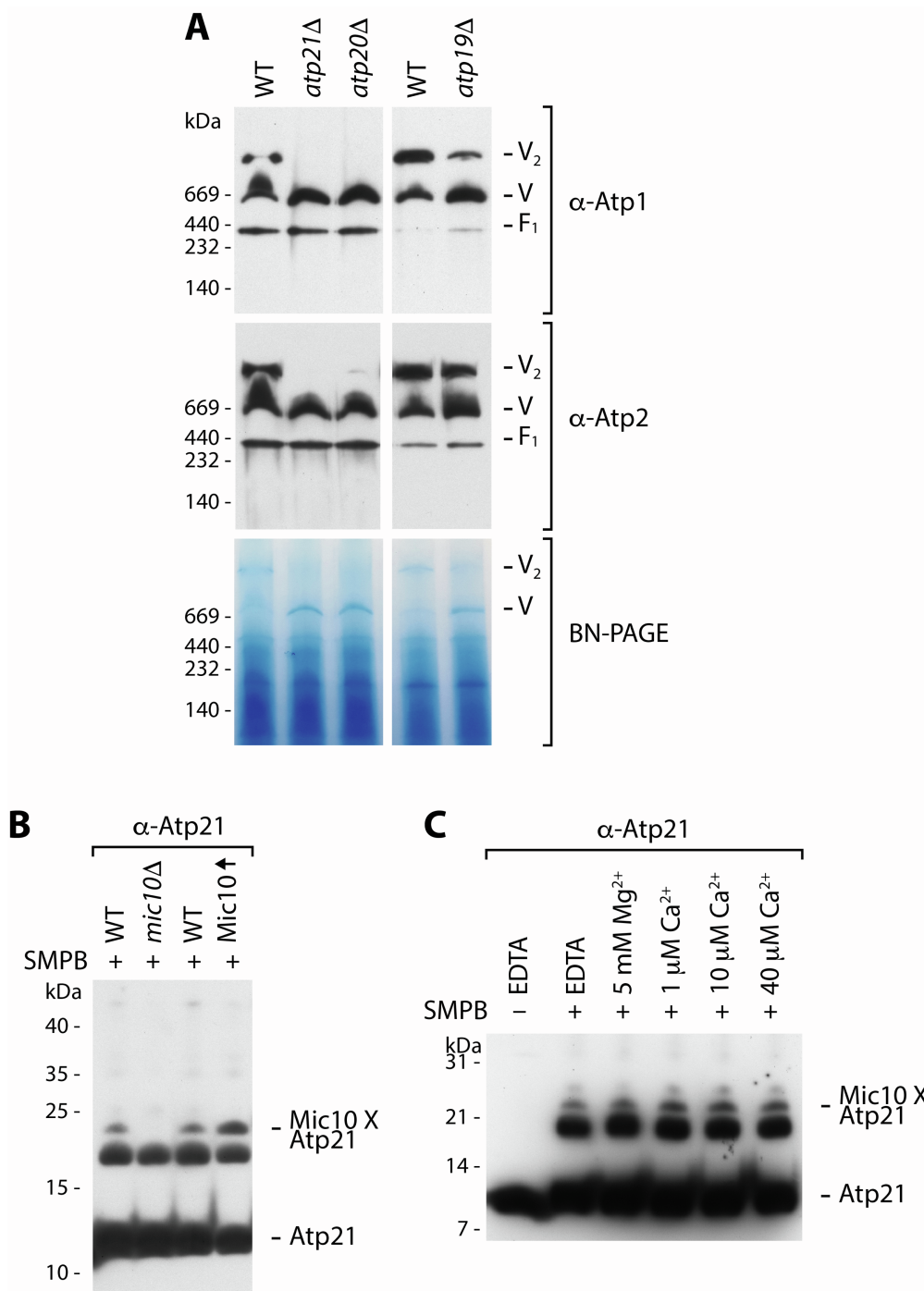


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**Supplemental information**

**Dual role of Mic10 in mitochondrial cristae  
organization and ATP synthase-linked  
metabolic adaptation and respiratory growth**

