**Fig. S3.** Characterization of mitochondria affected in TIM23 or TIM22 translocases.  

**a-b** Loading controls. Radiolabeled Mpc2 and Mpc3 were imported into mitochondria isolated from the indicated strains as described in Fig. 3a+c, import reactions were analyzed by BN-PAGE and Western blotting, and immunodecorated for the TOM
complex (α-Tom40) or stained with Coomassie to control for equal loading. Representative import experiments are shown. c Radiolabeled Mpc2 and Mpc3 were imported into wild-type, tim18Δ or tim22-14 mitochondria for the indicated periods. Mitoplasts were generated by hypo-osmotic swelling and treated with proteinase K. The samples were analyzed by SDS-PAGE and autoradiography. In all import experiments, non-imported precursors were degraded with proteinase K. d Steady-state protein levels of TIM22 mutant mitochondria. Mitochondria (10, 20 and 40 μg total mitochondrial protein) isolated from wild-type, tim22-14 or tim12-21 yeast strains were analyzed by SDS-PAGE and Western blotting with the indicated antisera. Tim22, Tim54, Tim18, Tim12, TIM22 components; Tim23, TIM23 translocase component; Yhm2, citrate/oxoglutarate carrier (canonical mitochondrial carrier); Ssc1, mitochondrial Hsp70, component of PAM; Tom70, Tom40, components of the TOM translocase. e-f Loading controls. Radiolabeled Mpc3 was imported into mitochondria isolated from the indicated strains as described in Fig. 3e+f, import reactions were analyzed by BN-PAGE and Western blotting, and immunodecorated for the TOM complex (α-Tom40) or stained with Coomassie to control for equal loading. Representative import experiments are shown.