they reflect a

broader effort to study human brain ageing with greater resolution, at multiple molecular levels, and in more relevant human cellular contexts.

Acknowledgements

A PhD is often described as a solitary endeavour, but looking back, I realise that nothing about this journey has been truly solitary. It has been shaped, supported and enriched by an extraordinary number of people, and I am deeply grateful to each of them.

First and foremost, I want to thank my main supervisor, Karolina. You have been nothing short of a pillar throughout this journey, your unwavering belief in my abilities, your boundless positive energy, and your constant encouragement have meant more than I can express. Whether it was responding to my messages at any hour of the day, welcoming my endless questions and ideas with your characteristic enthusiasm, or lifting me up when doubt crept in, you have been a supervisor in the truest and finest sense of the word. I am immensely proud to have been your mentee, and even prouder to know you as a person. You are not only a remarkable scientist, but a genuinely kind human being, and that combination is rare.

Johan and Yogita, my co-supervisors, have each played a role that went far beyond what most co-supervisors would. Johan, despite a schedule that would overwhelm most, always made time to meet, discuss and engage with my work. Your feedback and perspectives have been instrumental in shaping me as a researcher, and I am grateful for every conversation. Yogita, I owe much of my growth as a bioinformatician to your patient guidance and expertise. But beyond supervision, you have been a friend – someone I could turn to for advice on matters of both career and life, and that has meant the world to me.

To all present and past members of the Jakobsson lab – Raquel, Laura, Sara, Patricia, Giorgia, Giulia, Vivien, Diahann, Emily, Pia, Marie J, Jenny J, Anita, Ofelia, Nino, Carrie, Vilma, Filipa, Francesca – and the Pircs lab – Agi, Attila, Palma, Lea, Kinga, Balazs, Anna, Roli, Vivi, Bendeguz, Lisa, Sophie, and Ali – thank you for your guidance, generosity and support throughout my PhD, and for making it genuinely fun. I consider it a privilege to have worked among such extraordinary scientists and warm human beings.

Beyond my immediate research environment, I have also been fortunate to collaborate with generous and inspiring colleagues across projects. Professor Palkovits, your lifelong dedication to science and the depth of your contributions to the field command the highest respect; I have been profoundly inspired by your commitment. Eva, your thoughtful input and engagement have been equally

appreciated. Melinda and Zsuzsa, I have learnt so much from you about mass spectrometry and proteomics, and I owe much of my development in these areas to your expertise and patience. Csaba, it has been a true pleasure working with you – your insight, combined with your warmth and the understated humour that so often brightened our discussions, made our collaboration both productive and genuinely enjoyable. Enikő and Brigi, working with you on the clocks has been a delight; I am grateful for your collaboration and wish you all the very best for what lies ahead.

My PhD has also been shaped in important ways by the Professional Development Program, and I am deeply grateful to Christine and Jenny H for coordinating it with such dedication and care. Christine, your self-leadership coaching gave me tools I will carry well beyond this thesis. Jenny H, your mentorship has been invaluable, thank you for your wisdom and your time.

To my wonderful colleagues, past and present at BMC A11 – Ulla, Bengt, Petter, Maria T, Kerstin, Jana, Alice, Clara, Sara C, Maria G, Ronja, Daniel, Veronika, Malin Å, Malin P, Anna F, Anna H, Cilla, Elin, Luis, Marie Vejgården, Ling, Daniella, Christina, Gigi, Constanza, Andreas, Fredrik, Efrain, Srisayini, Claire P, Jenny N, Tamina, German, Leo, Millie and Eva, thank you for creating such a friendly and stimulating environment. Every conversation, small or big, has meant a great deal to me.

A special thank you to Linda and Paulina, whose administrative support has been exceptional throughout my time here. Paulina, I am particularly grateful for the effort you put into pushing forward my PhD admission process during what was a complicated time, your help made a real and tangible difference, and I have not forgotten it.

To Chris and the EPIC-CD group, thank you for always treating me as one of your own. Your warmth, friendliness and support throughout my PhD have meant a great deal, and I have cherished being an honorary member at many of your group activities.

To my friends, Pavitra, Tushar, Jonas, Sneha, Sudhir, Rohith, Sharmada, Szilvia, Arthur, Fereshteh, Giorgia, Melina, Sivani, Caterina, Agnes, Sylvie, and Lena, thank you for your unconditional love and support. A PhD tests you in ways that are difficult to explain to those who have not lived it, and having people who stand by you regardless makes all the difference. I could not have done this without you.

And finally, to my family – Appa, Amma, Sai, Manni, Chithi, Appu, and Viju. You have been my foundation. Your unwavering belief in me, your endless support, and your certainty that I would find my way have carried me through every difficult moment of this journey. Everything I do, I do knowing that you are behind me, and that knowledge is worth more than I can put into words.

To my late grandparents, Chithappa and Athai – I wish you could have seen this. I know you would have been proud, and that thought has stayed with me throughout.

