

Expanded View Figures

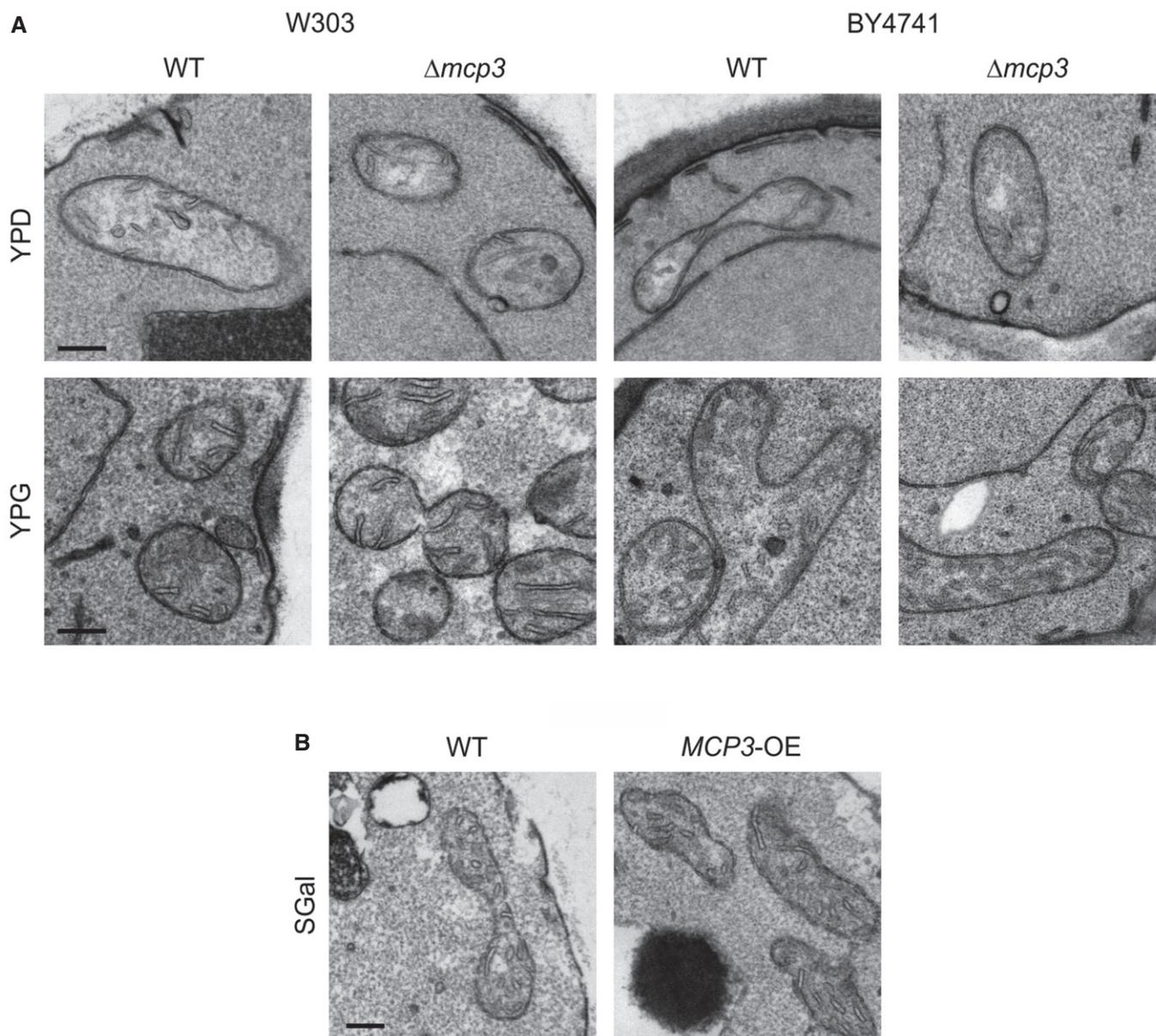


Figure EV1. Altered levels of Mcp3 have no effect on mitochondrial ultrastructure.

A WT and *mcp3* Δ cells in two different genetic backgrounds (W303 and BY4741) were grown in glucose (YPD) or glycerol (YPG) containing medium and analysed by thin-section transmission electron microscopy. Representative images of mitochondria are shown. Scale bars, 200 nm.

B WT cells carrying an empty plasmid (WT) and cells overexpressing *MCP3* from a plasmid under control of the *TPI* promoter (*MCP3*-OE) were grown in galactose-containing minimal medium (SGal) and analysed by thin-section transmission electron microscopy. Representative images of mitochondria are shown. Scale bar, 200 nm.

