Supplemental Figures to

S. Stold et al.

The inner-mitochondrial distribution of Oxa1 depends on the growth conditions and on the availability of substrates

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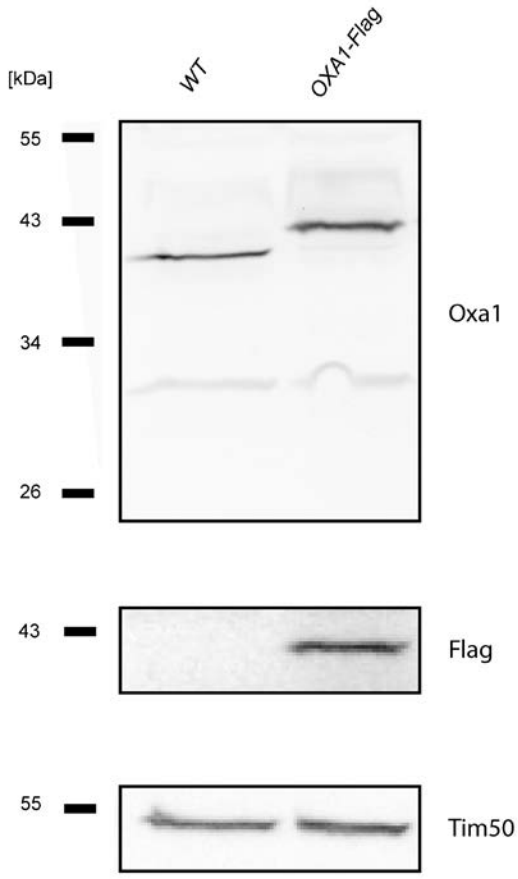

FIGURE S1: The full length Flag-tagged Oxa1 fusion protein is expressed at close to endogenous levels. Whole cell extracts were analysed by immunoblotting using an Oxa1-specific antiserum. As controls, Flag and Tim50 specific antisera were used.

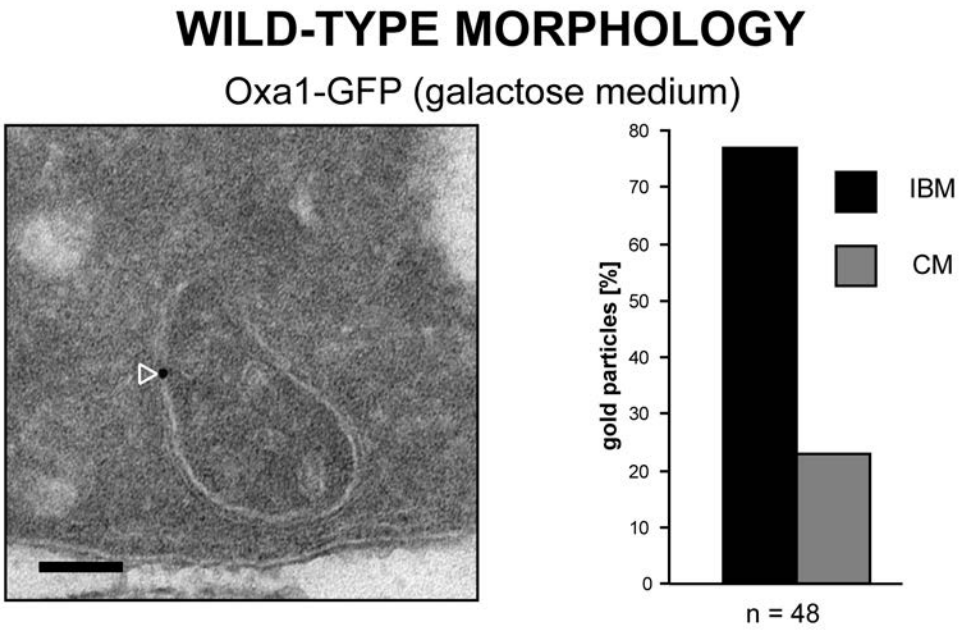

FIGURE S2: Oxa1-GFP exhibits a predominant localization in the inner boundary membrane in cells grown on the fermentable carbon source galactose. The immuno-EM sections were decorated with a GFP-specific antiserum. Left: representative image. The arrowhead points to a gold particle. Right: quantification of the Oxa1-GFP distribution between the IBM and CM. Scale bar: 100 nm.