References

1 Lopez-Otin, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. The hallmarks of aging. *Cell* **153**, 1194-1217 (2013). https://doi.org:10.1016/j.cell.2013.05.039

2 Lopez-Otin, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. Hallmarks of aging: An expanding universe. *Cell* **186**, 243-278 (2023). https://doi.org:10.1016/j.cell.2022.11.001

3 Guo, J. *et al.* Aging and aging-related diseases: from molecular mechanisms to interventions and treatments. *Signal Transduct Target Ther* **7**, 391 (2022). https://doi.org:10.1038/s41392-022-01251-0

4 Cohen, A. A. *et al.* Lack of consensus on an aging biology paradigm? A global survey reveals an agreement to disagree, and the need for an interdisciplinary framework. *Mech Ageing Dev* **191**, 111316 (2020). https://doi.org:10.1016/j.mad.2020.111316

5 Gaillard, J. M. & Lemaitre, J. F. The Williams' legacy: A critical reappraisal of his nine predictions about the evolution of senescence. *Evolution* **71**, 2768-2785 (2017). https://doi.org:10.1111/evo.13379

6 Kinzina, E. D., Podolskiy, D. I., Dmitriev, S. E. & Gladyshev, V. N. Patterns of Aging Biomarkers, Mortality, and Damaging Mutations Illuminate the Beginning of Aging and Causes of Early-Life Mortality. *Cell Rep* **29**, 4276-4284 e4273 (2019). https://doi.org:10.1016/j.celrep.2019.11.091

7 Williams, G. C. Pleiotropy, Natural Selection, and the Evolution of Senescence. *Evolution* **11** (1957). https://doi.org:10.2307/2406060

8 Bektas, A., Schurman, S. H., Sen, R. & Ferrucci, L. Aging, inflammation and the environment. *Exp Gerontol* **105**, 10-18 (2018). https://doi.org:10.1016/j.exger.2017.12.015

9 Partridge, L., Deelen, J. & Slagboom, P. E. Facing up to the global challenges of ageing. *Nature* **561**, 45-56 (2018). https://doi.org:10.1038/s41586-018-0457-8

10 Collaborators, G. B. D. D. F. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. *Lancet Public Health* **7**, e105-e125 (2022). https://doi.org:10.1016/S2468-2667(21)00249-8

11 Hou, Y. *et al.* Ageing as a risk factor for neurodegenerative disease. *Nat Rev Neurol* **15**, 565-581 (2019). https://doi.org:10.1038/s41582-019-0244-7

12 Finch, C. E. Evolution in health and medicine Sackler colloquium: Evolution of the human lifespan and diseases of aging: roles of infection, inflammation, and nutrition. *Proc Natl Acad Sci U S A* **107 Suppl 1**, 1718-1724 (2010). https://doi.org:10.1073/pnas.0909606106

13 Brayne, C., Gill, C., Paykel, E. S., Huppert, F. & O'Connor, D. W. Cognitive decline in an elderly population--a two wave study of change. *Psychol Med* **25**, 673-683 (1995). https://doi.org:10.1017/s0033291700034930

14 Aigbogun, M. S., Stellhorn, R., Krasa, H. & Kostic, D. Severity of memory impairment in the elderly: Association with health care resource use and functional limitations in the United States. *Alzheimers Dement (Amst)* **8**, 51-59 (2017). https://doi.org:10.1016/j.dadm.2017.04.001

15 Brito, D. V. C. *et al.* Assessing cognitive decline in the aging brain: lessons from rodent and human studies. *NPJ Aging* **9**, 23 (2023). https://doi.org:10.1038/s41514-023-00120-6

16 Crimmins, E. M. Lifespan and Healthspan: Past, Present, and Promise. *Gerontologist* **55**, 901-911 (2015). https://doi.org:10.1093/geront/gnv130

17 Vollset, S. E. *et al.* Fertility, mortality, migration, and population scenarios for 195 countries and territories from 2017 to 2100: a forecasting analysis for the Global Burden of Disease Study. *Lancet* **396**, 1285-1306 (2020). https://doi.org:10.1016/S0140-6736(20)30677-2

18 Economic, U. N. D. o. I. *World population ageing, 2013*. (UN, 2014).

19 Lutz, W., Sanderson, W. & Scherbov, S. The coming acceleration of global population ageing. *Nature* **451**, 716-719 (2008). https://doi.org:10.1038/nature06516

20 World Health Organization. *Ageing: Global Population*, <https://www.who.int/news-room/questions-and-answers/item/population-ageing> (2025).

21 World Health Organization. *Ageing and Health*, <https://www.who.int/news-room/fact-sheets/detail/ageing-and-health> (2025).

22 Moguilner, S. *et al.* Brain clocks capture diversity and disparities in aging and dementia across geographically diverse populations. *Nat Med* (2024). https://doi.org:10.1038/s41591-024-03209-x

23 Yang, Z. *et al.* Brain aging patterns in a large and diverse cohort of 49,482 individuals. *Nat Med* **30**, 3015-3026 (2024). https://doi.org:10.1038/s41591-024-03144-x

24 Dillman, A. A. *et al.* Transcriptomic profiling of the human brain reveals that altered synaptic gene expression is associated with chronological aging. *Sci Rep* **7**, 16890 (2017). https://doi.org:10.1038/s41598-017-17322-0

25 Mattson, M. P. & Arumugam, T. V. Hallmarks of Brain Aging: Adaptive and Pathological Modification by Metabolic States. *Cell Metab* **27**, 1176-1199 (2018). https://doi.org:10.1016/j.cmet.2018.05.011

26 Andres-Benito, P. *et al.* Proteostatic modulation in brain aging without associated Alzheimer's disease-and age-related neuropathological changes. *Aging (Albany NY)* **15**, 3295-3330 (2023). https://doi.org:10.18632/aging.204698

27 Soreq, L. *et al.* Major Shifts in Glial Regional Identity Are a Transcriptional Hallmark of Human Brain Aging. *Cell Rep* **18**, 557-570 (2017). https://doi.org:10.1016/j.celrep.2016.12.011

