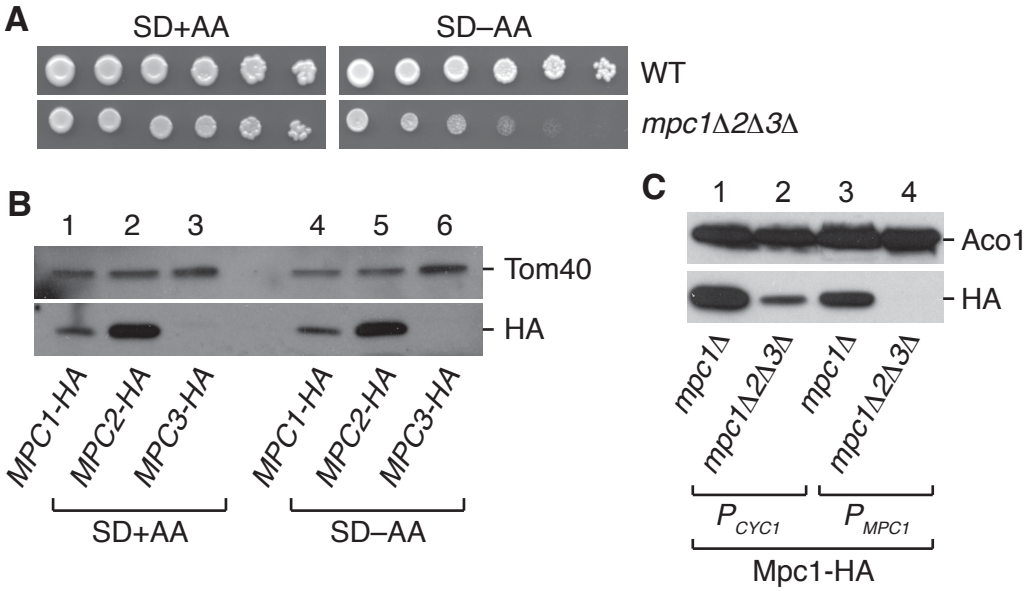
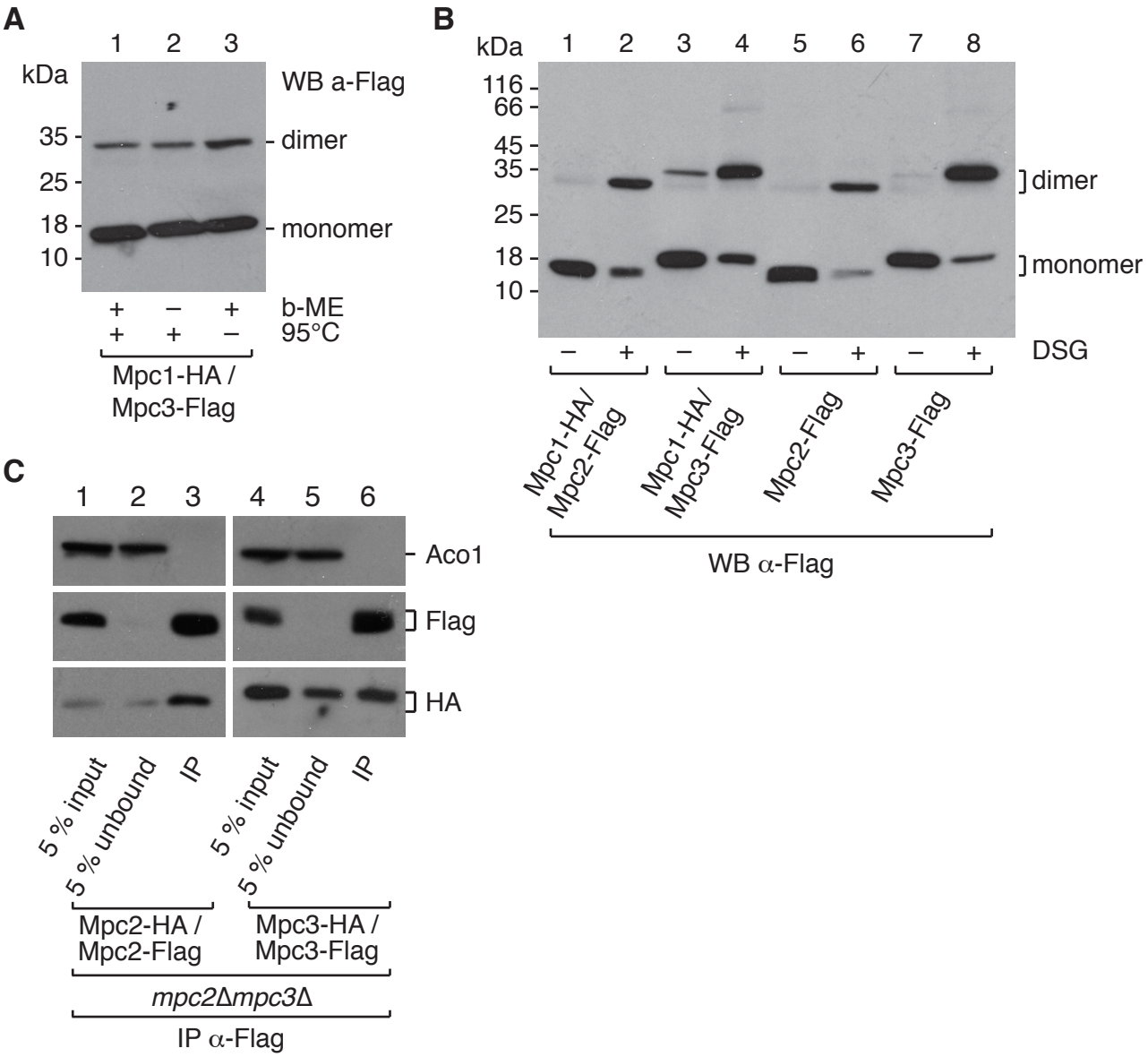