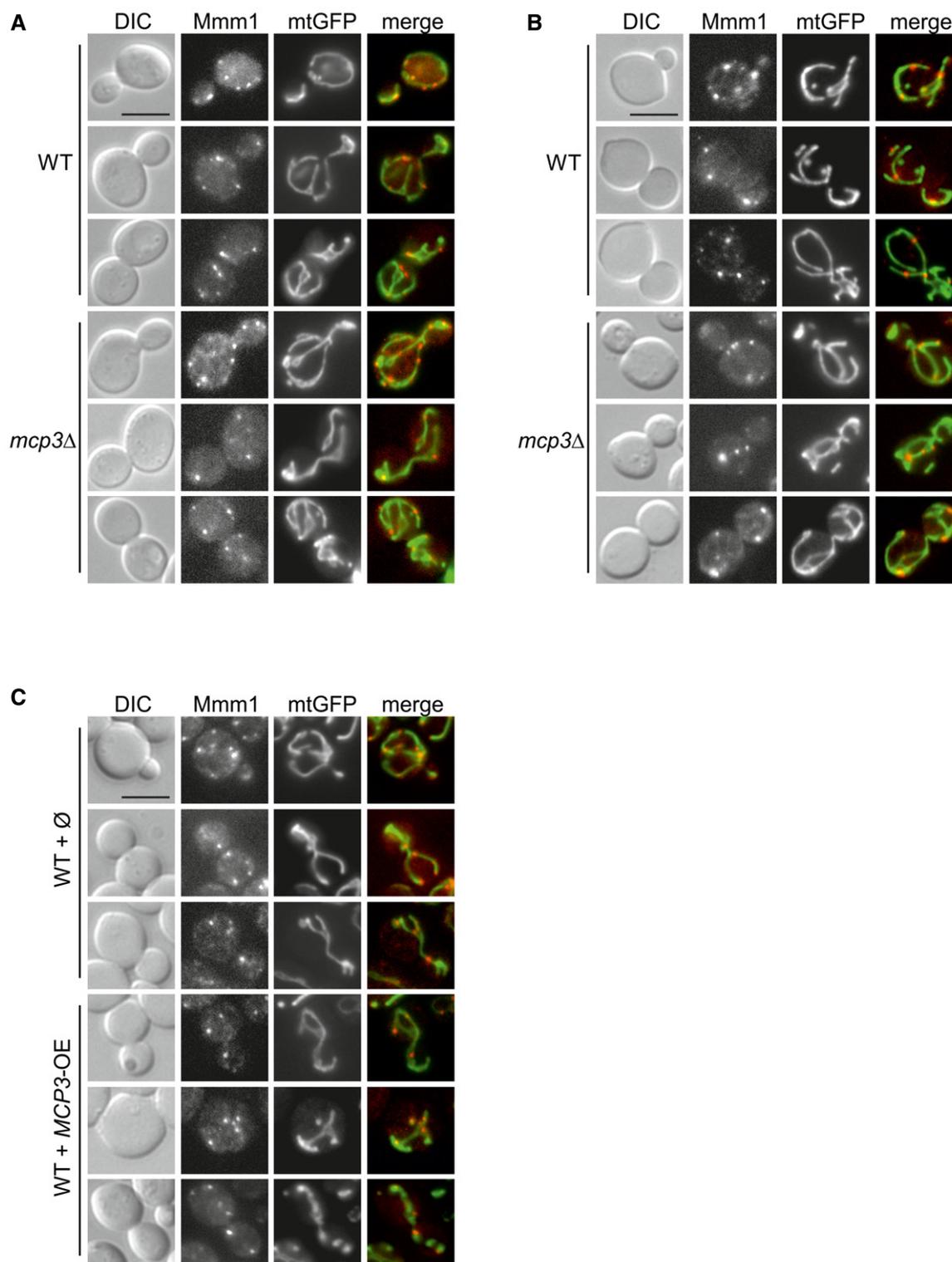


Figure EV2. Altered levels of Mcp3 have no effect on ERME5 foci formation and colocalization with mitochondria.

A, B WT and *mcp3*Δ cells (from BY4741 (A) or W303 (B) background) expressing mitochondrially targeted GFP (mtGFP) and RFP-tagged Mmm1 were grown to mid-logarithmic phase and then analysed by fluorescence microscopy. Representative images are shown. Scale Bars, 5 μm.

C WT cells harbouring an empty plasmid or a plasmid overexpressing Mcp3 (BY4741 background) were transformed with plasmids encoding mitochondrially targeted GFP (mtGFP) and RFP-tagged Mmm1 and were analysed as in (A). Scale Bar, 5 μm.