28 Frohlich, A. S. *et al.* Single-nucleus transcriptomic profiling of human orbitofrontal cortex reveals convergent effects of aging and psychiatric disease. *Nat Neurosci* (2024). https://doi.org:10.1038/s41593-024-01742-z

29 Emani, P. S. *et al.* Single-cell genomics and regulatory networks for 388 human brains. *Science* **384**, eadi5199 (2024). https://doi.org:10.1126/science.adi5199

30 Ling, E. *et al.* A concerted neuron-astrocyte program declines in ageing and schizophrenia. *Nature* **627**, 604-611 (2024). https://doi.org:10.1038/s41586-024-07109-5

31 Muralidharan, C. *et al.* Human Brain Cell-Type-Specific Aging Clocks Based on Single-Nuclei Transcriptomics. *Adv Sci (Weinh)* **12**, e06109 (2025). https://doi.org:10.1002/advs.202506109

32 Jeffries, A. M. *et al.* Single-cell transcriptomic and genomic changes in the ageing human brain. *Nature* **646**, 657-666 (2025). https://doi.org:10.1038/s41586-025-09435-8

33 Omar, O. M. F. *et al.* Endothelial TDP-43 depletion disrupts core blood-brain barrier pathways in neurodegeneration. *Nat Neurosci* **28**, 973-984 (2025). https://doi.org:10.1038/s41593-025-01914-5

34 Yang, H. *et al.* A lifespan transcriptomic atlas of the human prefrontal cortex at single-cell resolution. *medRxiv* (2025). https://doi.org:10.1101/2024.11.06.24316592

35 Sloan, N. X. *et al.* Uncovering the signatures of aging and senescence in the human dorsolateral prefrontal cortex. *Cell Genom*, 101127 (2026). https://doi.org:10.1016/j.xgen.2025.101127

36 Seeker, L. A. *et al.* Brain matters: unveiling the distinct contributions of region, age, and sex to glia diversity and CNS function. *Acta Neuropathol Commun* **11**, 84 (2023). https://doi.org:10.1186/s40478-023-01568-z

37 Chien, J. F. *et al.* Cell-type-specific effects of age and sex on human cortical neurons. *Neuron* **112**, 2524-2539 e2525 (2024). https://doi.org:10.1016/j.neuron.2024.05.013

38 Adams, L., Song, M. K., Yuen, S., Tanaka, Y. & Kim, Y. S. A single-nuclei paired multiomic analysis of the human midbrain reveals age- and Parkinson's disease-associated glial changes. *Nat Aging* **4**, 364-378 (2024). https://doi.org:10.1038/s43587-024-00583-6

39 Mesecar, M. E. *et al.* Region-Specific Transcriptional Signatures of Brain Aging in the Absence of Neuropathology at the Single-cell Level. *bioRxiv* (2025). https://doi.org:10.1101/2023.07.31.551097

40 Serrano-Pozo, A. *et al.* Astrocyte transcriptomic changes along the spatiotemporal progression of Alzheimer's disease. *Nat Neurosci* **27**, 2384-2400 (2024). https://doi.org:10.1038/s41593-024-01791-4

41 Cao, K., Chen-Plotkin, A. S., Plotkin, J. B. & Wang, L. S. Age-correlated gene expression in normal and neurodegenerative human brain tissues. *PLoS One* **5** (2010). https://doi.org:10.1371/journal.pone.0013098

42 Hipp, M. S., Kasturi, P. & Hartl, F. U. The proteostasis network and its decline in ageing. *Nat Rev Mol Cell Biol* **20**, 421-435 (2019). https://doi.org:10.1038/s41580-019-0101-y

43 Huuki-Myers, L. A. *et al.* A data-driven single-cell and spatial transcriptomic map of the human prefrontal cortex. *Science* **384**, eadh1938 (2024). https://doi.org:10.1126/science.adh1938

44 Siletti, K. *et al.* Transcriptomic diversity of cell types across the adult human brain. *Science* **382**, eadd7046 (2023). https://doi.org:10.1126/science.add7046

45 Langseth, C. M. *et al.* Comprehensive in situ mapping of human cortical transcriptomic cell types. *Commun Biol* **4**, 998 (2021). https://doi.org:10.1038/s42003-021-02517-z

46 Reu, P. *et al.* The Lifespan and Turnover of Microglia in the Human Brain. *Cell Rep* **20**, 779-784 (2017). https://doi.org:10.1016/j.celrep.2017.07.004

47 Anda, F. C. *et al.* Cortical neurons gradually attain a post-mitotic state. *Cell Res* **26**, 1033-1047 (2016). https://doi.org:10.1038/cr.2016.76

48 Jobson, D. D., Hase, Y., Clarkson, A. N. & Kalaria, R. N. The role of the medial prefrontal cortex in cognition, ageing and dementia. *Brain Commun* **3**, fcab125 (2021). https://doi.org:10.1093/braincomms/fcab125

49 Vickery, S. *et al.* The uniqueness of human vulnerability to brain aging in great ape evolution. *Sci Adv* **10**, eado2733 (2024). https://doi.org:10.1126/sciadv.ado2733

50 Douaud, G. *et al.* A common brain network links development, aging, and vulnerability to disease. *Proc Natl Acad Sci U S A* **111**, 17648-17653 (2014). https://doi.org:10.1073/pnas.1410378111

51 Guevara, E. E. *et al.* Epigenetic ageing of the prefrontal cortex and cerebellum in humans and chimpanzees. *Epigenetics* **17**, 1774-1785 (2022). https://doi.org:10.1080/15592294.2022.2080993

