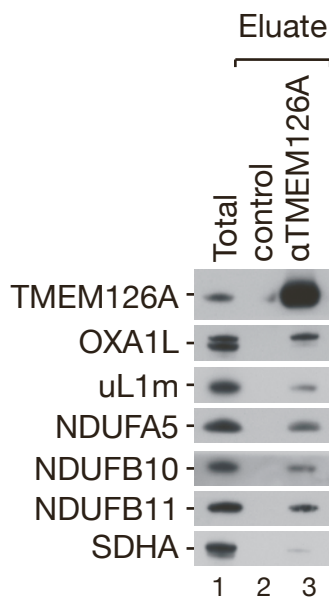
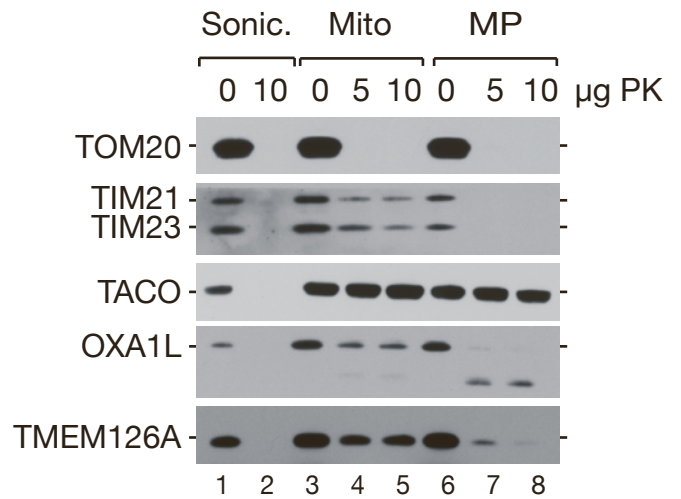
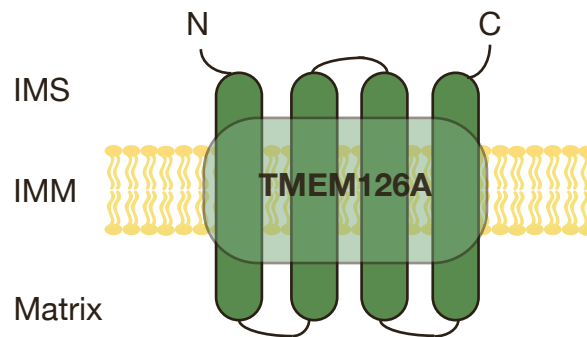


Supplemental information

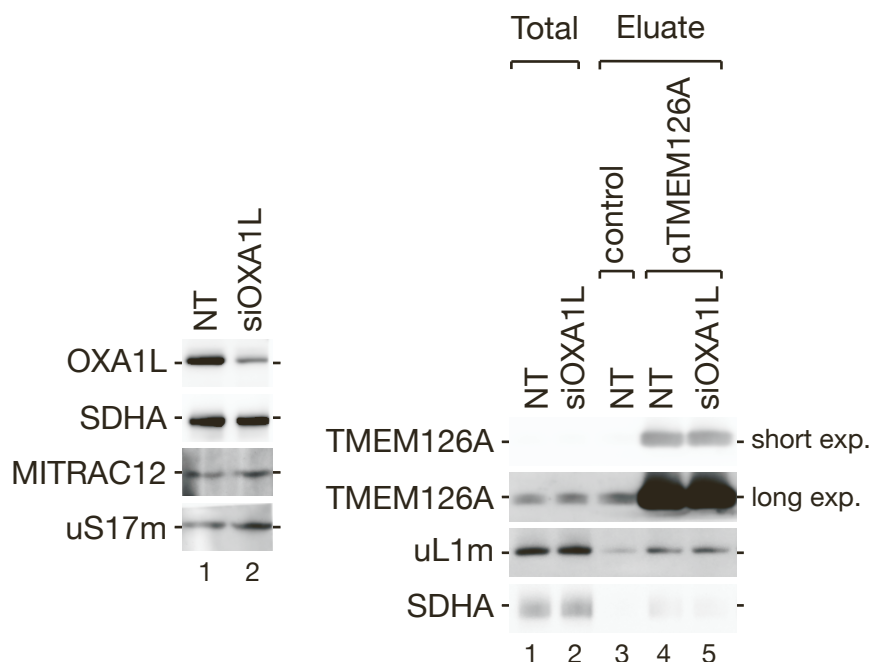
**Identification of TMEM126A
as OXA1L-interacting protein reveals
cotranslational quality control in mitochondria**