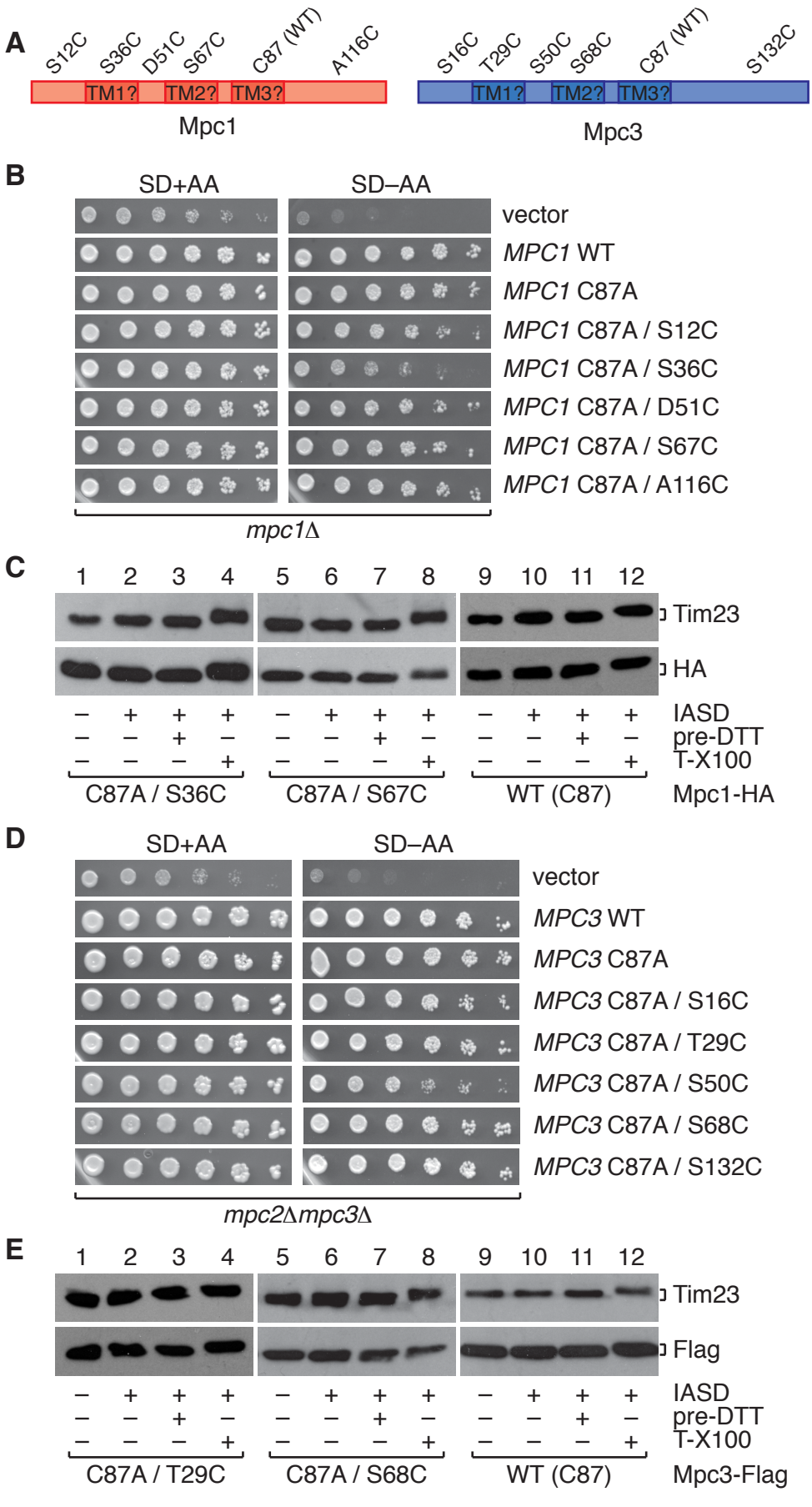