52 Lu, A. T. *et al.* Genetic architecture of epigenetic and neuronal ageing rates in human brain regions. *Nat Commun* **8**, 15353 (2017). https://doi.org:10.1038/ncomms15353

53 Svennerholm, L., Bostrom, K. & Jungbjer, B. Changes in weight and compositions of major membrane components of human brain during the span of adult human life of Swedes. *Acta Neuropathol* **94**, 345-352 (1997). https://doi.org:10.1007/s004010050717

54 Raz, N. *et al.* Selective aging of the human cerebral cortex observed in vivo: differential vulnerability of the prefrontal gray matter. *Cereb Cortex* **7**, 268-282 (1997). https://doi.org:10.1093/cercor/7.3.268

55 Azam, S., Haque, M. E., Balakrishnan, R., Kim, I. S. & Choi, D. K. The Ageing Brain: Molecular and Cellular Basis of Neurodegeneration. *Front Cell Dev Biol* **9**, 683459 (2021). https://doi.org:10.3389/fcell.2021.683459

56 Liu, Y. *et al.* The interaction between ageing and Alzheimer's disease: insights from the hallmarks of ageing. *Transl Neurodegener* **13**, 7 (2024). https://doi.org:10.1186/s40035-024-00397-x

57 Raket, L. L. *et al.* Impact of age at onset on symptom profiles, treatment characteristics and health-related quality of life in Parkinson's disease. *Sci Rep* **12**, 526 (2022). https://doi.org:10.1038/s41598-021-04356-8

58 Machiela, E. & Southwell, A. L. Biological Aging and the Cellular Pathogenesis of Huntington's Disease. *J Huntingtons Dis* **9**, 115-128 (2020). https://doi.org:10.3233/JHD-200395

59 Fu, H., Hardy, J. & Duff, K. E. Selective vulnerability in neurodegenerative diseases. *Nat Neurosci* **21**, 1350-1358 (2018). https://doi.org:10.1038/s41593-018-0221-2

60 Kampmann, M. Molecular and cellular mechanisms of selective vulnerability in neurodegenerative diseases. *Nat Rev Neurosci* **25**, 351-371 (2024). https://doi.org:10.1038/s41583-024-00806-0

61 Latumalea, D., Unfried, M., Barardo, D., Gruber, J. & Kennedy, B. K. DoliClock: a lipid-based aging clock reveals accelerated aging in neurological disorders. *Aging (Albany NY)* **17**, 1405-1428 (2025). https://doi.org:10.18632/aging.206266

62 Horvath, S. *et al.* Huntington's disease accelerates epigenetic aging of human brain and disrupts DNA methylation levels. *Aging (Albany NY)* **8**, 1485-1512 (2016). https://doi.org:10.18632/aging.101005

63 Thrush, K. L. *et al.* Aging the brain: multi-region methylation principal component based clock in the context of Alzheimer's disease. *Aging (Albany NY)* **14**, 5641-5668 (2022). https://doi.org:10.18632/aging.204196

64 Levine, M. E., Lu, A. T., Bennett, D. A. & Horvath, S. Epigenetic age of the pre-frontal cortex is associated with neuritic plaques, amyloid load, and Alzheimer's disease related cognitive functioning. *Aging (Albany NY)* **7**, 1198-1211 (2015). https://doi.org:10.18632/aging.100864

65 Lee, J. *et al.* Deep learning-based brain age prediction in normal aging and dementia. *Nat Aging* **2**, 412-424 (2022). https://doi.org:10.1038/s43587-022-00219-7

66 Cullen, N. C., Malarstig, A. N., Stomrud, E., Hansson, O. & Mattsson-Carlgren, N. Accelerated inflammatory aging in Alzheimer's disease and its relation to amyloid, tau, and cognition. *Sci Rep* **11**, 1965 (2021). https://doi.org:10.1038/s41598-021-81705-7

67 Gaser, C. *et al.* BrainAGE in Mild Cognitive Impaired Patients: Predicting the Conversion to Alzheimer's Disease. *PLoS One* **8**, e67346 (2013). https://doi.org:10.1371/journal.pone.0067346

68 Gonneaud, J. *et al.* Accelerated functional brain aging in pre-clinical familial Alzheimer's disease. *Nat Commun* **12**, 5346 (2021). https://doi.org:10.1038/s41467-021-25492-9

69 Lin, C. W. *et al.* Older molecular brain age in severe mental illness. *Mol Psychiatry* **26**, 3646-3656 (2021). https://doi.org:10.1038/s41380-020-0834-1

70 Teeuw, J. *et al.* Accelerated aging in the brain, epigenetic aging in blood, and polygenic risk for schizophrenia. *Schizophr Res* **231**, 189-197 (2021). https://doi.org:10.1016/j.schres.2021.04.005

71 Yusupov, N. *et al.* Transdiagnostic evaluation of epigenetic age acceleration and burden of psychiatric disorders. *Neuropsychopharmacology* **48**, 1409-1417 (2023). https://doi.org:10.1038/s41386-023-01579-3

72 Nagy, C. *et al.* Single-nucleus transcriptomics of the prefrontal cortex in major depressive disorder implicates oligodendrocyte precursor cells and excitatory neurons. *Nat Neurosci* **23**, 771-781 (2020). https://doi.org:10.1038/s41593-020-0621-y

73 Knopman, D. S. *et al.* Alzheimer disease. *Nat Rev Dis Primers* **7**, 33 (2021). https://doi.org:10.1038/s41572-021-00269-y