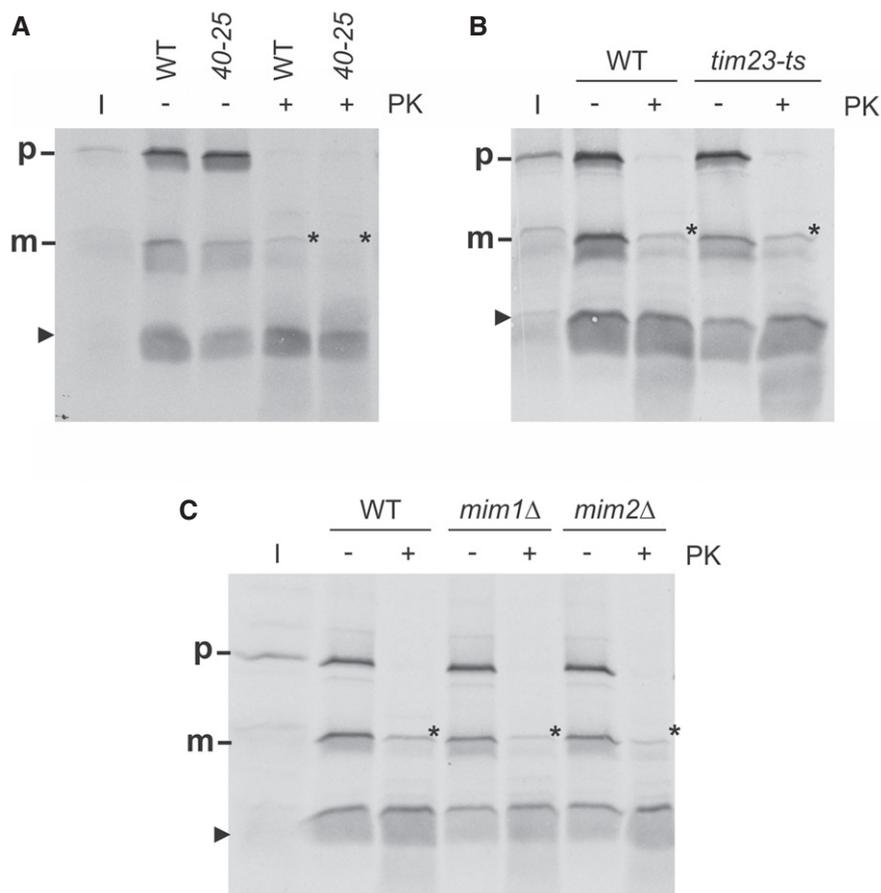


Figure EV3. Mcp3 precursor is degraded by PK *in vitro* if it does not reach the inter membrane space.

A Mitochondria from wild-type and *tom40-25* cells were isolated and incubated with radiolabelled Mcp3 for 15 min. After import, mitochondria were reisolated, left untreated or incubated with proteinase K (PK) and analysed by SDS-PAGE and autoradiography.

B Mitochondria from wild-type and *tim23ts* cells were isolated and incubated with radiolabelled Mcp3 for 15 min. Analysis was performed as in (A).

C Mitochondria from wild-type and *mim1Δ* or *mim2Δ* cells were isolated and incubated with radiolabelled Mcp3 for 15 min. Analysis was performed as in (A).

Data information: The mature (m) and precursor (p) form of Mcp3 are indicated. The arrowhead marks an additional band of the size of the cleaved N-terminus of Mcp3. The asterisk depicts a fragment of the size of mature Mcp3 that is PK protected.

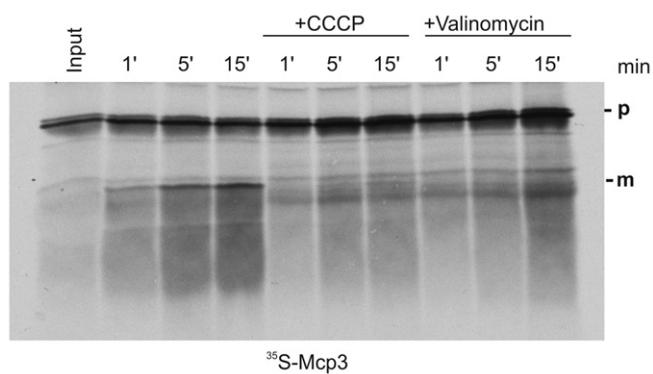


Figure EV4. Import of Mcp3 is dependent on the mitochondrial membrane potential.

Mitochondria isolated from wild-type yeast cells were incubated with radiolabelled precursor protein Mcp3 for the indicated time periods (1, 5 or 15 min) in the absence or presence of CCCP or valinomycin. After import, mitochondria were reisolated and analysed by SDS-PAGE and autoradiography. Input, 20% of radiolabeled precursor applied in the reaction.