Supplementary Figure Legends

Supplementary Figure 1. Su9-DHFR import into wild type and *tim10*^{K68E} mitochondria.

^{35}S-Su9-DHFR was imported into non heat-shocked (A) and heat-shocked (B) mitochondria isolated from wild-type and *tim10*^{K68E} yeast strains in the presence and absence of a membrane potential ($\Delta\psi$). Where indicated, samples were treated with trypsin (50 μg/ml). All samples were analyzed using SDS-PAGE and phosphorimaging (p = precursor; m = mature).

Supplementary Figure 2. Analysis of Tim9 and Tim10 truncation mutants.

(A) Proteins from whole cell extracts of haploid yeast strain YPH499, *Tim10*^{WT} and *tim10*$^{\Delta N12}$, were separated on SDS-PAGE and subjected to western blotting. Tim10 levels in each strain were quantified relative to Mia40 (error bars represent S.D., n=3).

(B) Growth of *tim9Δ* yeast strains expressing wild-type Tim9, Tim9$^{\Delta N10}$, Tim9$^{\Delta N15}$ or lacking Tim9 plated on minimal glucose media supplemented with 5-FOA at 24°C.

(C) Growth of yeast strains expressing wild-type Tim9, Tim9$^{\Delta C10}$, Tim9$^{\Delta C13}$, Tim9$^{\Delta C14}$ or lacking Tim9 plated on minimal glucose media supplemented with 5-FOA at 24°C.

(D) ^{35}S-Tim9$^{\Delta N15}$, ^{35}S-Tim9$^{\Delta C14}$, ^{35}S-Tim10$^{\Delta N21}$ and ^{35}S-Tim10$^{\Delta C10}$ were imported into wild type mitochondria. Following import, all samples were treated with trypsin (50 μg/ml) before analysis by SDS-PAGE and phosphorimaging.

Supplementary Figure 3. Analysis of steady state protein levels in mitochondria isolated from yeast strains.

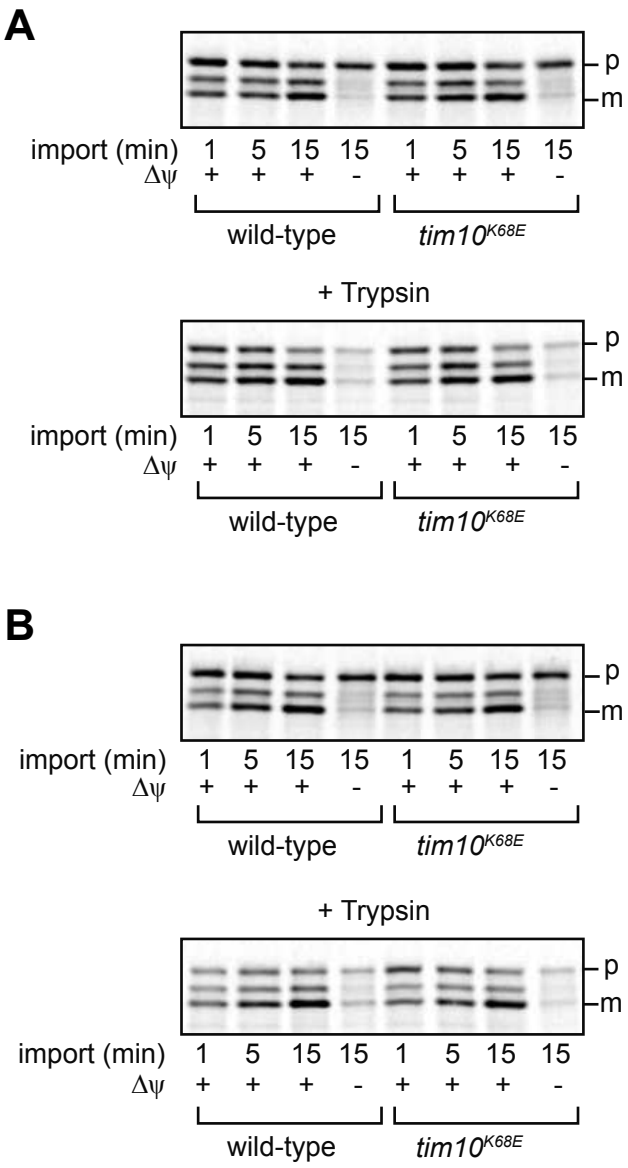
(A - C) Equal amounts of mitochondria isolated from yeast strains as labeled were analyzed using SDS-PAGE and immunodecoration with antibodies against mitochondrial marker proteins.