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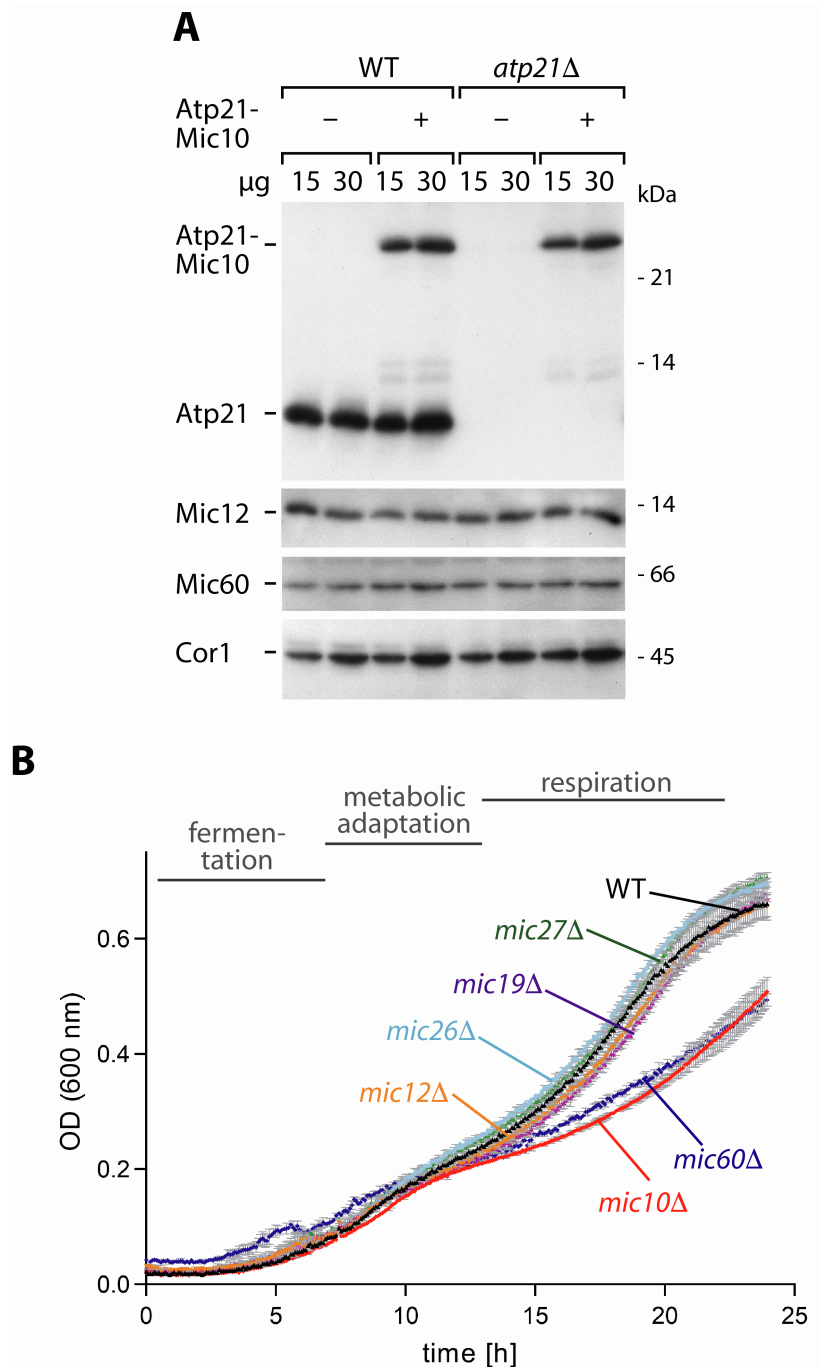
**Figure S1. ATP synthase mutant strains and Mic10 crosslinking to Atp21. Related to Figures 1 and 4**

(A) Characterization of ATP synthase complexes in mitochondria lacking Atp19 (Su k), Atp20 (Su g) or Atp21 (Su e) by BN-PAGE analysis. Upper and middle panels: Western blots using antisera against ATP synthase subunits Atp1 ( $F_1\alpha$ ) and Atp2 ( $F_1\beta$ ), respectively. Lower panel: Untreated BN-PAGE gel with native complexes stained by residual Coomassie from the electrophoresis. Dimeric ATP synthase was absent in

mitochondria lacking Atp20 or Atp21, and destabilized in those lacking Atp19. WT, wild-type.

(B) *In organello* crosslinking of mitochondria from WT, *mic10* $\Delta$  or Mic10 overexpressing strains and analysis for Atp21 crosslinks. An  $\alpha$ -Atp21-reactive band at ~22 kDa was absent in mitochondria lacking Mic10 and its molecular weight is consistent with a direct crosslinking product between Atp21 and Mic10. This crosslink band was more abundant in mitochondria with increased Mic10 levels. SMPB, succinimidyl 4-(*p*-maleimido-phenyl)butyrate.

(C) *In organello* crosslinking of WT mitochondria in the presence or absence of EDTA or the divalent cations Mg<sup>2+</sup> or Ca<sup>2+</sup>. The crosslinking between Mic10 and Atp21 was unaffected by the treatments.

