

Expanded View Figures

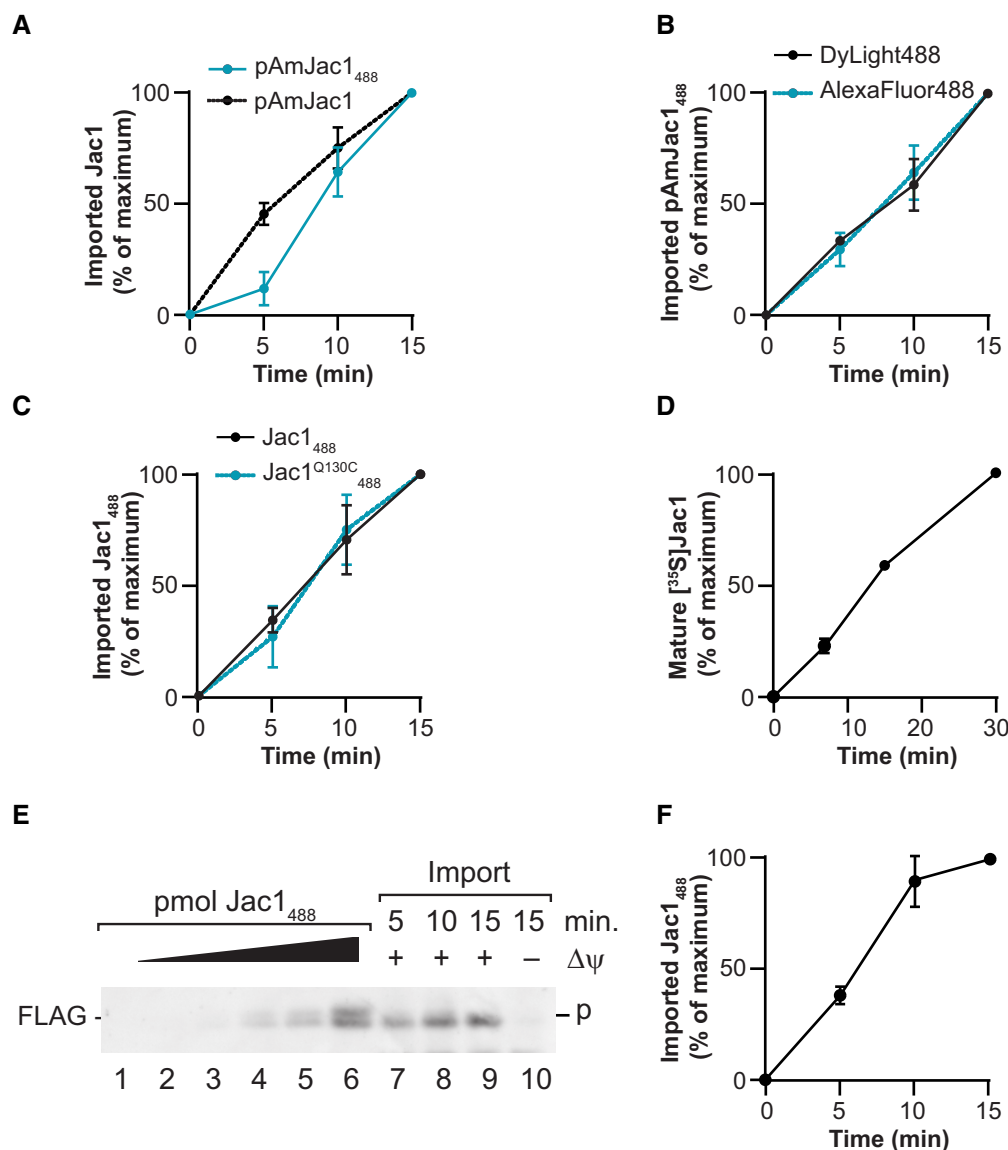
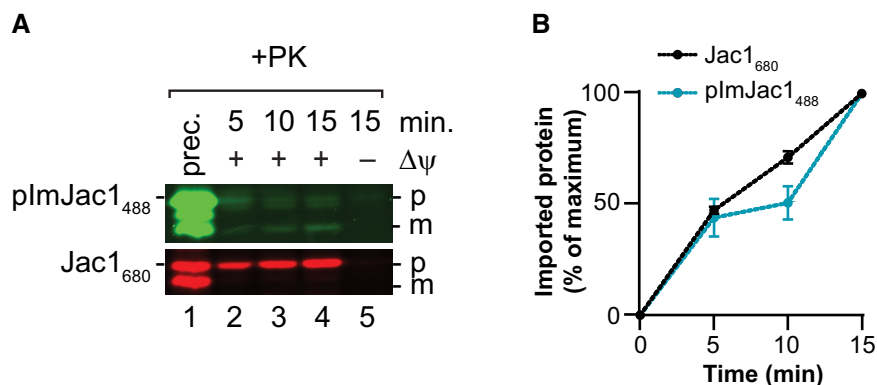


