# The Role of Sideroflexin-3 in Mitochondrial Dysfunction: Implications for Parkinson's Disease

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**Background and aims:** Neurodegenerative diseases (NDDs) involve the progressive loss of neuron function[1]. Parkinson's disease (PD), the most common NDD, affects over 6 million people globally, causing motor symptoms like rigidity and tremors, alongside non-motor signs such as cognitive impairment and sleep dysfunction[2]. PD is marked by α-synuclein aggregation, synaptic defects, and mitochondrial dysfunction, which leads to oxidative damage and impaired bioenergetics[3]. Currently, no disease-modifying therapies exist, emphasizing the need for novel diagnostic and therapeutic targets.

Sideroflexin 3 (SFXN3), a mitochondrial membrane protein, has emerged as a potential target due to its role in iron transport and influence on neurodegenerative pathways[4]. Studies in animal models suggest SFXN3 may have neuroprotective effects, though its absence does not directly trigger neurodegeneration. This study aimed to investigate the phenotypic effects of SFXN3 deletion in SH-SY5Y (neuronal-like) cells. We sought to understand the cellular and molecular consequences of losing SFXN3 function. This served as a proof of concept for exploring SFXN3 as a potential therapeutic target in Parkinson's disease, where mitochondrial dysfunction is a critical factor.

**Results and Discussion:** SFXN3 is ubiquitous is highly expressed in the brain[4,5]. Correspondingly, SH-SY5Y SFXN3 KO cells exhibited notable differences in mitochondrial and cellular behaviour compared to WT cells. The knockout led to reduced mitochondrial content and dense accumulation around the nucleus **(Fig. 1-a, b)**. At higher magnification, mitochondria appear to create a vast network, possibly suggesting changes in mitochondrial dynamics to recover function[6]. These two features are also present in PD, where mitochondria exhibit disturbed fusion and fission and altered distribution surrounding the nucleus[7,8]. However, further exploration using electron microscopy would be necessary to investigate these observations in more detail.

Additionally, we observed altered growth dynamics in absence of SFXN3 **(Fig. 1-c)**. SFXN3 KO cell number was 1.5 times lower than WT in F12 medium and even lower in regular DMEM. SFXN3 KO cells grow better in F12 than DMEM because F12's richer nutrients and growth factors[9] may help compensate for the metabolic defects caused by SFXN3 loss. In glucose-deprived DMEM, KO cells may have entered a quiescent state[10]. In contrast, in the absence of glucose, WT SH-SY5Y cells grow after 24h, possibly using leftover glucose storage[10], lowering again for 3 days showing how glucose