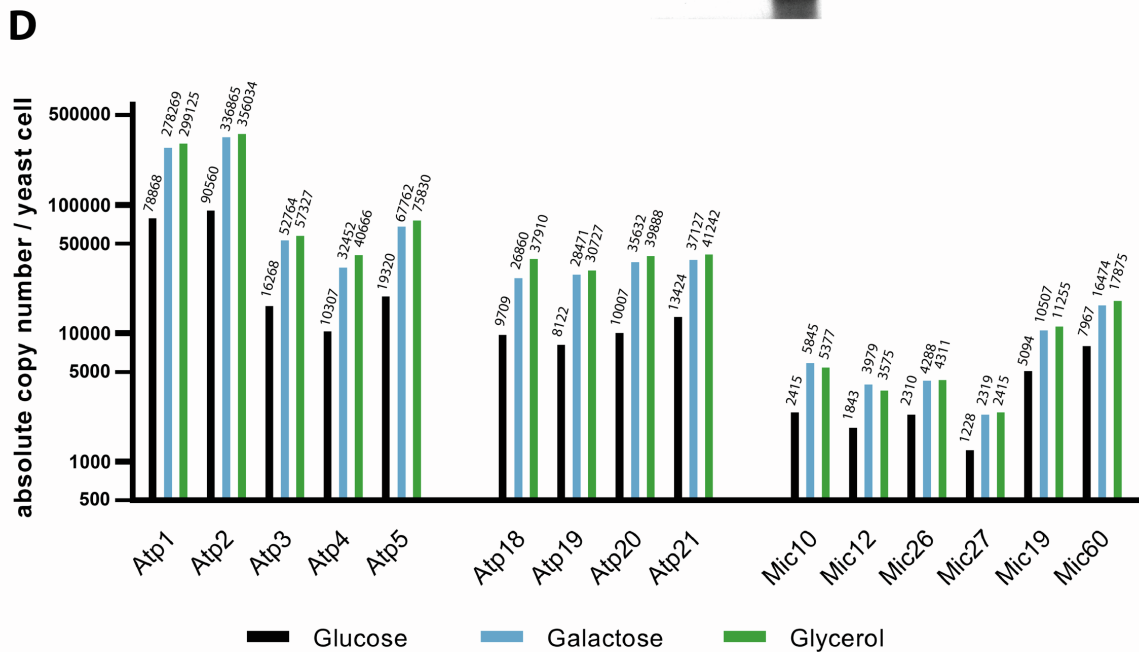
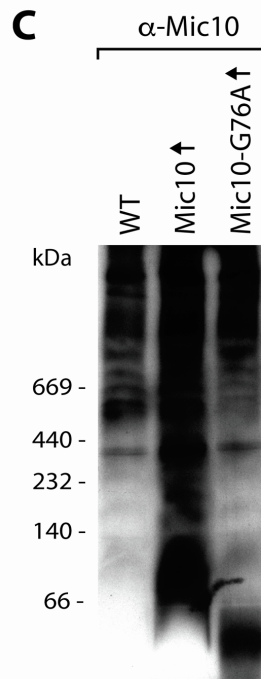
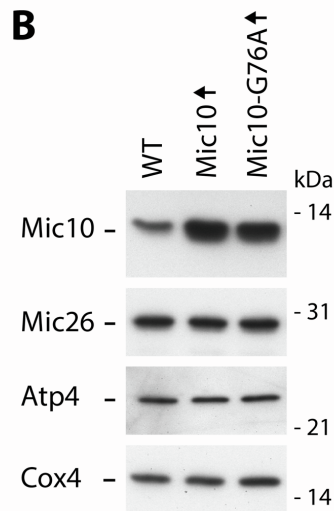
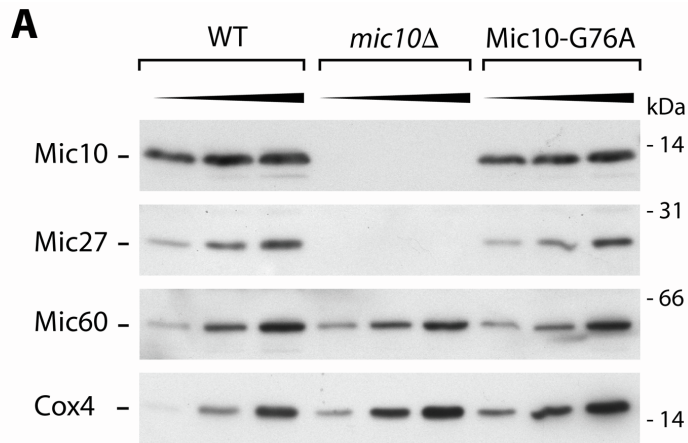


**Figure S2. Expression of Atp21-Mic10 and growth of MICOS mutant strains. Related to Figures 2 and 3**

(A) Protein levels in mitochondria from WT or *atp21Δ* cells with or without expression of Atp21-Mic10. Lanes were loaded with 15 or 30 μg mitochondrial protein, respectively. Cor1, component of complex III of the respiratory chain.

