

Cyclophilin D modulates mitochondrial bioenergetics but is dispensable for mitochondrial transplantation

Zamprogno Elisa¹, Coluccino Gabriele¹, Carpi Giada¹, Comelli Marina¹, Sponga Sandro², Beltrami Antonio P^{1,3}, Lippe Giovanna¹

¹Dept of Medicine, Univ of Udine, Udine, Italy; ²Division of Cardiac Surgery, Dept of Cardiothoracic and Vascular Disease, Univ of Siena, Siena, Italy; ³Academic Hospital of Udine, ASUFC, Udine, Italy

Contribution to [MiP2026](#) – Received 2026-09-28 – Online 2026-10-07

Cite: Zamprogno E, Coluccino G, Carpi G, Comelli M, Sponga S, Beltrami AP,3, Lippe G (2026) Cyclophilin D modulates mitochondrial bioenergetics but is dispensable for mitochondrial transplantation. BEC preprints 2026.11 <https://doi.org/10.26124/becprep2026-0011>

INTRODUCTION

The peptidylprolyl cis-trans isomerase Cyclophilin D (CyPD), encoded by the *PPIF* gene, is the only member of the Cyclophilin protein family present in the mitochondrial matrix. The human *PPIF* gene encodes a 207-residue protein; its 29-residue mitochondrial targeting sequence (MTS) is cleaved upon mitochondrial entry, yielding the mature 178-residue CyPD [1], which we have structurally characterized by NMR Spectroscopy [2]. CyPD is indeed a key protein in the mitochondrial proteome and interacts with numerous protein partners, thus exerting diverse biological functions in the mitochondrial homeostasis. Nevertheless, the best characterized function of CyPD is the promotion of the permeability transition pore (mPTP) opening by association with the actual pore component(s). The mPTP is a multi-conductance Ca^{2+} -dependent channel of the inner mitochondrial membrane, whose long-lasting opening disrupts bioenergetics parameters leading to mitochondrial swelling and ultimately cell death. The current consensus model is that the mPTP is mediated both by ATP synthase and the adenine nucleotide transporter, possibly associated in a complex where the channel would form at, or near, the c-ring of ATP synthase [1].

The mPTP involvement in pathology has been extensively documented, supporting the notion that CyPD could serve as a promising therapeutic target. Significant efforts are currently made to develop other CyPD-specific inhibitors, which hold promise for treatment of different pathologies [1]. Dysregulated mPTP opening is increasingly recognized as a pivotal contributor to cardiac injury, with mitochondrial transplantation emerging as a novel strategy for myocardial protection. This therapeutic approach involves isolating healthy, respiration-competent mitochondria from a donor and delivering them into the damaged cardiac tissue. The primary objective of our study was to test the efficacy of transplanting mitochondria derived from *PPIF*^{-/-} HEK293 cells into pericytes isolated from donor cardiac atria compared to HEK293 WT cells. We hypothesized that *PPIF*^{-/-} mitochondria, being less prone to mPTP opening under calcium overload conditions potentially encountered during transplantation, would also exhibit improved bioenergetic performance through reduced CyPD-mediated inhibition of ATP synthase. [3] Given the limited

understanding of CyPD's role in mitochondrial bioenergetics, this study also addresses this critical knowledge gap by characterizing respiratory function in isolated mitochondria from HEK293 WT and *PPIF*^{-/-} cells.

METHODS

Mitochondria were isolated from WT and *PPIF*^{-/-} HEK293 cells using the QIAGEN QMitochondria Isolation Kit. Oxygen consumption of freshly isolated mitochondria was evaluated through high-resolution respirometry (Oroboros Oxygraph-2K) adopting a substrate-uncoupler-inhibitor-titration protocol [4]. The maximal oligomycin-sensitive ATP hydrolytic capacity of ATP synthase was measured using the acidification channel (ECAR) of the Seahorse XFe24 Analyzer by sequentially adding 20 mM ATP and 5 μ M FCCP to respiring mitochondria [5].

Pericytes isolated from donor cardiac atria were cultured in DMEM and tested for viability using the MTT assay after a 24-hour treatment with 25 μ g/cm² of WT and *PPIF*^{-/-} mitochondria. Proliferation was assessed by cell counting of Hoechst-stained nuclei.