74 Mathys, H. *et al.* Single-cell multiregion dissection of Alzheimer's disease. *Nature* **632**, 858-868 (2024). https://doi.org:10.1038/s41586-024-07606-7

75 Olah, M. *et al.* Single cell RNA sequencing of human microglia uncovers a subset associated with Alzheimer's disease. *Nat Commun* **11**, 6129 (2020). https://doi.org:10.1038/s41467-020-19737-2

76 Morabito, S. *et al.* Single-nucleus chromatin accessibility and transcriptomic characterization of Alzheimer's disease. *Nat Genet* **53**, 1143-1155 (2021). https://doi.org:10.1038/s41588-021-00894-z

77 Grubman, A. *et al.* A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease reveals cell-type-specific gene expression regulation. *Nat Neurosci* **22**, 2087-2097 (2019). https://doi.org:10.1038/s41593-019-0539-4

78 Gabitto, M. I. *et al.* Integrated multimodal cell atlas of Alzheimer's disease. *Nat Neurosci* **27**, 2366-2383 (2024). https://doi.org:10.1038/s41593-024-01774-5

79 Saudou, F. & Humbert, S. The Biology of Huntingtin. *Neuron* **89**, 910-926 (2016). https://doi.org:10.1016/j.neuron.2016.02.003

80 A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. *Cell* **72**, 971-983 (1993). https://doi.org:10.1016/0092-8674(93)90585-e

81 Bates, G. P. *et al.* Huntington disease. *Nat Rev Dis Primers* **1**, 15005 (2015). https://doi.org:10.1038/nrdp.2015.5

82 Walker, F. O. Huntington's disease. *Lancet* **369**, 218-228 (2007). https://doi.org:10.1016/S0140-6736(07)60111-1

83 Labbadia, J. & Morimoto, R. I. Huntington's disease: underlying molecular mechanisms and emerging concepts. *Trends Biochem Sci* **38**, 378-385 (2013). https://doi.org:10.1016/j.tibs.2013.05.003

84 Hodge, R. D. *et al.* Conserved cell types with divergent features in human versus mouse cortex. *Nature* **573**, 61-68 (2019). https://doi.org:10.1038/s41586-019-1506-7

85 Bakken, T. E. *et al.* Comparative cellular analysis of motor cortex in human, marmoset and mouse. *Nature* **598**, 111-119 (2021). https://doi.org:10.1038/s41586-021-03465-8

86 Granzotto, A., Vissel, B. & Sensi, S. L. Lost in translation: Inconvenient truths on the utility of mouse models in Alzheimer's disease research. *Elife* **13** (2024). https://doi.org:10.7554/eLife.90633

87 Burns, T. C., Li, M. D., Mehta, S., Awad, A. J. & Morgan, A. A. Mouse models rarely mimic the transcriptome of human neurodegenerative diseases: A systematic bioinformatics-based critique of preclinical models. *Eur J Pharmacol* **759**, 101-117 (2015). https://doi.org:10.1016/j.ejphar.2015.03.021

88 Fisher, E. M. C. & Bannerman, D. M. Mouse models of neurodegeneration: Know your question, know your mouse. *Sci Transl Med* **11** (2019). https://doi.org:10.1126/scitranslmed.aaq1818

89 Jucker, M. The benefits and limitations of animal models for translational research in neurodegenerative diseases. *Nat Med* **16**, 1210-1214 (2010). https://doi.org:10.1038/nm.2224

90 Kanari, L. *et al.* Of mice and men: Dendritic architecture differentiates human from mouse neuronal networks. *iScience* **28**, 112928 (2025). https://doi.org:10.1016/j.isci.2025.112928

91 Javan, G. T., Singh, K., Finley, S. J., Green, R. L. & Sen, C. K. Complexity of human death: its physiological, transcriptomic, and microbiological implications. *Front Microbiol* **14**, 1345633 (2023). https://doi.org:10.3389/fmicb.2023.1345633

92 Glavan, D. *et al.* Identification of transcriptome alterations in the prefrontal cortex, hippocampus, amygdala and hippocampus of suicide victims. *Sci Rep* **11**, 18853 (2021). https://doi.org:10.1038/s41598-021-98210-6

93 Durrenberger, P. F. *et al.* Effects of antemortem and postmortem variables on human brain mRNA quality: a BrainNet Europe study. *J Neuropathol Exp Neurol* **69**, 70-81 (2010). https://doi.org:10.1097/NEN.0b013e3181c7e32f

94 Stan, A. D. *et al.* Human postmortem tissue: what quality markers matter? *Brain Res* **1123**, 1-11 (2006). https://doi.org:10.1016/j.brainres.2006.09.025

95 Danner, B. *et al.* Brain banking in the United States and Europe: Importance, challenges, and future trends. *J Neuropathol Exp Neurol* **83**, 219-229 (2024). https://doi.org:10.1093/jnen/nlae014

96 Deribe, Y. L., Pawson, T. & Dikic, I. Post-translational modifications in signal integration. *Nat Struct Mol Biol* **17**, 666-672 (2010). https://doi.org:10.1038/nsmb.1842

97 Ardito, F., Giuliani, M., Perrone, D., Troiano, G. & Lo Muzio, L. The crucial role of protein phosphorylation in cell signaling and its use as targeted therapy (Review). *Int J Mol Med* **40**, 271-280 (2017). https://doi.org:10.3892/ijmm.2017.3036

98 Hunter, T. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. *Cell* **80**, 225-236 (1995). https://doi.org:10.1016/0092-8674(95)90405-0

