Figure 2

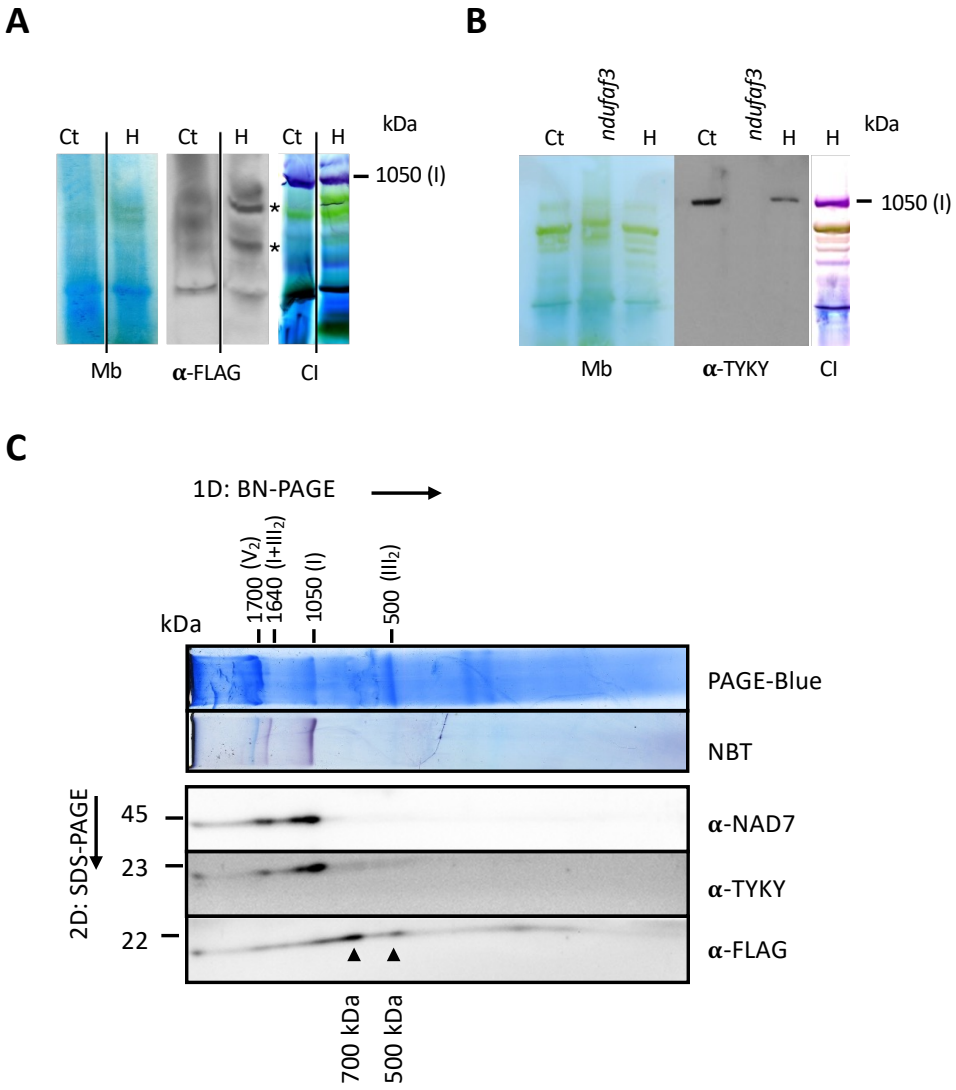

Figure 2. Analysis of complex I assembly by 2D BN/SDS PAGE reveals that NDUFAF3 co-migrates with two intermediates.

A. Membrane extracts from control (Ct) and H strain were resolved in native conditions (BN-PAGE). The left part (Mb) represents the blotted membrane before incubation with antibodies against the FLAG tag of NDUFAF3 (α-FLAG), the middle panel represents the immunoblot, and the right panel (CI) represents NADH staining for complex I activity. The asterisks indicate the position of the NDUFAF3 containing complexes (~700 and 500 kDa). The black lines indicate the separation on the membrane/blot/gel between the loadings of Ct and H transformant, signifying some samples unrelated to the study **B.** Membrane extracts from control (Ct), *ndufaf3* and H strain were resolved in native conditions (BN-PAGE). The left part (Mb) represents the blotted membrane before incubation with antibodies against the TYKY antibodies, the middle panel represents the immunoblot, the right panel (CI) represents NADH staining for complex I activity. **C.** Purified mitochondria samples were resolved in native conditions (left to right) and denaturing conditions (top to bottom). Starting at the top, the panels display an enriched mitochondrial fraction, NADH/NBT staining of the mitochondrial fraction, and immunoblots against the NAD7 and TYKY complex I subunits and the FLAG tag of NDUFAF3. V_2, I+III_2, I, and III_2, correspond to respiratory chain complexes V (dimeric state), supercomplex I+ III_2, complex I, and complex III (dimeric state), respectively (Cardol et *al.*, 2006; Cardol et *al.*, 2008, Waltz *et al.*, 2025). The grey arrows identify the position of the NDUFAF3 containing complexes (~700 and 500 kDa).