RESULTS AND DISCUSSION

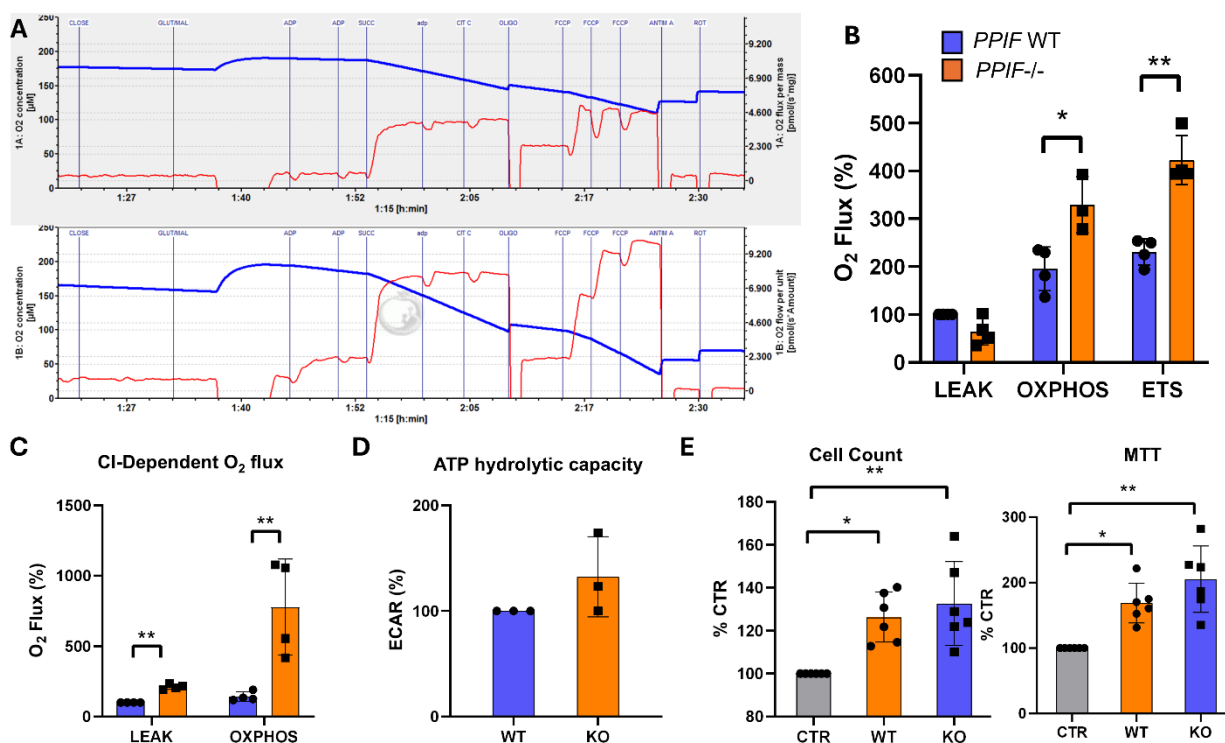


Figure 1: **A)** Representative high-resolution respirometry traces of mitochondria from WT (top trace) and *PPIF*^{-/-} HEK293 cells (bottom trace). Experiments were performed in air-saturated respiration medium (MIR05) at 37°C [4]. **B)** Respiration rates in A, fueled with malate (4 mM), glutamate (10 mM) and succinate (10 mM), were corrected for residual O₂ consumption, normalized to protein content, and expressed relative to WT LEAK-state respiration, obtained after 4 μ M oligomycin addition (100% for each biological replicate). **C)** Respiration values from A obtained with malate and glutamate only. Data are expressed as in B. **D)** The maximal oligomycin-sensitive ATP hydrolytic capacity of WT and *PPIF*^{-/-} mitochondria was measured as acidification rates using the Seahorse XFe24 Analyzer. **E)** WT and *PPIF*^{-/-} mitochondria were co-incubated with pericytes for 24h, then MTT assay or cell counting through Hoechst staining were performed. Statistical analysis was performed using paired two-tailed t-tests in B-D (WT vs KO), and the Kruskal-Wallis test followed by Dunn's test in E (* $p < 0.05$; ** $p < 0.01$).