99 Tenreiro, S., Eckermann, K. & Outeiro, T. F. Protein phosphorylation in neurodegeneration: friend or foe? *Front Mol Neurosci* **7**, 42 (2014). https://doi.org:10.3389/fnmol.2014.00042

100 Sambataro, F. & Pennuto, M. Post-translational Modifications and Protein Quality Control in Motor Neuron and Polyglutamine Diseases. *Front Mol Neurosci* **10**, 82 (2017). https://doi.org:10.3389/fnmol.2017.00082

101 Drouin-Ouellet, J., Pircs, K., Barker, R. A., Jakobsson, J. & Parmar, M. Direct Neuronal Reprogramming for Disease Modeling Studies Using Patient-Derived Neurons: What Have We Learned? *Front Neurosci* **11**, 530 (2017). https://doi.org:10.3389/fnins.2017.00530

102 Aversano, S., Caiazza, C. & Caiazzo, M. Induced pluripotent stem cell-derived and directly reprogrammed neurons to study neurodegenerative diseases: The impact of aging signatures. *Front Aging Neurosci* **14**, 1069482 (2022). https://doi.org:10.3389/fnagi.2022.1069482

103 Chanoumidou, K. *et al.* One-step Reprogramming of Human Fibroblasts into Oligodendrocyte-like Cells by SOX10, OLIG2, and NKX6.2. *Stem Cell Reports* **16**, 771-783 (2021). https://doi.org:10.1016/j.stemcr.2021.03.001

104 Quist, E. *et al.* Transcription factor-based direct conversion of human fibroblasts to functional astrocytes. *Stem Cell Reports* **17**, 1620-1635 (2022). https://doi.org:10.1016/j.stemcr.2022.05.015

105 Pfisterer, U. *et al.* Direct conversion of human fibroblasts to dopaminergic neurons. *Proc Natl Acad Sci U S A* **108**, 10343-10348 (2011). https://doi.org:10.1073/pnas.1105135108

106 Bruzelius, A. *et al.* Reprogramming Human Adult Fibroblasts into GABAergic Interneurons. *Cells* **10** (2021). https://doi.org:10.3390/cells10123450

107 Colasante, G. *et al.* Rapid Conversion of Fibroblasts into Functional Forebrain GABAergic Interneurons by Direct Genetic Reprogramming. *Cell Stem Cell* **17**, 719-734 (2015). https://doi.org:10.1016/j.stem.2015.09.002

108 Richner, M., Victor, M. B., Liu, Y., Abernathy, D. & Yoo, A. S. MicroRNA-based conversion of human fibroblasts into striatal medium spiny neurons. *Nat Protoc* **10**, 1543-1555 (2015). https://doi.org:10.1038/nprot.2015.102

109 Liu, M. L. *et al.* Small molecules enable neurogenin 2 to efficiently convert human fibroblasts into cholinergic neurons. *Nat Commun* **4**, 2183 (2013). https://doi.org:10.1038/ncomms3183

110 Caiazzo, M. *et al.* Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. *Nature* **476**, 224-227 (2011). https://doi.org:10.1038/nature10284

111 Son, E. Y. *et al.* Conversion of mouse and human fibroblasts into functional spinal motor neurons. *Cell Stem Cell* **9**, 205-218 (2011). https://doi.org:10.1016/j.stem.2011.07.014

112 Chambers, S. M. *et al.* Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol* **27**, 275-280 (2009). https://doi.org:10.1038/nbt.1529

113 Kriks, S. *et al.* Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. *Nature* **480**, 547-551 (2011). https://doi.org:10.1038/nature10648

114 Vera, E. & Studer, L. When rejuvenation is a problem: challenges of modeling late-onset neurodegenerative disease. *Development* **142**, 3085-3089 (2015). https://doi.org:10.1242/dev.120667

115 Wang, H., Yang, Y., Liu, J. & Qian, L. Direct cell reprogramming: approaches, mechanisms and progress. *Nat Rev Mol Cell Biol* **22**, 410-424 (2021). https://doi.org:10.1038/s41580-021-00335-z

116 Mertens, J. *et al.* Directly Reprogrammed Human Neurons Retain Aging-Associated Transcriptomic Signatures and Reveal Age-Related Nucleocytoplasmic Defects. *Cell Stem Cell* **17**, 705-718 (2015). https://doi.org:10.1016/j.stem.2015.09.001

117 Huh, C. J. *et al.* Maintenance of age in human neurons generated by microRNA-based neuronal conversion of fibroblasts. *Elife* **5** (2016). https://doi.org:10.7554/eLife.18648

118 Pfisterer, U. *et al.* Efficient induction of functional neurons from adult human fibroblasts. *Cell Cycle* **10**, 3311-3316 (2011). https://doi.org:10.4161/cc.10.19.17584

119 Pircs, K. *et al.* Distinct subcellular autophagy impairments in induced neurons from patients with Huntington's disease. *Brain* **145**, 3035-3057 (2022). https://doi.org:10.1093/brain/awab473

120 Mertens, J. *et al.* Age-dependent instability of mature neuronal fate in induced neurons from Alzheimer's patients. *Cell Stem Cell* **28**, 1533-1548 e1536 (2021). https://doi.org:10.1016/j.stem.2021.04.004

121 Drouin-Ouellet, J. *et al.* Age-related pathological impairments in directly reprogrammed dopaminergic neurons derived from patients with idiopathic Parkinson's disease. *Stem Cell Reports* **17**, 2203-2219 (2022). https://doi.org:10.1016/j.stemcr.2022.08.010

122 Plikus, M. V. *et al.* Fibroblasts: Origins, definitions, and functions in health and disease. *Cell* **184**, 3852-3872 (2021). https://doi.org:10.1016/j.cell.2021.06.024