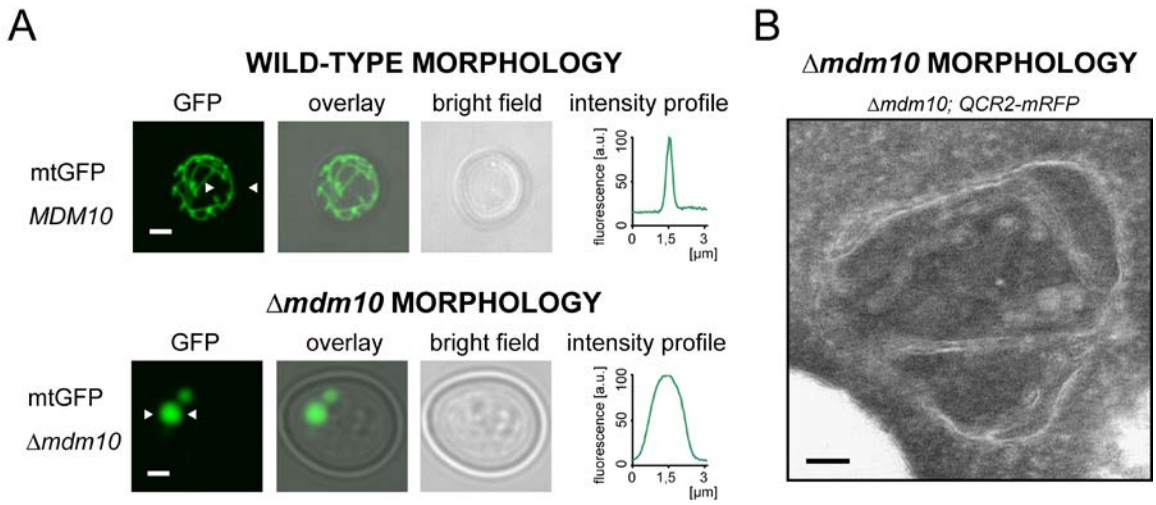

FIGURE S3: The deletion of *MDM10* leads to enlarged, spherical mitochondria containing cristae. (**A**) Live-cell fluorescence microscopy of cells expressing matrix targeted GFP (mtGFP). Top: wild-type cells exhibit a typical mitochondrial network structure. Bottom: cells featuring enlarged mitochondria due to the lack of Mdm10. Single confocal sections are displayed. The intensity profiles show the fluorescence intensity of mtGFP (green) between the two arrowheads. (**B**) Cryo-EM section of a *Δmdm10* cell showing an enlarged mitochondrion. Scale bars: 2 µm (A) and 100 nm (B).