(B) Growth of WT cells as well as cells lacking each MICOS subunit individually in minimal media with 3% glycerol + 0.1% glucose. After initial fermentation on glucose, the cells undergo diauxic shift to adapt to respiration. Only *mic10Δ* and *mic60Δ* deletion

strains displayed growth defects during diauxic shift as well as during respiration. In contrast, the other MICOS deletion strains underwent metabolic adaptation and grew under respiratory condition like WT. Data are represented as mean  $\pm$  SD, n = 3.



**Figure S3. Characterization of oligomerization-deficient Mic10 and protein abundance. Related to Figure 4**

(A) Protein levels in mitochondria analyzed also in Fig. 4A. Expression of Mic10-G76A restored Mic27 levels which are dependent on Mic10. Lanes were loaded with 10, 20 or 40  $\mu$ g mitochondrial protein, respectively. Cox4, subunit of complex IV of the respiratory chain.

(B) Protein levels in mitochondria from cells overexpressing Mic10 (WT form) or Mic10-G76A in WT background. Levels of both Mic10 and Mic10-G76A were significantly and similarly elevated upon overexpression as compared to the WT level. Atp4 (Su b), ATP synthase subunit.

(C) Mic10-containing native complexes in mitochondria as analyzed also in (B). In line with its oligomerization defect, overexpressed Mic10-G76A was severely impaired in forming blue native complexes compared to Mic10 (WT form).

(D) Absolute copy numbers of ATP synthase and MICOS subunits in yeast grown on the fermentable carbon source glucose (black), galactose (blue) or the non-fermentable carbon source glycerol (green); all values are derived from Morgenstern et al. (2017). Atp1 ( $F_1\alpha$ ), Atp2 ( $F_1\beta$ ), Atp3 ( $F_1\gamma$ ):  $F_1$  subunits of the ATP synthase; Atp4 (Su b), Atp5 (Su 5/OSCP): subunits of the peripheral stalk of the ATP synthase; Atp18 (Su i), Atp19 (Su k), Atp20 (Su g), Atp21 (Su e): ATP synthase subunits involved in dimerization, oligomerization and promoting membrane curvature; Mic10, Mic12, Mic26, Mic27, Mic19, Mic60: MICOS components. The protein levels of ATP synthase subunits are significantly higher than those of MICOS subunits, indicating that the interaction of Mic10 with the ATP synthase is substoichiometric.

Table S1. Oligonucleotides used in this study. Related to STAR Methods

Atp21-His-F	TTGAAAGAGTTATTCTTAACGCCGTTGA ATCCCTGAAGGAAGCTTCAACAGGACC CGGACACCACCAT	This paper	2412
Atp21-His-R	GTGCGAGCTAATGTGCATTTTTAGTATC CTATTTATGTTGAAGCTTCTATATCGAT GAATTCGAGCTCG	This paper	2413
Atp19-His-F	GAAAAATTCATTGAAAACACTTAAAG AAACATTCGGAAAAGCAAGATGCGGGA CCCGGACACCACCAT	This paper	2414
Atp19-His-R	ATTTATTGTATGTACAAAAGATCTTCAA CCGCGCAGCAATCAAGCTATATATCGAT GAATTCGAGCTCG	This paper	2415
Atp5-His fwd	GCATTTCTACAAAGATTCAAAAACGTAA TAAGGTCTTAGAGGACAGCATTTCGTAT CGCGCAGGTCGAC	This paper	LS01
Atp5-His rev	CAAAGAAAGTAACATCAAACGAGTTGA GCATATCCAACCTATATTATTAACGATCG ATGAATTCGAGCTCG	This paper	LS02
Mic10-1-F	ATGTCCGAACAAGCACAAAC	This paper	2416
Met25P-R	GGATCCGATGGGGGTAATAG	This paper	2417
Met25P-Atp21-F	CAGATACATAGATACAATTCTATTACCCCATC GGATCCATGTTCGACAGTTAATG	This paper	2418
Fusion-Tim-Mic-R	GTTGTTGTGTTTGTGCTTGTTCCGACATTGTTG AAGCTTCCTTCAGGGATTCAACG	This paper	2419
Mic10-G76A-F	GGCATTGGATTTGGTGTGCA AGAGGCTACGCCGAGG	This paper	2420
Mic10-G76A-R	CCTCGGCGTAGCCTCTTGCAAC ACCAAATCCAATGCC	This paper	2421
SP6-Mic10-1-F	TCGATTTAGGTGACACTATAGAAGCGGCCACC ATGTCCGAACAAGCAC	This paper	1220
Mic10-rv	CTGCACGGATCCCTAAACCTTCGAGGATCTGA	Bohnert et al., 2015	865