123 Belair-Hickey, J. J. *et al.* Neural crest precursors from the skin are the primary source of directly reprogrammed neurons. *Stem Cell Reports* **19**, 1620-1634 (2024). https://doi.org:10.1016/j.stemcr.2024.10.003

124 Zhang, L. *et al.* Small Molecules Efficiently Reprogram Human Astroglial Cells into Functional Neurons. *Cell Stem Cell* **17**, 735-747 (2015). https://doi.org:10.1016/j.stem.2015.09.012

125 Corti, S. *et al.* Direct reprogramming of human astrocytes into neural stem cells and neurons. *Exp Cell Res* **318**, 1528-1541 (2012). https://doi.org:10.1016/j.yexcr.2012.02.040

126 Kim, K. M., Thaqi, M., Peterson, D. A. & Marr, R. A. Induced Neurons for Disease Modeling and Repair: A Focus on Non-fibroblastic Cell Sources in Direct Reprogramming. *Front Bioeng Biotechnol* **9**, 658498 (2021). https://doi.org:10.3389/fbioe.2021.658498

127 Sramko, B. *et al.* The Wisdom in Teeth: Neuronal Differentiation of Dental Pulp Cells. *Cell Reprogram* **25**, 32-44 (2023). https://doi.org:10.1089/cell.2022.0102

128 Chai, Y. *et al.* Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. *Development* **127**, 1671-1679 (2000). https://doi.org:10.1242/dev.127.8.1671

129 Hollands, P., Aboyeji, D. & Orcharton, M. Dental pulp stem cells in regenerative medicine. *Br Dent J* (2018). https://doi.org:10.1038/sj.bdj.2018.348

130 Lewandowski, S. A., Kular, L. & Jagodic, M. Epigenetic age acceleration as a biomarker of amyotrophic lateral sclerosis severity? *EBioMedicine* **110**, 105470 (2024). https://doi.org:10.1016/j.ebiom.2024.105470

131 Kular, L. *et al.* Epigenetic clock indicates accelerated aging in glial cells of progressive multiple sclerosis patients. *Front Aging Neurosci* **14**, 926468 (2022). https://doi.org:10.3389/fnagi.2022.926468

132 Teschendorff, A. E. & Horvath, S. Epigenetic ageing clocks: statistical methods and emerging computational challenges. *Nat Rev Genet* **26**, 350-368 (2025). https://doi.org:10.1038/s41576-024-00807-w

133 Min, M., Egli, C., Dulai, A. S. & Sivamani, R. K. Critical review of aging clocks and factors that may influence the pace of aging. *Front Aging* **5**, 1487260 (2024). https://doi.org:10.3389/fragi.2024.1487260

134 Shireby, G. L. *et al.* Recalibrating the epigenetic clock: implications for assessing biological age in the human cortex. *Brain* **143**, 3763-3775 (2020). https://doi.org:10.1093/brain/awaa334

135 Meyer, D. H. & Schumacher, B. Aging clocks based on accumulating stochastic variation. *Nat Aging* **4**, 871-885 (2024). https://doi.org:10.1038/s43587-024-00619-x

136 Zheng, G. X. *et al.* Massively parallel digital transcriptional profiling of single cells. *Nat Commun* **8**, 14049 (2017). https://doi.org:10.1038/ncomms14049

137 Buckley, M. T. *et al.* Cell-type-specific aging clocks to quantify aging and rejuvenation in neurogenic regions of the brain. *Nat Aging* **3**, 121-137 (2023). https://doi.org:10.1038/s43587-022-00335-4

138 Ochoa, D. *et al.* The functional landscape of the human phosphoproteome. *Nat Biotechnol* **38**, 365-373 (2020). https://doi.org:10.1038/s41587-019-0344-3

139 Shrigley, S., Pircs, K., Barker, R. A., Parmar, M. & Drouin-Ouellet, J. Simple Generation of a High Yield Culture of Induced Neurons from Human Adult Skin Fibroblasts. *J Vis Exp* (2018). https://doi.org:10.3791/56904

140 Drouin-Ouellet, J. *et al.* REST suppression mediates neural conversion of adult human fibroblasts via microRNA-dependent and -independent pathways. *EMBO Mol Med* **9**, 1117-1131 (2017). https://doi.org:10.15252/emmm.201607471

141 Butler, A., Hoffman, P., Smibert, P., Papalexi, E. & Satija, R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat Biotechnol* **36**, 411-420 (2018). https://doi.org:10.1038/nbt.4096

142 Korsunsky, I. *et al.* Fast, sensitive and accurate integration of single-cell data with Harmony. *Nat Methods* **16**, 1289-1296 (2019). https://doi.org:10.1038/s41592-019-0619-0

143 Germain, P. L., Lun, A., Garcia Meixide, C., Macnair, W. & Robinson, M. D. Doublet identification in single-cell sequencing data using scDblFinder. *F1000Res* **10**, 979 (2021). https://doi.org:10.12688/f1000research.73600.2

144 Yaron-Barir, T. M. *et al.* The intrinsic substrate specificity of the human tyrosine kinome. *Nature* **629**, 1174-1181 (2024). https://doi.org:10.1038/s41586-024-07407-y

145 Johnson, J. L. *et al.* An atlas of substrate specificities for the human serine/threonine kinome. *Nature* **613**, 759-766 (2023). https://doi.org:10.1038/s41586-022-05575-3