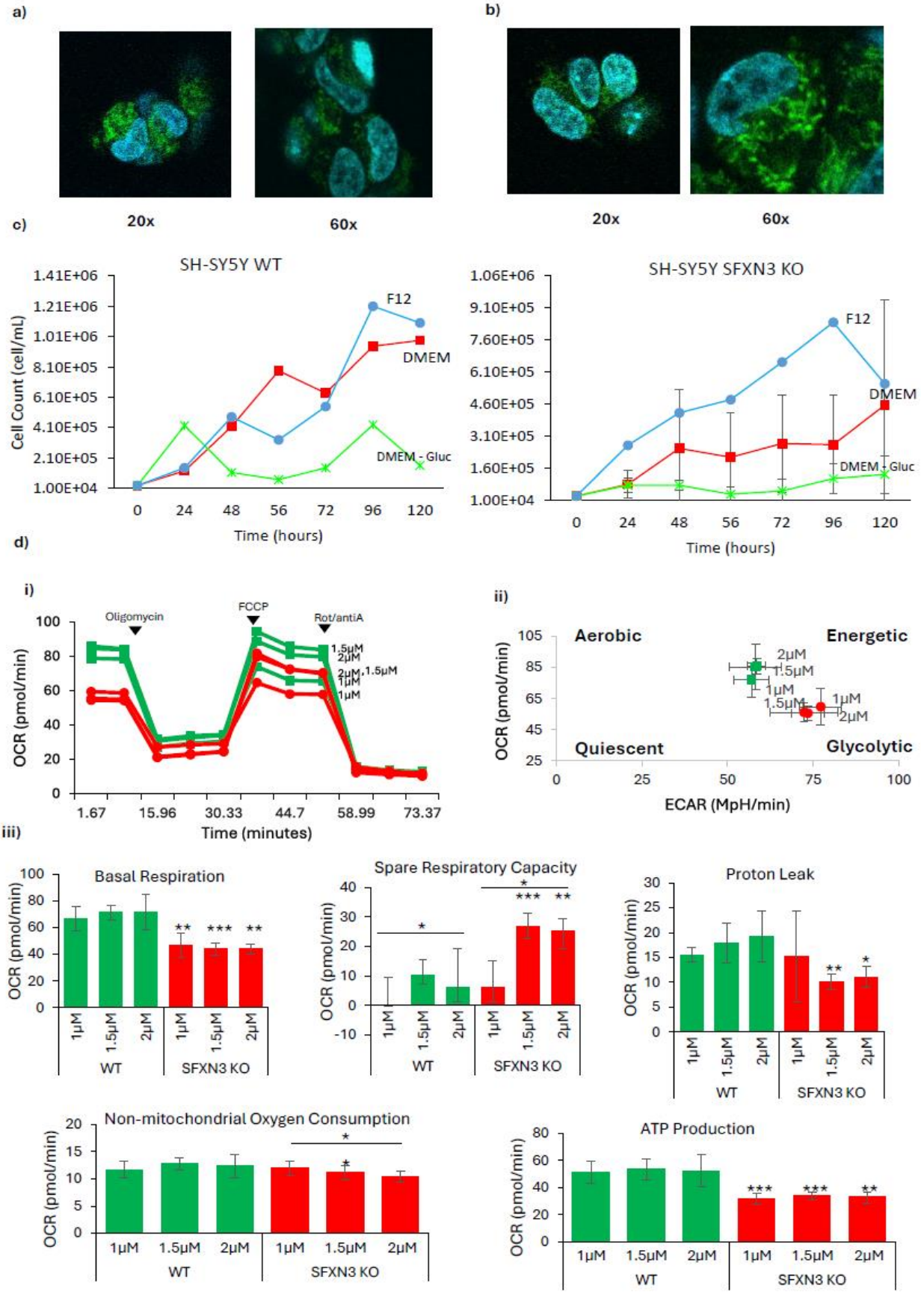

a)
20x
60x
b)
20x
60x
c)
SH-SY5Y WT
Cell Count (cell/mL)
Time (hours)
F12
DMEM
DMEM - Gluc
SH-SY5Y SFXN3 KO
d)
i)
Oligomycin
FCCP
Rot/antiA
OCR (pmol/min)
Time (minutes)
ii)
Aerobic
Energetic
Quiescent
Glycolytic
ECAR (MpH/min)
iii)
Basal Respiration
Spare Respiratory Capacity
Proton Leak
Non-mitochondrial Oxygen Consumption
ATP Production
WT
SFXN3 KO

*Figure 1.* ***Phenotypical analysis of SFXN3 KO SH-SY5Y. a-b)*** *Confocal microscopy images acquired using a Nikon A1-HR Confocal with a 20x and 60x oil-immersion objective, the cells are stained with DAPI (Nunc Blue™ (blue)) and Mito Tracker Green (green), allowing for a comparative analysis of nuclear and mitochondrial structures between* ***a)*** *wild-type SH-SY5Y cells and* ***b)*** *SH-SY5Y SFXN3 KO to identify any phenotypic changes resulting from the gene knockout****. c)*** *Cell growth curve: WT and KO cells were grown in 6-well plates in three different media – F12 (blue dot), DMEM (red square) and DMEM minus glucose (green cross). Wells were seeded with 3.00 x10$^4$ cells and stained with Trypan Blue and counted every 24h with Countess™ II Automated cell counter. Error bars (standard deviation) showed when three independent repeats were performed.* ***d)*** *Mitochondrial Stress Test Analysis Using Seahorse X96 Analyzer of SH-SY5Y WT and SFXN3 KO at different FCCP concentrations (1μM, 1.5μM and 2μM).* ***i)*** *Oxygen Consumption Rate (OCR) profile of SH-SY5Y WT and KO). Key injections were made at the indicated time points to analyse different parameters of mitochondrial function: 1 μM Oligomycin (ATP synthase inhibitor) to measure ATP-linked respiration, FCCP (uncoupler) to assess the maximum respiratory capacity, and 0.5 μM Rotenone/Antimycin A (complex I and III inhibitors) to determine non-mitochondrial respiration****. ii)*** *Representative traces and quantification of extracellular acidification rate (ECAR) measured simultaneously with OCR, indicating the glycolytic function. Injection of oligomycin was made to evaluate glycolytic capacity.* ***iii)*** *Bar graph summarizing the basal respiration, spare respiratory capacity, proton leak, non-mitochondrial oxygen consumption, and ATP production in SFXN3 KO compared to SH-SY5Y WT. Data are presented as mean ± S. D. Statistical significance was determined using one-way ANOVA with Tukey post-test within groups and unpaired t-test to compare FCCP treatment in SFXN3 KO versus control (WT). *P<0.05, **P<0.01, ***P<0.0001. Data not normalised to cell count.*

suggesting an adaptive mechanism perhaps switching the energy sources, activating fatty acid oxidation mimicking the central nervous system energy reserve[11] and inhibiting anabolic processes.

