Figure S1.  Cox14 and Coa3 are integral inner membrane proteins exposing the C termini to the IMS.  (A) Schematic representation of Coa3 and Cox14. Numbers indicate amino acids; TM indicates the predicted transmembrane segment. Both proteins possess predicted presequences that appear to be not processed in mitochondria. (B) Proteolytic fragments of Coa3 or Cox14 are not detected with antibodies directed against the C termini. Protease protection analysis was performed as described in Fig. 1 E. Images represent the section of the blots below the Coa3 and Cox14 signals. TX-100, Triton X-100; PK, proteinase K.
Figure S2. A fraction of Coa3 is associated with respiratory chain supercomplexes. (A) 75 µg of wild-type (WT) and Coa3^HA mitochondria was solubilized in digitonin buffer and analyzed by BN-PAGE and Western blotting with antibodies against the HA epitope, Cox4 (complex IV), and F1β (F1,F0-ATPase). (B) Native protein complexes containing Cor1^TAP or Tim18^ProtA (control) were purified by IgG chromatography. Samples were separated by SDS-PAGE and analyzed by Western blotting. (C) Isolation of Mss51 and Cox14 complexes by IgG chromatography of solubilized mitochondria from wild type, Mss51^TAP, or Cox14^TAP. Bound proteins were eluted with SDS sample buffer, and samples were analyzed by SDS-PAGE and Western blotting. TAP* indicates free TAP tag that is generated by an uncharacterized proteolytic event and enriched by IgG chromatography. (B and C) The amounts of protein loaded in the total samples correspond to 5% of the eluate.
Figure S3. Cox1 stability of double mutants is unchanged compared with loss of Coa3 or Cox14 alone. (A) In vivo labeling of mitochondrial translation products in the indicated mutants. Extended exposure of gels displayed in Fig. 5 C is shown. (B) Pulse-chase experiments as in Fig. 5 C were performed by labeling mitochondrial translation products in vivo in the indicated strains. Three independent experiments were quantified and plotted as in Fig. 5 D. Mean ratios of Cox1/Cob relative to 0-min chase (100%) are shown. Error bars indicate SEM (n = 3). WT, wild type.