146 Hornbeck, P. V. *et al.* PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. *Nucleic Acids Res* **43**, D512-520 (2015). https://doi.org:10.1093/nar/gku1267

147 Zakar-Polyak, E., Csordas, A., Palovics, R. & Kerepesi, C. Profiling the transcriptomic age of single-cells in humans. *Commun Biol* **7**, 1397 (2024). https://doi.org:10.1038/s42003-024-07094-5

148 Blair, L. J. *et al.* Accelerated neurodegeneration through chaperone-mediated oligomerization of tau. *J Clin Invest* **123**, 4158-4169 (2013). https://doi.org:10.1172/JCI69003

149 Criado-Marrero, M. *et al.* Hsp90 and FKBP51: complex regulators of psychiatric diseases. *Philos Trans R Soc Lond B Biol Sci* **373** (2018). https://doi.org:10.1098/rstb.2016.0532

150 Matosin, N. *et al.* Associations of psychiatric disease and ageing with FKBP5 expression converge on superficial layer neurons of the neocortex. *Acta Neuropathol* **145**, 439-459 (2023). https://doi.org:10.1007/s00401-023-02541-9

151 Zhou, X. *et al.* Integrated proteomics reveals autophagy landscape and an autophagy receptor controlling PKA-RI complex homeostasis in neurons. *Nat Commun* **15**, 3113 (2024). https://doi.org:10.1038/s41467-024-47440-z

152 Chun, B. Y. *et al.* Protective Role of Limitrin in Experimental Autoimmune Optic Neuritis. *Invest Ophthalmol Vis Sci* **62**, 8 (2021). https://doi.org:10.1167/iovs.62.9.8

153 Yonezawa, T. *et al.* Limitrin, a novel immunoglobulin superfamily protein localized to glia limitans formed by astrocyte endfeet. *Glia* **44**, 190-204 (2003). https://doi.org:10.1002/glia.10279

154 Yeo, S. Y. *et al.* Transglutaminase 2 contributes to a TP53-induced autophagy program to prevent oncogenic transformation. *Elife* **5**, e07101 (2016). https://doi.org:10.7554/eLife.07101

155 Eskelinen, E. L. Roles of LAMP-1 and LAMP-2 in lysosome biogenesis and autophagy. *Mol Aspects Med* **27**, 495-502 (2006). https://doi.org:10.1016/j.mam.2006.08.005

156 Chen, X. *et al.* Activation of the Wnt/beta-catenin/CYP1B1 pathway alleviates oxidative stress and protects the blood-brain barrier under cerebral ischemia/reperfusion conditions. *Neural Regen Res* **19**, 1541-1547 (2024). https://doi.org:10.4103/1673-5374.386398

157 Wu, J. *et al.* CYP1B1 affects the integrity of the blood-brain barrier and oxidative stress in the striatum: An investigation of manganese-induced neurotoxicity. *CNS Neurosci Ther* **30**, e14633 (2024). https://doi.org:10.1111/cns.14633

158 Wang, H., Penaloza, T., Manea, A. J. & Gao, X. PFKP: More than phosphofructokinase. *Adv Cancer Res* **160**, 1-15 (2023). https://doi.org:10.1016/bs.acr.2023.03.001

159 Janer, A. *et al.* ESYT1 tethers the ER to mitochondria and is required for mitochondrial lipid and calcium homeostasis. *Life Sci Alliance* **7** (2024). https://doi.org:10.26508/lsa.202302335

160 Magistretti, P. J. & Allaman, I. A cellular perspective on brain energy metabolism and functional imaging. *Neuron* **86**, 883-901 (2015). https://doi.org:10.1016/j.neuron.2015.03.035

161 Gupta, R. *et al.* Post-translational modifications: Regulators of neurodegenerative proteinopathies. *Ageing Res Rev* **68**, 101336 (2021). https://doi.org:10.1016/j.arr.2021.101336

162 Needham, E. J., Parker, B. L., Burykin, T., James, D. E. & Humphrey, S. J. Illuminating the dark phosphoproteome. *Sci Signal* **12** (2019). https://doi.org:10.1126/scisignal.aau8645

163 De Munter, S., Kohn, M. & Bollen, M. Challenges and opportunities in the development of protein phosphatase-directed therapeutics. *ACS Chem Biol* **8**, 36-45 (2013). https://doi.org:10.1021/cb300597g

164 Fahs, S., Lujan, P. & Kohn, M. Approaches to Study Phosphatases. *ACS Chem Biol* **11**, 2944-2961 (2016). https://doi.org:10.1021/acschembio.6b00570

165 Storm, P. *et al.* Lineage tracing of stem cell-derived dopamine grafts in a Parkinson's model reveals shared origin of all graft-derived cells. *Sci Adv* **10**, eadn3057 (2024). https://doi.org:10.1126/sciadv.adn3057

166 AN, H. W., M, I. A., W, A. S. & Mohammed, W. K. Histological changes in dental pulp tissue with age: a comparative study. *Cell Mol Biol (Noisy-le-grand)* **71**, 75-79 (2025). https://doi.org:10.14715/cmb/2025.71.6.10

167 Iezzi, I., Cerqueni, G., Licini, C., Lucarini, G. & Mattioli Belmonte, M. Dental pulp stem cells senescence and regenerative potential relationship. *J Cell Physiol* **234**, 7186-7197 (2019). https://doi.org:10.1002/jcp.27472

168 Maeda, H. Aging and Senescence of Dental Pulp and Hard Tissues of the Tooth. *Front Cell Dev Biol* **8**, 605996 (2020). https://doi.org:10.3389/fcell.2020.605996