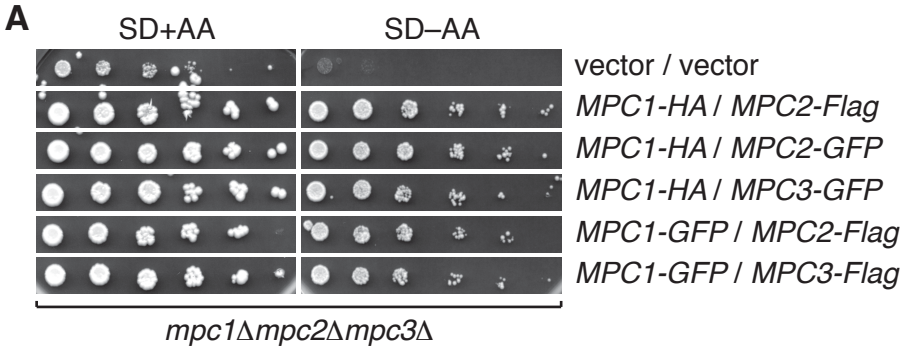
(Bender et al., Suppl. Figure S1)

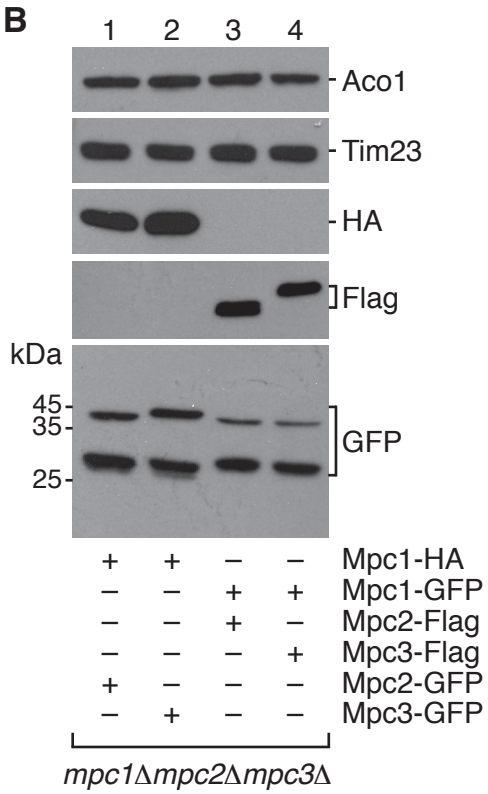


(Bender et al., Suppl. Figure S2)