Focusing on metabolism, the SFXN3 KO cells exhibit a more glycolytic phenotype compared to WT cells **(Fig. 1-d)**. characterised by reduced metabolic parameters expect for a higher spare respiratory capacity. The reduced mitochondrial (basal) respiration in KO cells could reflect a compensatory mechanism where glycolysis provides sufficient ATP, potentially reducing the need for mitochondrial ATP production and influencing various metabolic pathways. Under normal conditions, WT cells appear to operate closer to their maximum capacity, while KO cells do not, potentially indicating a metabolic adjustment in the KO cells, which seemed contradictory. However, the apparent higher spare respiratory capacity in KO cells might not reflect a true increase but could be an artefact of the short-term assay period, as it may not be sustained over extended times. Additionally, it is crucial to normalise data for cell count and protein content to ensure accurate comparisons, which wasn't possible due to low concentrations of Hoesch stain injected.

Our data suggested that loss of SFXN3 influences mitochondrial bioenergetics, contrasting with the mice model data[12]. Conversely, the SH-SY5Y SFXN3 KO cells show a similar phenotype to the mitochondrial impairment seen in PD patients where synaptic mitochondria show a decrease in ATP production[13], inhibited enzyme activity in the electron transport chain[14] and complex I deficiency[15]. Using a human cell model to study SFXN3 provides more accurate insights into its role in the human genome and proteome than rodent models. This is key for understanding how SFXN3 loss, seen in PD patients. Further experiments suggested in **Figure 2** are required to get a full picture of the impact of the KO.

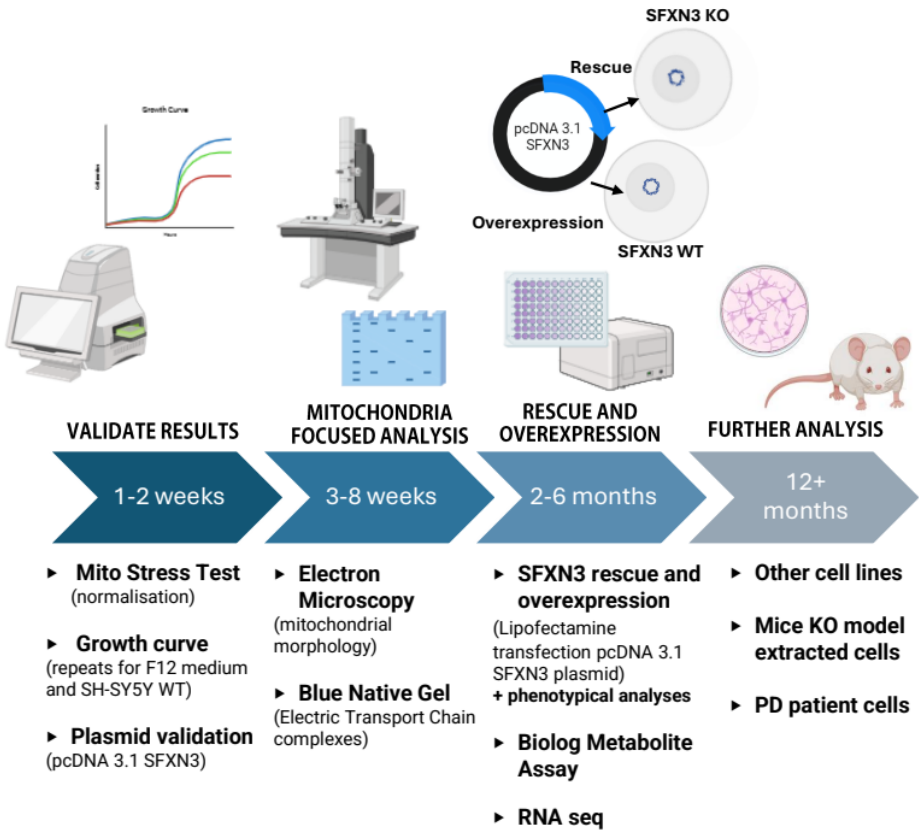
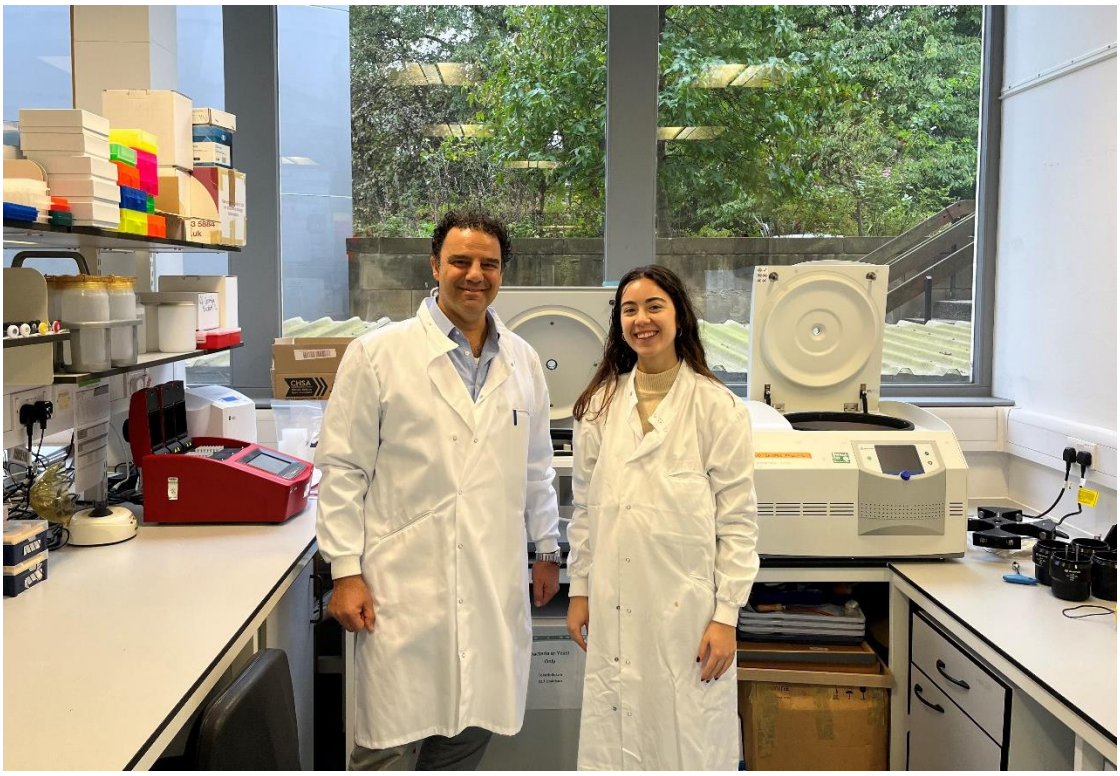



