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Supplementary information

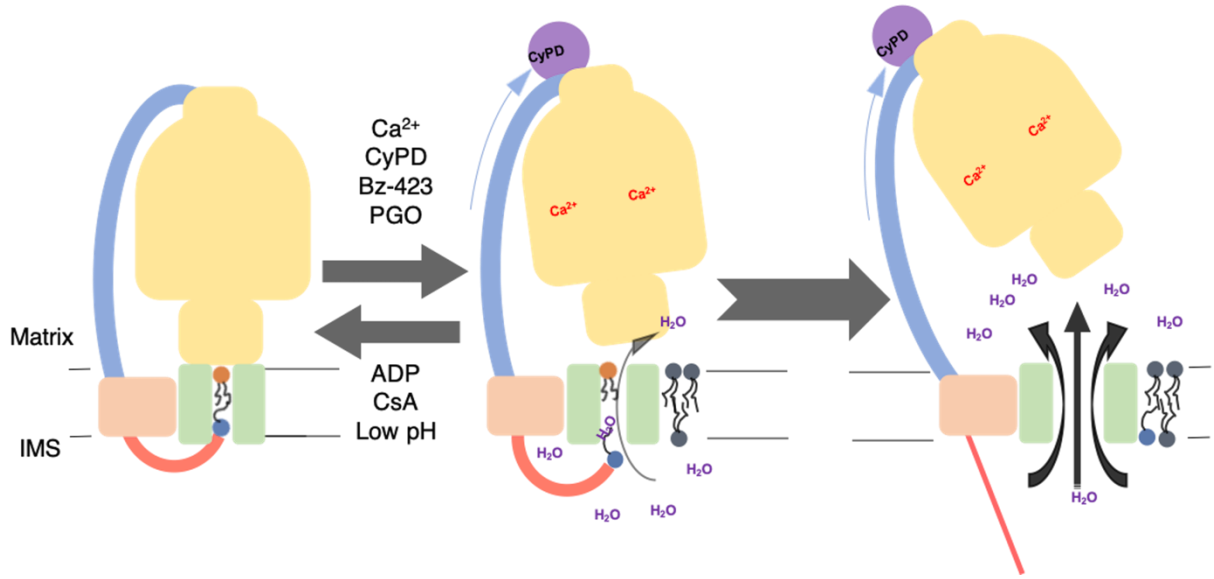
The assembly, regulation and function of the mitochondrial respiratory chain

In the format provided by the authors and unedited

Supplementary Box 1 | **Physiological roles of complex V outside the OXPHOS chain**

In addition to being the main producer of ATP in eukariotic cells, complex V also has other crucial roles in cellular homeostasis, such as assisting mitochondrial cristae formation and the regulation of apoptosis via the PTP. Mitochondrial membrane dynamics are involved in the response to multiple external stimuli, not only related to metabolism, but also to signalling pathways, as has been recently extensively reviewed[1]. Mitochondrial cristae are the main structural feature of the inner mitochondrial membrane, representing invaginations that greatly expand the membrane surface and therefore the space to host the components of the OXPHOS system. ATP synthase dimers have long been known to drive the formation of mitochondrial cristae[2,3], by imposing a curvature of the membrane and dimer rows have been observed to line the cristae ridges, while the other OXPHOS chain complexes are located in the flat regions of the cristae (Fig 1)[4–6]. Interestingly, recent microscopy analysis revealed that in human mitochondria ATP synthase dimers contribute to the correct localization of the Mitochondrial contact site and Cristae Organizing System (MICOS) complex in the inner membrane, via direct interactions with the Mic10 component[7]. MICOS is responsible for the formation of cristae junctions and the specific interaction with ATP synthase dimers turns out to be a crucial event in lamellar cristae formation, thereby expanding the role of complex V in shaping the inner mitochondrial membrane. As mentioned above, ATP synthase also regulates apoptosis: Ca^{2+} and cyt *c* are well-known triggers of cell death[8] and the permeability transition pore (PTP) has been proposed to be the vehicle for the massive release of cyt *c* in the cytosol, in response to calcium binding to complex V[9]. Recent structural data coming from the cryo-EM analysis of ovine ATP synthase, extracted from the membranes of heart mitochondria with the mild detergent digitonin, to preserve its native conformation, has for the first time proposed a detailed mechanism for the opening of the PTP[10]. The structures revealed that subunit e is bound to a lipid on the IMS side, which is inserted into the c-ring cavity. Interestingly, this lipid does not seem to rotate with the ring, but rather stays fixed to subunit e. On the opposite side of the leaflet, conversely, another lipid molecule was observed, which was rotating with the ring. Cryo-EM data collected from a sample exposed to calcium then showed that the connection of subunit e to the c-ring was modified and that the ring itself was progressively losing definition until complete disappearance. These data suggest that PTP opening is driven by destabilization of the c-ring upon removal of the subunit e-linked lipid in the cavity. This in turn has been proposed to be triggered by a conformational change in the F_1 domain, which would be propagated to subunit e via the peripheral stalk. The figure below (reproduced from ref[10]) depicts the suggested model based on these structures, starting with reversible opening (middle) which eventually becomes irreversible (right). Compounds either promoting or inhibiting the PTP are listed along the arrows. The F_1 module is in yellow, peripheral stalk in blue, subunit a in salmon, c-ring in green, and subunit e, connected to the lipid plug, is in red. Cyclophilin D (CyPD) attaches to the top of F_1 to promote PTP opening. In this scenario the ATP synthase monomer is responsible for the PTP, in contrast to earlier suggestions that PTP might be formed at the dimer interface[11]. However, the debate on whether a monomer[12] or a dimer[13] is necessary for PTP still continues. Further studies on Ca^{2+}-induced

conformational changes in ATP synthase are necessary to verify these proposals, both on detergent-extracted enzyme, but at higher resolution, and also in situ in membranes by cryogenic electron tomography.



Supplementary note | **Antibody usage in two recent publications**[14,15]

In the two indicated references, the method section refers to "Invitrogen 35-8100" as the antibody used to detect MT-CO1. As can be verified by checking the catalog number on the vendor's website (https://www.thermofisher.com/antibody/product/COX1-Antibody-clone-COX-111-Monoclonal/35-8100), this antibody actually recognises COX1 (i.e. cyclooxygenase 1, a.k.a. PTGS1) and not subunit 1 of cytochrome c oxidase, or complex IV.

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