Supplementary Figure 4. Mitochondrial protein import analysis.

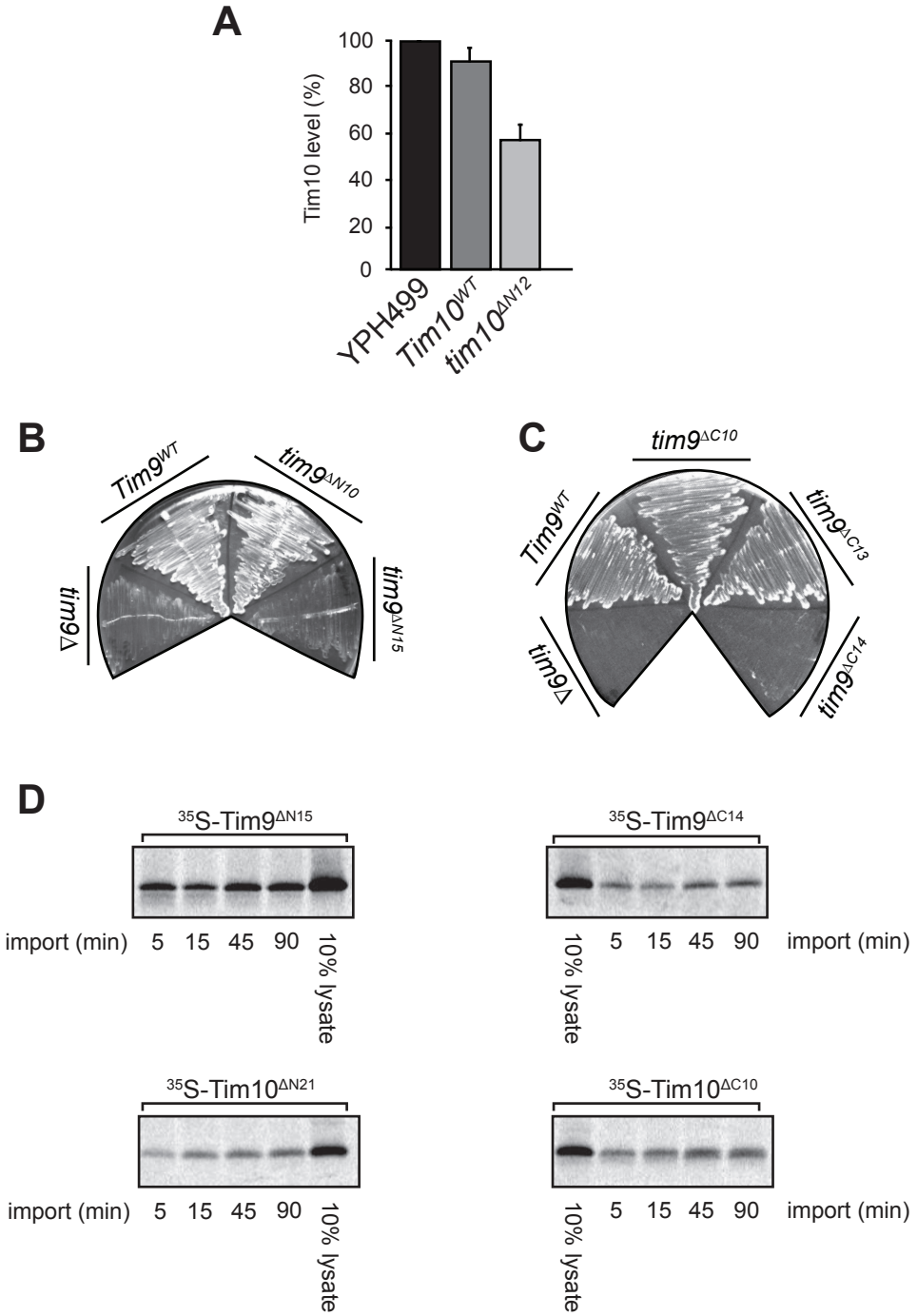
(A) ^{35}S-AAC was imported at 24°C into mitochondria isolated from wild-type, *tim10*$^{\Delta C10}$ and *tim10*$^{\Delta N12}$ yeast strains for the time points indicated in the presence and absence of a membrane potential ($\Delta\psi$). Mitochondria were solubilized and analyzed via BN-PAGE and phosphorimaging (AAC_{II} - AAC dimer).