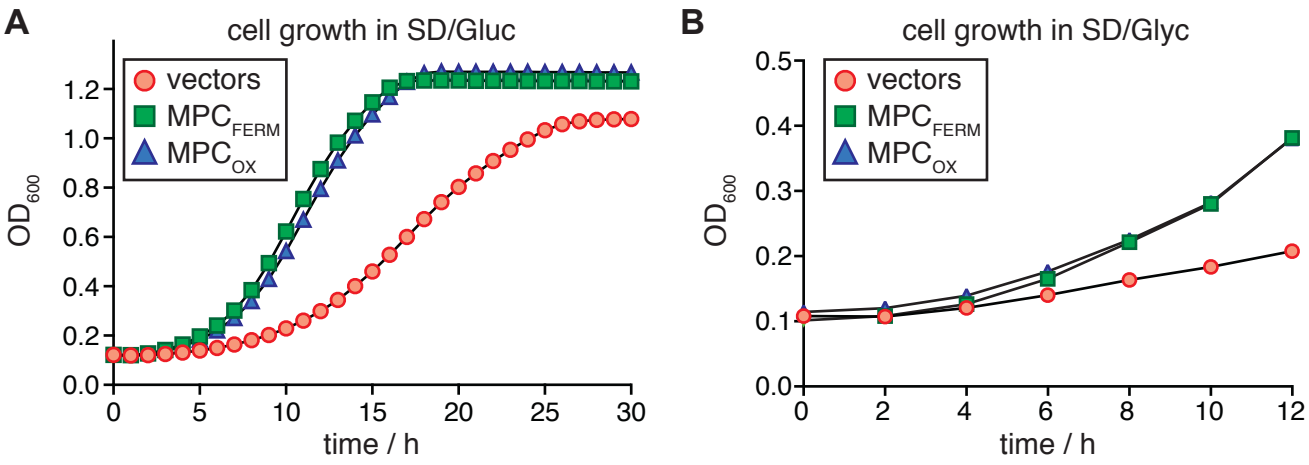


(Bender et al., Suppl. Figure S3)

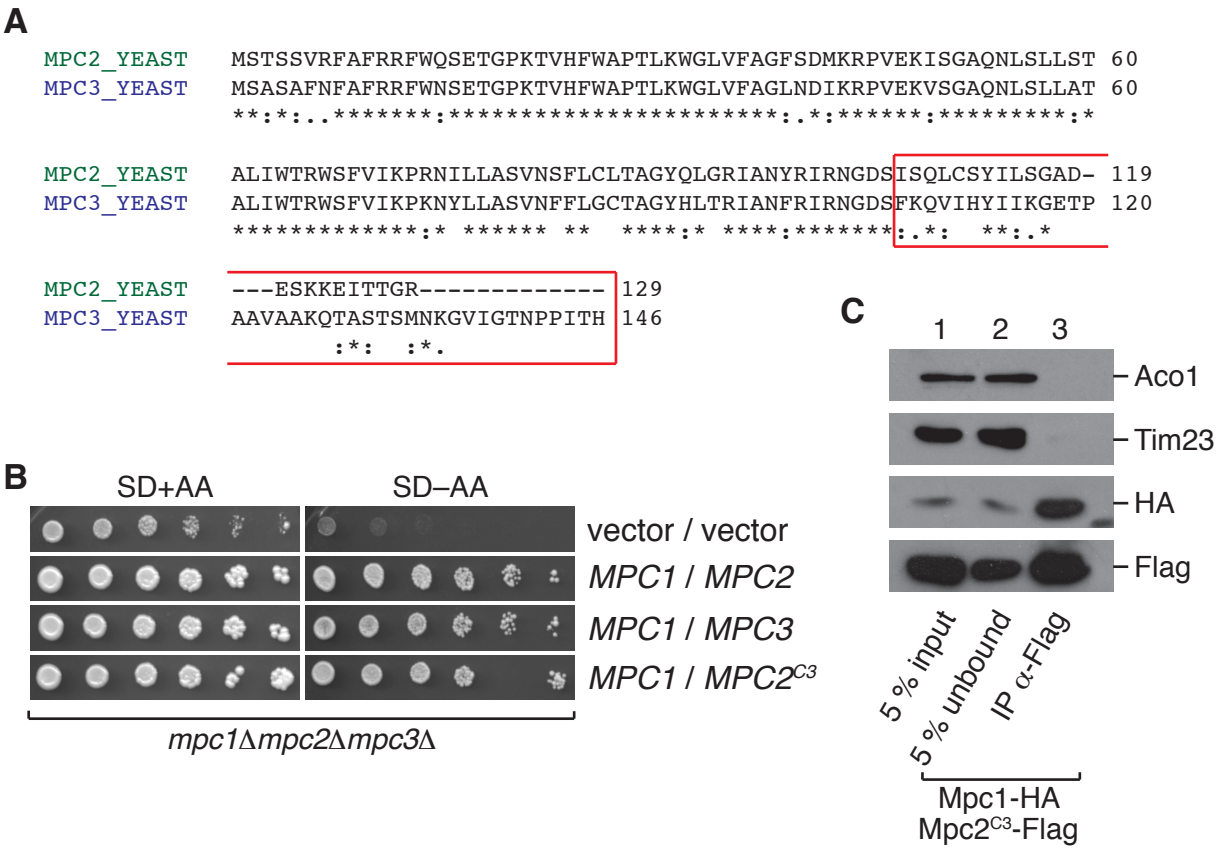




(Bender et al., Suppl. Figure S4)