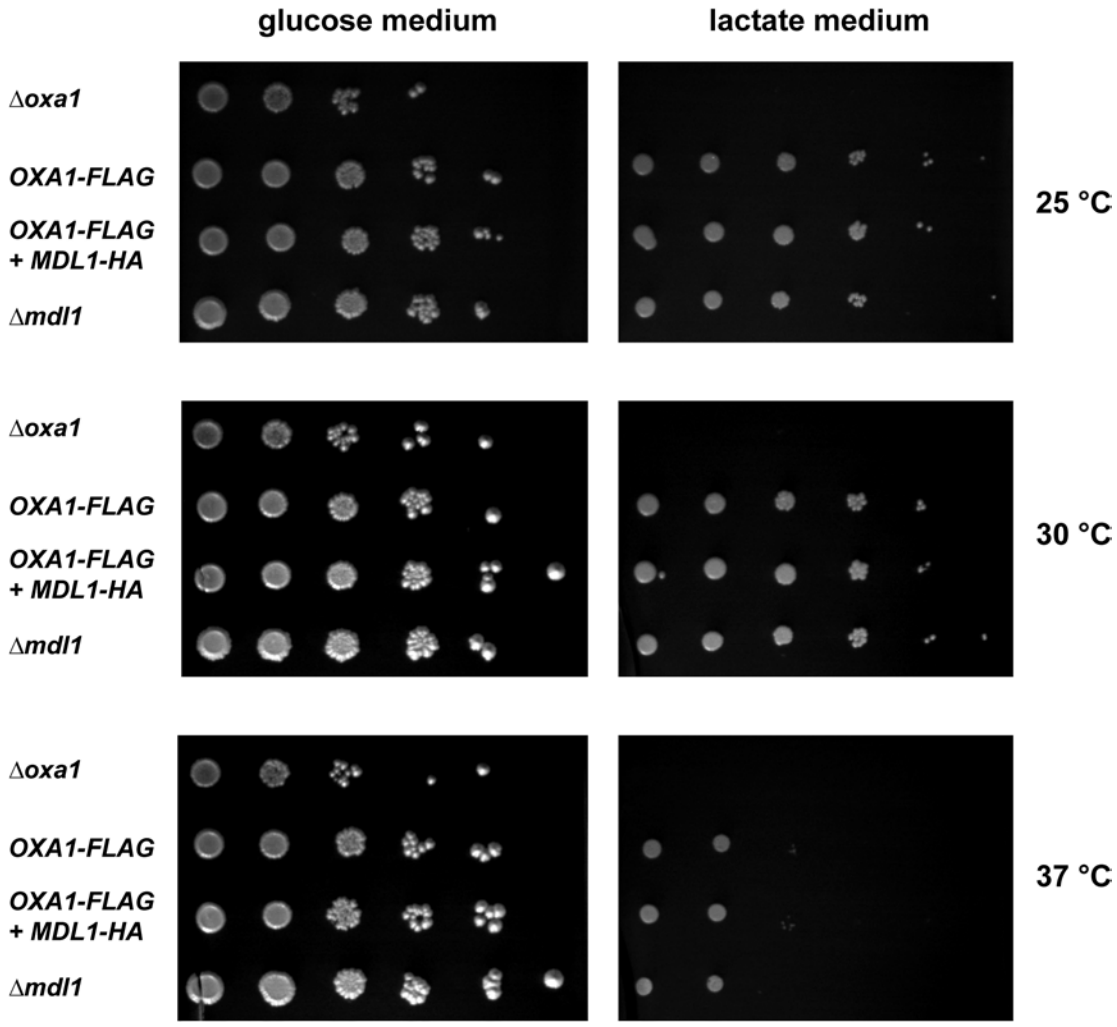

FIGURE S4: Influence of the deletion or overexpression of *MDL1* on the growth of yeast cells grown at different temperatures and on different carbon sources. Tenfold serial dilutions of logarithmically growing cultures were spotted onto plates containing glucose (fermentable) or lactate (non-fermentable) as sole carbon sources and incubated for 7 d at 25 °C, 30 °C or 37 °C. The Oxa1-Flag expressing strains were devoid of a chromosomal copy of the endogenous *OXA1* gene and expressed Oxa1-Flag from a centromeric plasmid using the native OXA1 promoter.