(B) ^{35}S-Tom40 was imported at 24°C into mitochondria isolated from wild-type, *tim10*$^{\Delta C10}$ and *tim10*$^{\Delta N12}$ yeast strains for the time points indicated. Mitochondria were solubilized and analyzed via BN-PAGE and phosphorimaging (SAM intermediate – Tom40 intermediate associated with the SAM complex; Intermediate II – Tom40 integrated in the mitochondrial outer membrane).

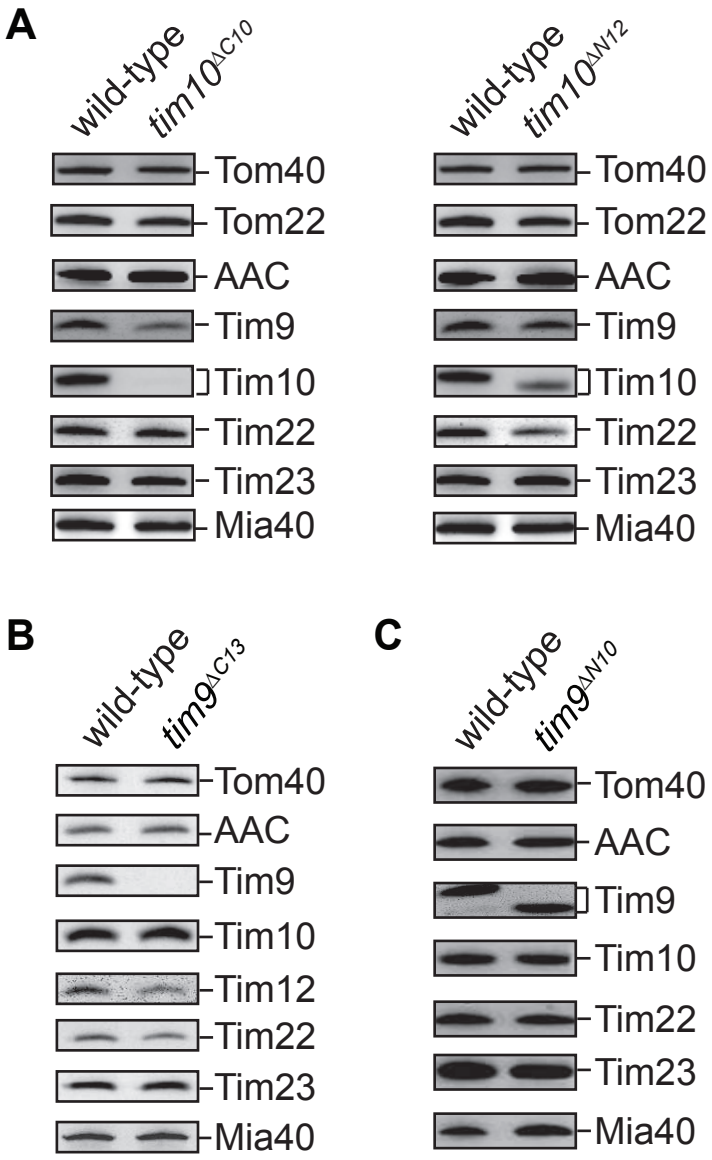
(C and D) ^{35}S-Su9-DHFR was imported into mitochondria isolated from wild-type, *Tim9*$^{\Delta C13}$ and *Tim9*$^{\Delta N10}$ yeast strains in the presence and absence of a membrane potential ($\Delta\psi$), and subjected to SDS-PAGE and autoradiography (p - precursor; m - mature).