(Bender et al., Suppl. Figure S5)



(Bender et al., Suppl. Figure S6)

Supplementary figure legends

Figure S1. Expression of MPC proteins. (A) The mpc1Δmpc2Δmpc3Δ strain shows a slow growth phenotype on glucose-containing minimal medium with (SD+AA) or without (SD–AA) amino acids. (B) No difference in expression levels of Mpc1, Mpc2 and Mpc3 between minimal medium with (SD+AA) and without (SD–AA) amino acids. (C) Mpc1 is only moderately overexpressed when the *CYC1* promoter is used, as compared to the endogenous *MPC1* promoter.

Figure S2. Crosslink of MPC components. (A) A fraction of Mpc3 forms an SDS-resistant dimer. While the amount of dimer does not increase in non-reducing SDS-PAGE conditions (lane 2), there is more dimer if the sample is not heated prior to electrophoresis (lane 3). (B) Mpc2 and Mpc3 form homodimers in the absence of Mpc1. Chemical crosslinking with DSG in isolated *mpc1*Δ*mpc2*Δ*mpc3*Δ mitochondria expressing Mpc2 or Mpc3. (C) Homomeric interaction of both Mpc2 and Mpc3 in the presence of Mpc1. Mpc2 and Mpc3 were expressed with two different epitope tags (Flag and HA) in the presence of endogenous Mpc1, the Flag-tagged protein was immunoprecipitated and co-pulldown of the HA-tagged subunit confirmed by western blot.

Figure S3. IASD labeling in single cysteine variants of Mpc1 and Mpc3. (A) Scheme showing the cysteine variants of Mpc1 and Mpc3. TM, putative transmembrane segment according to prediction algorithms (Herzig et al, 2012). (B) Growth test of Mpc1 cysteine variants. (C) IASD labeling of additional Mpc1 variants C87A/S36C, C87A/S67C and WT (C87). (D) Growth test of Mpc3 cysteine variants. (E) IASD labeling of additional Mpc3 variants C87A/T29C, C87A/S68C and WT (C87).

Figure S4. GFP fusions of MPC proteins. (A) Growth test of Mpc1-GFP, Mpc2-GFP, Mpc3-GFP. (B) Expression of GFP fusion proteins in isolated mitochondria.

Figure S5. Growth curves after MPC_{FERM} and MPC_{OX} expression. While the strain carrying empty vectors shows moderately delayed growth, no difference can be observed between MPC_{FERM}- and MPC_{OX}-expressing strains. Growth in glucose-containing medium (A) and glycerol-containing medium (B) was measured as increase in OD_{600}. Growth curves in (A) were obtained in 96-well format using a plate

reader, while curves in (B) were determined manually from shaking cultures to ensure availability of oxygen.

Figure S6. C-terminal exchange chimera Mpc2^{C3} and Mpc3^{C2} (A) Alignment of yeast Mpc2 and Mpc3. Identical amino acids are indicated by asterisks (*). The red box shows the divergent C-terminal region. (B) Growth test of yeast cells expressing Mpc1 and the chimeric protein Mpc2^{C3}. (C) Co-Immunoprecipitation of Mpc1-HA with Mpc2^{C3}-Flag.

Name	Genotype	Source
BY4741	*MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	EUROSCARF
SHY3	*MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; MPC1-3HA::HIS3MX6*	Herzig et al, 2012
SHY4	*MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; MPC2-3HA::HIS3MX6*	Herzig et al, 2012
SHY5	*MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; MPC3-3HA::HIS3MX6*	Herzig et al, 2012
RL285-16C	*MATa; his3∆1; ura3∆0*	Herzig et al, 2012
SHY9	*MATa; his3Δ1; ura3Δ0; mpc1∆::KanMX*	Herzig et al, 2012
SHY14	*MATa; his3Δ1; ura3Δ0; mpc2∆::NatMX; mpc3∆::HphMX*	Herzig et al, 2012
SHY15	*MATa; his3Δ1; ura3Δ0; mpc1∆::KanMX; mpc2∆::NatMX; mpc3∆::HphMX*	Herzig et al, 2012

Table S1. Yeast strains. Strains used in this study and their genotypes are listed.