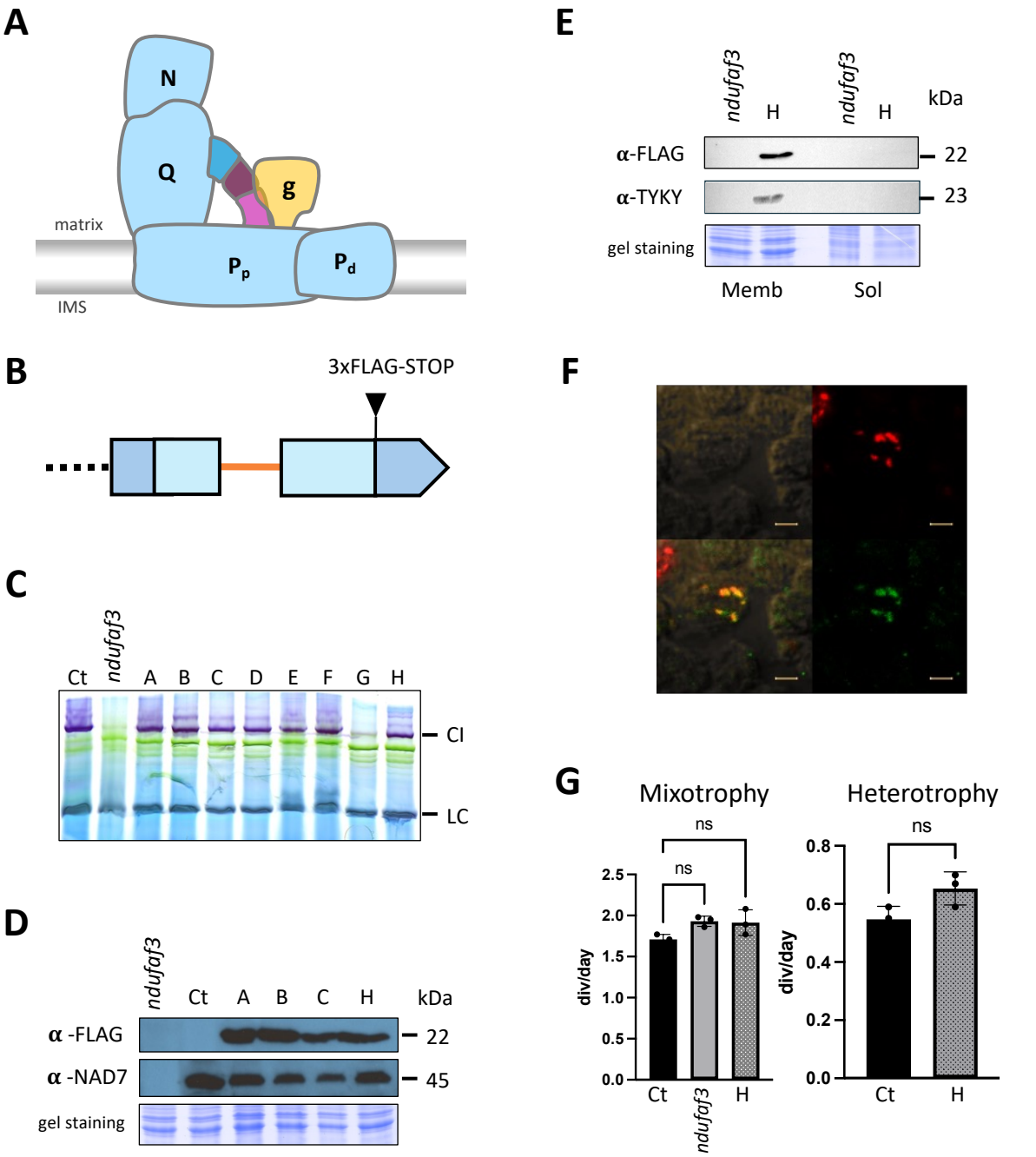

Figure 1. The *NDUFAF3-3xFLAG* gene restores complex I activity in a *ndufaf3* mutant and its corresponding gene product is addressed to mitochondria.

A. Modular structure of complex I. Schematic representation of the complex I structure, including the ferredoxin bridge composed, from left to right, of the B14 (blue), SDAP (dark red), C1-FDX (pink), and γ carbonic anhydrase domain (yellow) subunits. The modules of the matrix arm (N and Q modules) and the membrane arm (P_p and P_d modules) are shown in blue. IMS: intermembrane space. **B.** Schematic representation of *NDUFAF3-3xFLAG* gene (Cre12.g496800) synthetic gene. Dark blue boxes represent endogenous 5' and 3' UTRs, while light blue boxes depict exons. The 3xFLAG tag insertion site is indicated by the black arrow. The orange line represents the *RBCS2i*1 intron engineered in the construct, and the dotted grey line depicts the endogenous promoter (200 bp) (see **Fig. S1** for details). **C.** NADH/NBT staining of native protein complexes from membrane extracts of eight NDUFAF3-3xFLAG-expressing transformants (A-H), the recipient mutant (*ndufaf 3*), and the wild-type (Control, Ct) strain (600 µg of proteins per lane) separated by BN-PAGE. CI: complex I, LC: loading control. **D.** Immunoblots of membrane fractions (20 µg of proteins per lane) incubated with antibodies against the FLAG tag of NDUFAF3-3xFLAG and NAD7. A Coomassie blue-stained gel loaded with the same extracts, is included as a loading control. **E.** Immunoblots of membrane fractions (Memb: 20 µg of proteins per lane) and soluble fractions (Sol: 20 µg of proteins per lane) incubated with FLAG or TYKY antibodies. A Coomassie blue staining of a gel loaded with the same extracts is included as a loading control. **F.** Immunofluorescence staining of fixed cells of the H transformant visualized by confocal microscopy: upper left: brightfield image; upper right: MitoTracker Orange staining; lower right: Alexa Fluor 488 conjugated FLAG-tag antibodies; lower left: merged image. Scale: 2 µm. **G.** Growth rates (number of divisions/24h) in mixotrophy and heterotrophy. n = 3, error bars are standard deviations, ns = non-significant. Statistical analysis was performed using Ordinary one-way ANOVA for growth in mixotrophy and an unpaired t-test for growth in heterotrophy.