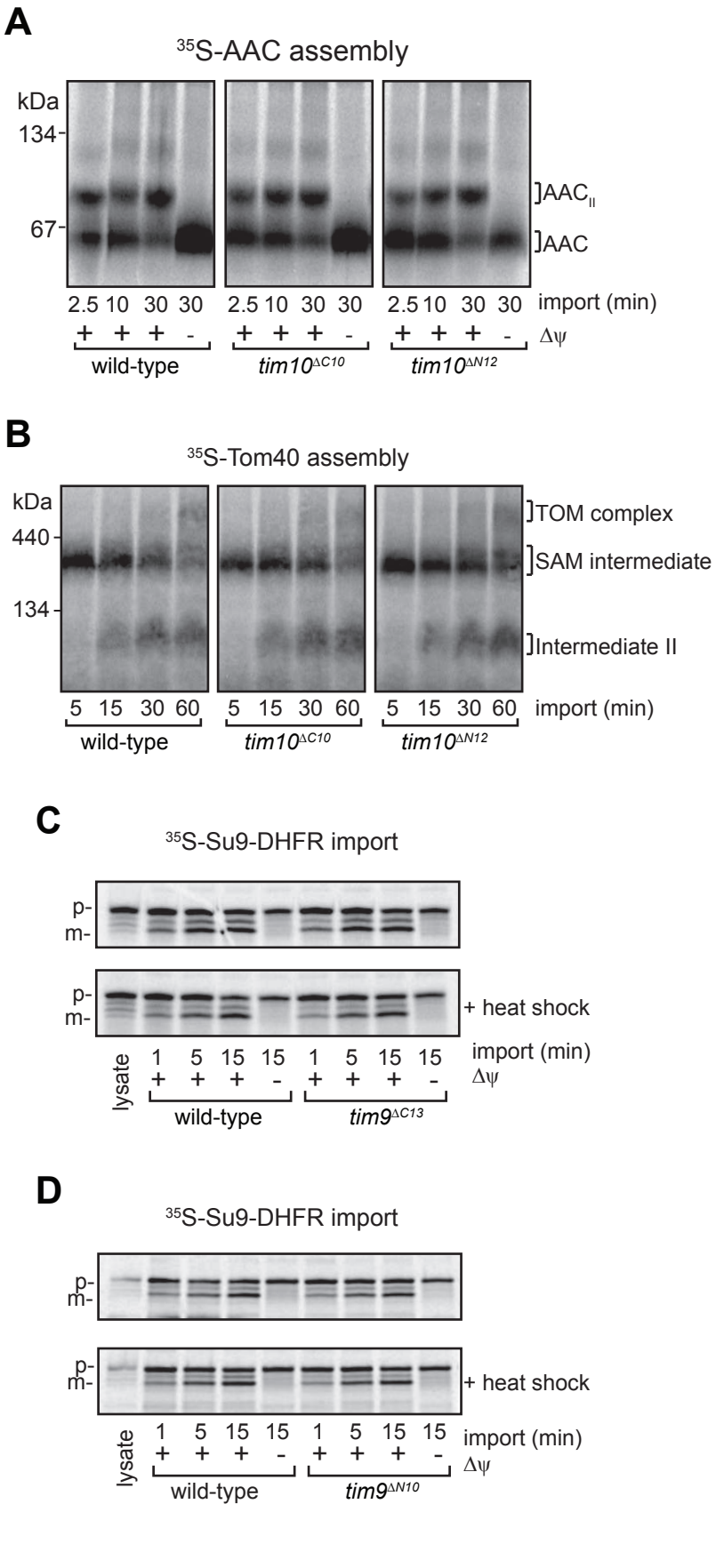
Baker et al. Fig. S1



Baker et al. Fig. S2



Baker et al. Fig. S3



Baker et al. Fig. S4