*Figure 2.* ***Proposed future work.*** *This diagram outlines the proposed future experimental plan for studying the role of SFXN3 in mitochondrial function and Parkinson's disease (PD) pathology.* ***Validation of Results*** *(1-2 weeks): This phase involves confirming current data through repeating Mito Stress Tests with proper normalisation and growth curve experiments, specifically in F12 medium and SH-SY5Y wild-type (WT) cells. Additionally, validation of the pcDNA 3.1 SFXN3 plasmid will be carried out.* ***Mitochondria-Focused Analysis*** *(3-8 weeks): Mitochondrial dynamics will be assessed via Electron Microscopy to examine morphological changes, while Blue Native Gel Electrophoresis will be used to analyse the integrity and function of mitochondrial electron transport chain complexes.* ***Rescue and Overexpression*** *(2-6 months): In this phase, the impact of SFXN3 rescue and overexpression will be explored using Lipofectamine transfection with the pcDNA 3.1 SFXN3 plasmid, followed by detailed phenotypic analysis. This stage also includes a Biolog Metabolite Assay and RNA sequencing to investigate metabolic changes and transcriptional alterations caused by SFXN3 manipulation.* ***Further Analysis*** *(12+ months): Longer-term experiments will focus on testing additional cell lines, analysing extracted neuronal cells from SFXN3 knockout (KO) mice, and using PD patient cells to further explore the role of SFXN3 in neurodegeneration.*

**Impact of the work:** This research advances understanding of SFXN3's role in Parkinson's disease, potentially identifying it as a new therapeutic target for PD and other neurodegenerative disorders. Addressing mitochondrial dysfunction, it offers promising avenues for treatment development and could significantly improve patient care. By promoting and sharing knowledge through publications and collaborations with clinicians and pharmaceutical companies, this work aligns with the Biochemical Society's strategy to disseminate molecular bioscience advancements. It fosters wider dialogue among molecular bioscientists, supporting innovation and the translation of research into clinical applications, contributing to the fight against neurodegenerative diseases.

**Studentship value:** This scholarship enabled me to effectively develop key soft skills, including literature analysis, critical evaluation of research, and synthesising information for presentations. The program also improved my time management and collaboration abilities, allowing me to establish valuable academic connections. Mentorship throughout the project offered insights and fostered collaborations, significantly strengthening my future Ph.D. applications. This experience equipped me with the tools to excel in biological research and beyond.



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