Figure EV1. Effect of precursor modification with a fluorophore on protein import.

- A Quantification of import of pAmJac1 modified (pAmJac1₄₈₈) and non-modified (pAmJac1). The amount of imported protease-protected samples at 15 min was set to 100% in each case; error bars indicate SEM (*n* = 4).
- B Quantification of import of pAmJac1 conjugated to DyLight488 or Alexa Fluor 488. The amount of imported protease-protected samples at 15 min was set to 100% in each case; error bars indicate SEM (*n* = 3).
- C Quantification of import of Jac1 modified with DyLight488 at the C-terminus (Jac1₄₈₈) and internally (Jac1^{Q130C}₄₈₈). The amount of imported protease-protected samples at 15 min was set to 100% in each case; error bars indicate SEM (*n* = 3).
- D Quantification of import of radioactive [³⁵S]Jac1. The amount of imported protease-protected samples at 30 min was set to 100%; error bars indicate SEM (*n* = 3).
- E Immunoblot-based analysis of Jac1₄₈₈ import into mitochondria from Fig 1C. The PDVF membranes were incubated with anti-FLAG antibody for Jac1 detection.
- F Quantification by immunoblot-based detection of Jac1₄₈₈ import shown in (E). The amount of imported protease-protected samples at 15 min was set to 100%; error bars indicate SEM (*n* = 3).

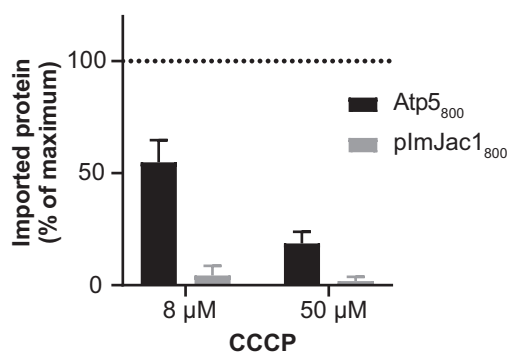
Data information: The values represented in the graphs correspond to the arithmetic mean $\pm$ standard error of the mean (SEM). The number of biological independent replicates (*n*) for each experiment is indicated for each assay.

**Figure EV2. Parallelization of import.**

A Co-import of Jac1₆₈₀ and plmJac1₄₈₈ into wild-type mitochondria for indicated times. Samples were treated with Proteinase K. Prec., purified precursor protein; p, precursor; m, mature.

B Quantification of co-import shown in (A). The amount of imported protease-protected samples at 15 min was set to 100% in each case; error bars indicate SEM ($n = 3$).

Data information: The values represented in the graphs correspond to the arithmetic mean $\pm$ standard error of the mean (SEM). The number of biological independent replicates (n) for each experiment is indicated for each assay.

**Figure EV3. Membrane potential influence over the import of different precursors.**

Quantification of import of Atp5₈₀₀ and plmJac1₈₀₀ over 15 min into mitochondria treated with 8 and 50 μ M CCCP. The amount of imported protease-protected protein in the absence of CCCP was set to 100%; error bars indicate SEM ($n = 3$).

Data information: The values represented in the graphs correspond to the arithmetic mean $\pm$ standard error of the mean (SEM). The number of biological independent replicates (n) for each experiment is indicated for each assay.