Sabine Poerschke, Silke Oeljeklaus, Luis Daniel Cruz-Zaragoza, Alexander Schenzielorz, Drishan Dahal, Hauke Sven Hillen, Hirak Das, Laura Sophie Kremer, Anusha Valpadashi, Mirjam Breuer, Johannes Sattmann, Ricarda Richter-Dennerlein, Bettina Warscheid, Sven Dennerlein, and Peter Rehling

A**B****C**

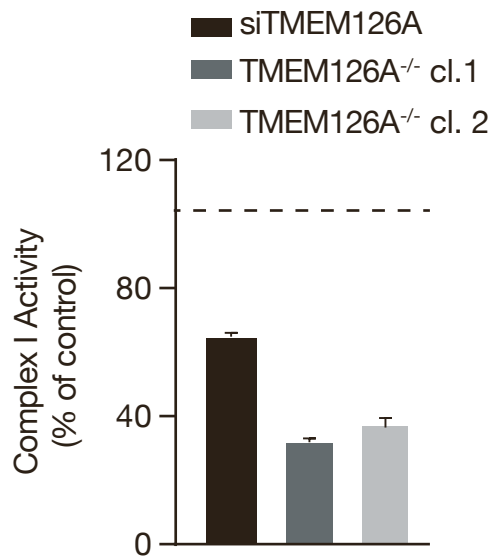
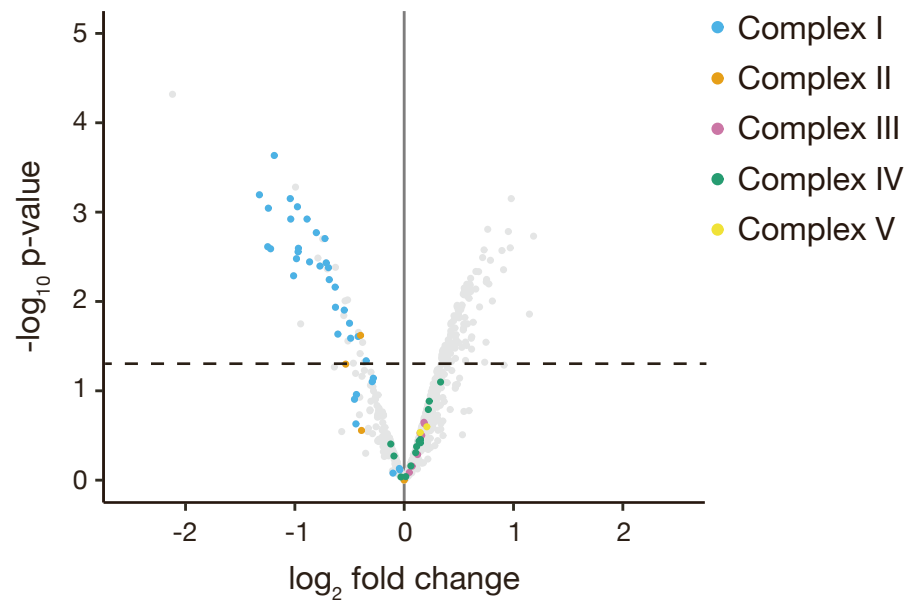
Supplemental Figure S1: TMEM126A is an IMM protein, with its N- and C-terminus facing the IMS.

Related to Figure 1. (A) Immunoprecipitation of TMEM126A using an antibody against TMEM126A. Eluates were analysed by western blot. (B) Mitochondria were isolated and the submitochondrial localization of TMEM126A determined. Proteinase K (PK) was added to intact mitochondria (Mito) or hypotonically swollen mitochondria (MP). Further, mitochondria were subjected to sonication in the presence of PK (Sonic). (C) Scheme of the topology of TMEM126A.