Although it is well established that *PPIF*^{-/-} mitochondria display a much lower propensity for mPTP opening [6], their respiration pattern remains poorly characterized. Oxygen consumption profiles obtained by high-resolution respirometry show that, with both CI and CII substrates fueling respiration, mitochondria from *PPIF*^{-/-} cells exhibit much higher ADP-stimulated (OXPHOS) and maximal noncoupled (ETS) respiration. Moreover, KO samples tend to exhibit a reduced LEAK respiration compared with WT, which is consistent with the mPTP being significantly more sensitive to low Ca²⁺ in WT mitochondria (Fig. 1B). Such differential behavior could be explained by the following findings: i) In WT mitochondria, the NADH-linked pathway (through CI) is impaired (Fig. 1C), despite the similar CI protein levels in the two genotypes (data not shown), thus suggesting that the reduced CI-linked respiration is a consequence of NAD⁺ depletion associated with mPTP opening during mitochondria isolation [7], which ultimately affects all respiration states; ii) the relief of the inhibitory effect of CyPD on ATP synthase in *PPIF*^{-/-} mitochondria leads to a higher ADP-stimulated respiration. In accordance with this, the maximal ATP hydrolytic capacity (measured by changes in pH due to proton release by ATP synthase) is about 30% higher in *PPIF*^{-/-} samples than in WT mitochondria (Fig. 1D), in agreement with previous results in cardiac mitochondria [3].

Co-incubation of cardiac pericytes with mitochondria from both genotypes (Fig. 1E) leads to a significant increase in cell viability and proliferation (with *PPIF*^{-/-} mitochondria showing a higher level of significance compared to untreated pericytes than WT ones). Contrary to our hypothesis, however, the improved bioenergetic profile of *PPIF*^{-/-} mitochondria and their reduced sensitivity to Ca²⁺-induced mPTP opening did not translate into a significantly enhanced transplantation efficiency into pericytes compared to WT mitochondria. This intriguing discrepancy suggests that mitochondrial internalization or survival within recipient pericytes are not regulated by mechanisms strictly dependent on baseline bioenergetic fitness and/or mPTP opening.

ACKNOWLEDGMENTS

This work was supported by PRIN2022Z85H74 and by PRIN2022NBMBCH. We thank Dr. Michela Carraro (University of Padova, Italy) for providing *PPIF*^{-/-} HEK293 cells.

AUTHOR CONTRIBUTIONS

EZ, GCo, GCa performed the experiments and analyzed the data. MC contributed to data analysis and interpretation. GL, APB, and SS acquired funding. GL, GCo, and MC contributed to drafting the manuscript. GL conceptualized and supervised the work. The authors declare no conflicts of interest.

REFERENCES

1. Coluccino G et al. (2023) Cyclophilin D in Mitochondrial Dysfunction: A Key Player in Neurodegeneration? *Biomolecules*. 13(8):1265 <https://doi.org/10.3390/biom13081265>.
2. Coluccino G et al. (2024) N-terminal cleavage of cyclophilin D boosts its ability to bind F-ATP synthase. *Commun Biol*. 7(1):1486 <https://doi.org/10.1038/s42003-024-07172-8>.
3. Giorgio V et al. (2009) Cyclophilin D Modulates Mitochondrial F₀F₁-ATP Synthase by Interacting with the Lateral Stalk of the Complex. *Journal of Biological Chemistry*. 284(49):33982–33988 <https://doi.org/10.1074/jbc.M109.020115>.

4. Gnaiger E et al. (2020) Mitochondrial physiology. *Bioenergetics Communications*. 2020 <https://doi.org/10.26124/BEC:2020-0001.V1>.
5. Acin-Perez R et al. (2023) Inhibition of ATP synthase reverse activity restores energy homeostasis in mitochondrial pathologies. *EMBO J*. 42(10):EMBJ2022111699 <https://doi.org/10.15252/emj.2022111699>.
6. Carraro M et al. (2020) The Unique Cysteine of F-ATP Synthase OSCP Subunit Participates in Modulation of the Permeability Transition Pore. *Cell Reports*. 32(9):108095 <https://doi.org/10.1016/j.celrep.2020.108095>.
7. Di Lisa F et al. (2001) Opening of the Mitochondrial Permeability Transition Pore Causes Depletion of Mitochondrial and Cytosolic NAD⁺ and Is a Causative Event in the Death of Myocytes in Postischemic Reperfusion of the Heart. *Journal of Biological Chemistry*. 276(4):2571–2575 <https://doi.org/10.1074/jbc.M006825200>.

Copyright ©2026 The author. This is an Open Access preprint (not peer-reviewed) distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. © The author, who has granted BEC preprints an Open Access preprint license in perpetuity.