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Functional analysis of novel wing domain subunits in the *Toxoplasma gondii* ATP synthase complex

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ATP synthase, a membrane bound multi-subunit enzyme is an integral part of the eukaryotic mitochondrial oxidative phosphorylation. The general architecture and subunit composition of ATP synthase is well studied in common model organism, and the catalytic mechanism is considered conserved across eukaryotes, but little is known about this critical complex in Apicomplexa. Using cryo-electron microscopy, we have characterized the *Toxoplasma gondii* ATP synthase complex, which reveals its new structural features (Mühleip et al; Nat. Comm. 2021). *T. gondii* ATP synthase integrates 17 new subunits not seen in other systems and conserved across Myzozoa. Some are involved in forming a unique ATP synthase hexamer, which defines the apicomplexan bulbous cristae. But the role of the others is largely unknown. Curiously, three new subunits, ATPTG1, ATPTG4 and ATPTG14, form a distinct peripheral matrix-exposed “wing” region. We applied conditional gene knockdown approach to investigate their function. Our results show that these subunits are essential for tachyzoite parasite survival even in culture, yet the different mutants exhibit distinct phenotypic outcomes: ATPTG1 and ATPTG14 depletion results in an immediate arrest in the lytic cycle, while ATPTG4 depletion allows some replication before parasite fails to grow. Additionally, depletion of ATPTG1 and ATPTG4 disturbs ATP synthase complex stability, suggesting that these subunits have distinct roles despite the joint formation of the wing. In conclusion, our study aims to provide new insights into unique features of *T. gondii* ATP synthase, enhancing our understanding of its role in parasite’s mitochondrial biology and probable targets for therapeutic intervention.