A**B**

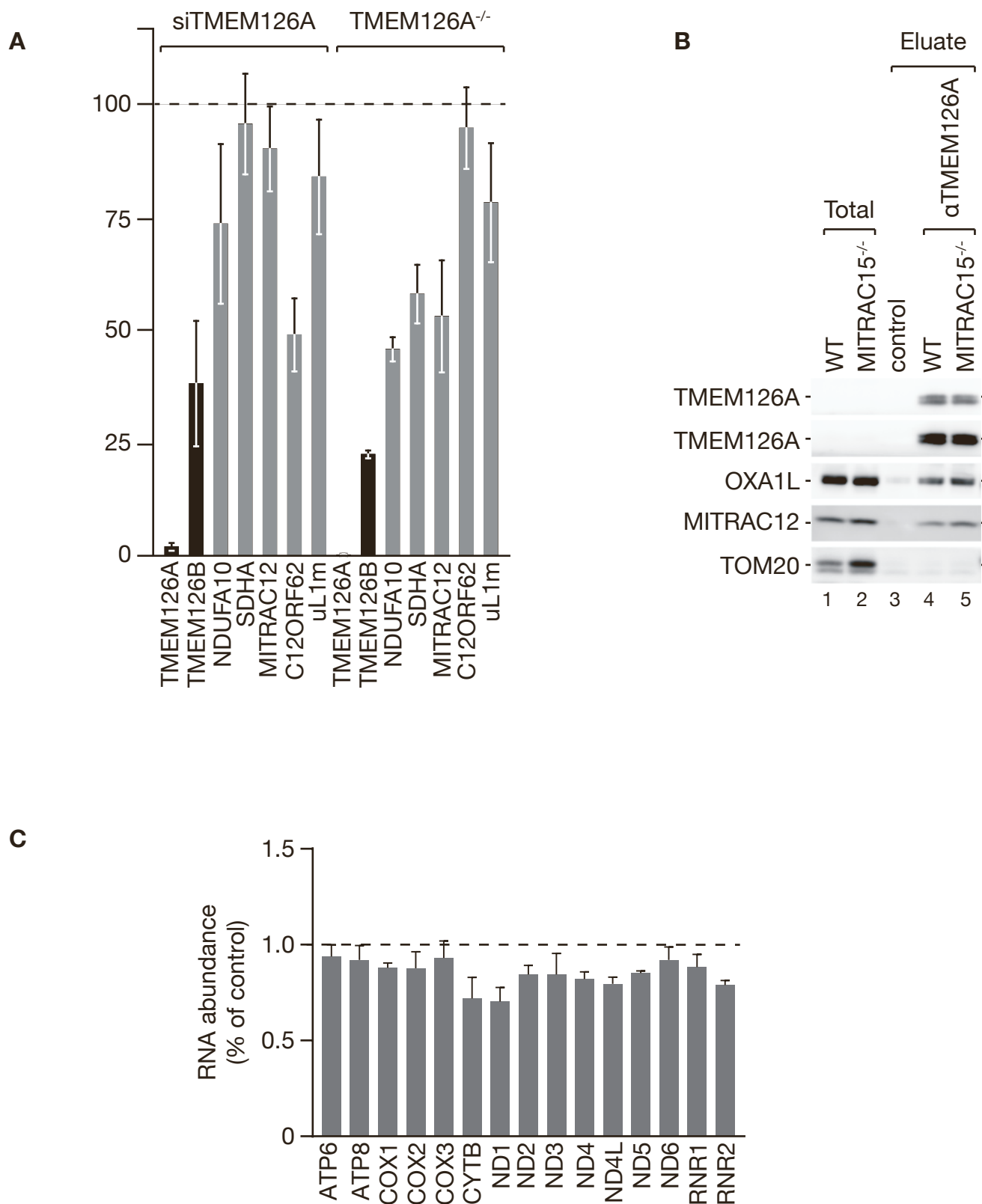
Supplemental Figure S2: TMEM126A interacts with the mitochondrial ribosome independently of OXA1L. Related to Figure 2. (A) OXA1L gets reduced upon siRNA application. OXA1L was reduced by siRNA treatment and whole cell lysates analyzed by western blot analysis.

(B) OXA1L was depleted by siRNA application and mitochondria isolated. A fraction (10 μ g) of these mitochondria was used to confirm the reduction of OXA1L by SDS-PAGE and western blotting (left panel). The majority (1.2 mg) were solubilized under mild conditions and subjected to TMEM126A antibody immunoprecipitation. Eluates were analyzed by western blot. (short exp. = short exposure; long exp.=long exposure).

A**B**

Supplemental Figure S3: Depletion of TMEM126A reduces complex I. Related to Figure 4.

(A) Measurement of the enzymatic activity of complex I in either siRNA treated cells against TMEM126A or TMEM126A^{-/-} cells. (mean = SEM, n = 6). (B) Quantitative mitochondrial proteome analysis of TMEM126A depleted cell. WT or TMEM126A^{-/-} cells were cultured in SILAC media, mitochondria isolated and subjected to quantitative SILAC mass spectrometry analysis.



Supplemental Figure S4: Interaction of TMEM126A and OXA1L during complex I reduction (A) and siRNA mediated reductions of TMEM126A does not affect mitochondrial RNA levels (B). Related to Figure 5. (A) Quantification of protein steady state amount after either siRNA depletion or the knockout of TMEM126A. n=3; SEM (A) Mitochondria from MITRAC15^{-/-} cells were isolated and subjected to immunoprecipitations (Total 0,75%; Eluate 100 %) (B) HEK293 cells were transfected with siRNA targeting TMEM126A. In the following RNA was extracted and NanoString analysis performed as previously described (Cruz-Zaragoza et al., 2021).