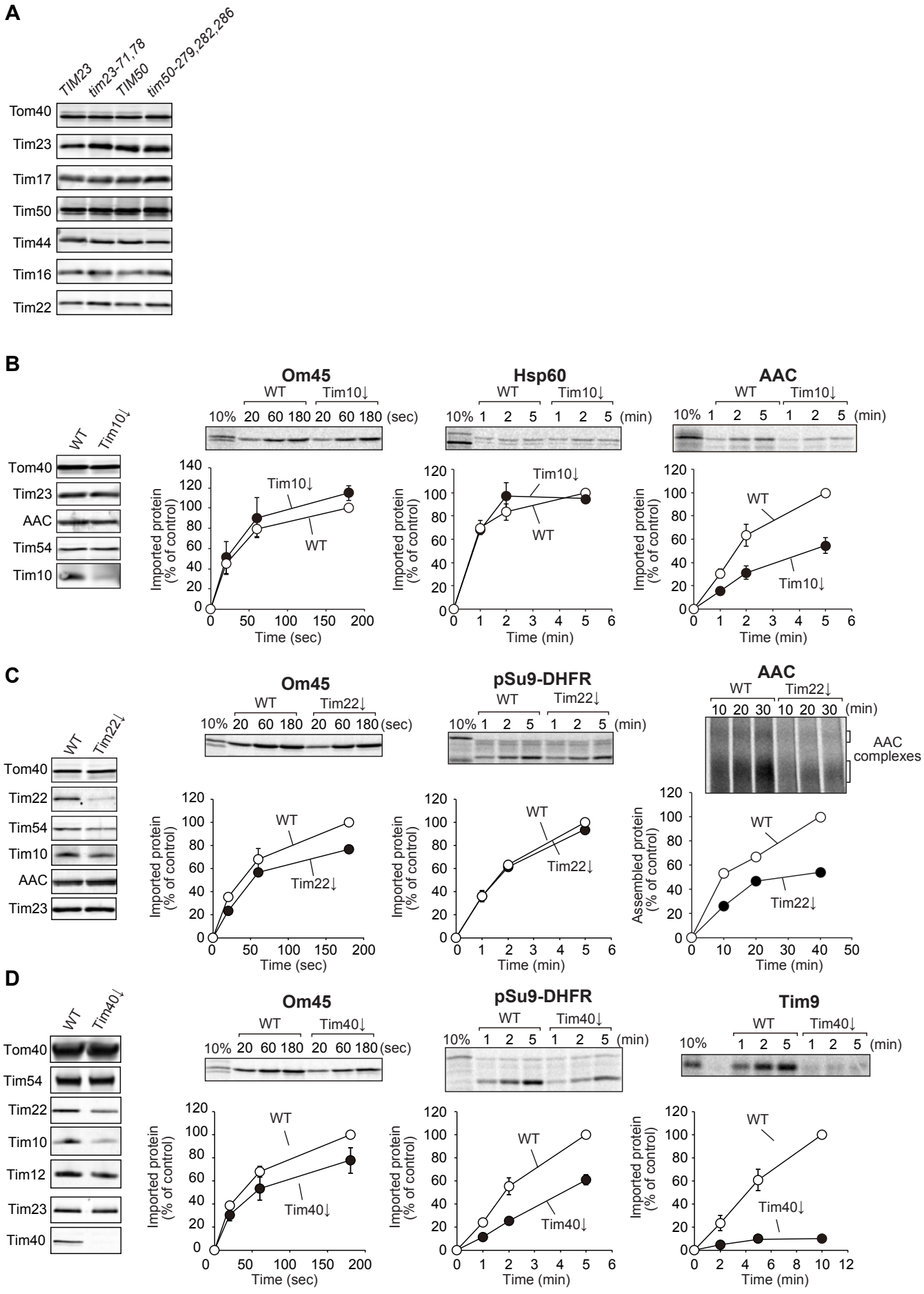

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Fig S4 | Tim23 and Tim50, but not Tim10, Tim22, or Tim40, are involved in Om45 import. (**A**) Immunoblotting of mitochondria isolated from *tim23-71,78*, *tim50-279,282,286*, and their corresponding wild-type cells (*TIM23* and *TIM50*) with the indicated antibodies against subunits of the translocator complexes (Tom40 for the TOM40 complex, Tim23, Tim17, Tim50, Tim44, and Tim16 for the TIM23 complex, and Tim22 for the TIM22 complex). (**B-D**) Dependence of Om45 import on Tim10 (TIM22 pathway), Tim22 (TIM22 pathway) and Tim40 (Tim40/MIA pathway) was tested. Mitochondria were isolated from (**B**) GAL-Tim10 (Tim10↓), (**C**) GAL-Tim22 (Tim22↓), (**D**) GAL-Tim40 (Tim40↓) and W303-1A (WT) cells that were cultivated in lactate medium containing 0.2% glucose at 23°C for 11 h, 6.5 h and 11.5 h, respectively, after shift from YPGal. Mitochondria were analyzed by SDS-PAGE and immunoblotting with the indicated antibodies (**B-D**, left panels). ^{35}S-labeled Om45, AAC, the Hsp60 precursor, Tim9, and pSu9-DHFR were imported into (**B**) Tim10↓ (**C**) Tim22↓ and (**D**) Tim40↓ mitochondria for the indicated time. The Hsp60 precursor and pSu9-DHFR are the substrates for the TIM23 pathway and for negative controls. Tim40 and Tim10 drive translocation of substrate proteins across the OM and Tim22 facilitates assembly of carrier proteins like AAC (ADP-ATP carrier) in the IM. After digestion of unimported proteins with PK, imported proteins were analyzed by SDS-PAGE followed by radioimaging. Amounts of the imported proteins at the longest incubation in wild-type mitochondria were set to 100%. Values are mean ± SEM (n=3). To analyze assembly of AAC via the TIM22 complex in Tim22↓ mitochondria, the mitochondria after import were solubilized with 1.0% digitonin and subjected to BN-PAGE followed by radioimaging. Sum of the amounts for the two bands representing the AAC complexes was plotted against time, and the amount at the longest incubation in wild-type mitochondria was set to 100%.