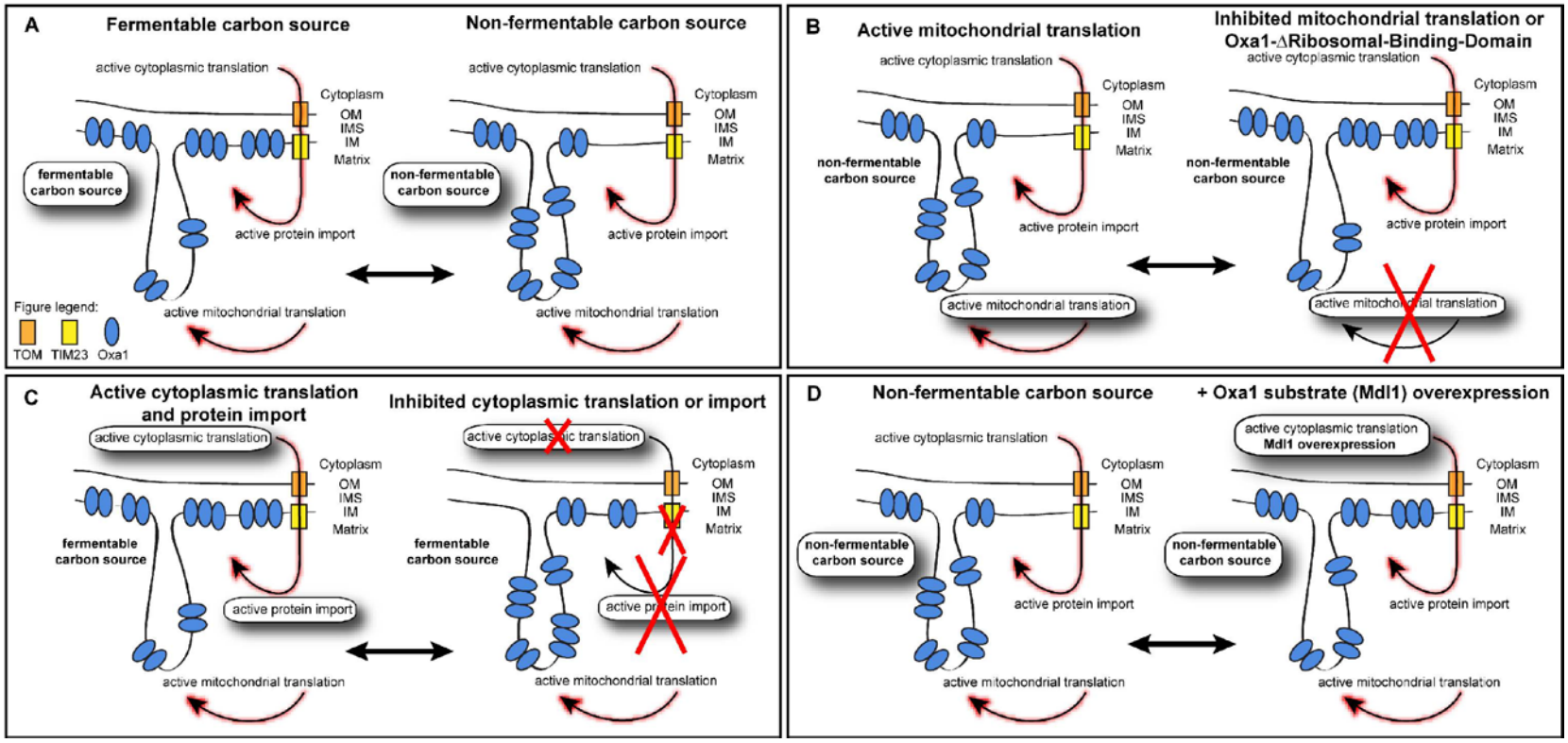
A
Fermentable carbon source
Non-fermentable carbon source
active cytoplasmic translation
Cytoplasm
OM
IMS
IM
Matrix
fermentable carbon source
non-fermentable carbon source
active protein import
active mitochondrial translation
Figure legend:
TOM
TIM23
Oxa1
B
Active mitochondrial translation
Inhibited mitochondrial translation or Oxa1-ΔRibosomal-Binding-Domain
non-fermentable carbon source
C
Active cytoplasmic translation and protein import
Inhibited cytoplasmic translation or import
D
Non-fermentable carbon source
+ Oxa1 substrate (Mdl1) overexpression
Mdl1 overexpression

FIGURE S5: Schematic representation of the observed factors that influence the distribution of Oxa1 between the IMB and the CM. (**A**) Oxa1 is enriched in the IBM when grown on a fermentable carbon source and redistributed to the CM upon a change to a non-fermentable carbon source. (**B**) The enrichment of Oxa1 in the CM when grown on a non-fermentable carbon source is abrogated when mitochondrial translation is inhibited by chloramphenicol. Likewise, Oxa1 without its C-terminal RBD is no longer enriched at the CM. (**C**) The preferential localisation of Oxa1 in the IBM when grown on a fermentable carbon source requires active cytoplasmic translation as well as active protein import into the mitochondrial matrix. (**D**) The preferential localisation of Oxa1 in the CM when grown on a non-fermentable carbon source can be inverted by the overexpression of the nuclear-encode Oxa1 substrate Mdl1.