



Supplemental figure legends

Supplemental Figure 1. Mic27 is required to stabilize the MICOS complex but not for formation of a high molecular weight complex containing Mic10. A, 2D BN-PAGE.

Isolated mitochondria of a wildtype and a $\Delta mic27$ yeast strain were solubilized with digitonin (ratio: digitonin to protein 2g/g) and separated by BN-PAGE (1st dimension) and excised gel strips were used for Tris-tricine SDS-PAGE (2nd dimension), and western blot analysis was performed. Monomers (M), dimers (D), and oligomers of the F₁F₀-ATP synthase (O) are indicated. **B**, Mic27 and other subunits were tested for their expression in MICOS deletion mutant strains and in $\Delta su e$ cells by western blot analysis. Indicated mitochondrial marker proteins were analyzed, Mgm1, Sam50/Tob55, F1 β and Su e, and subunits of the MICOS-complex, Mic60, Mic10, Mic26, and Mic27. **C**, Validation of Mic27 overexpression using a $\Delta mic27$ strain transformed with pYX242-Mic27. Mic27 and other subunits were tested for their expression in the indicated strains. A wild type and a $\Delta mic27$ yeast strain harboring the empty vector (pYX242) were used as controls. Indicated mitochondrial marker proteins were analyzed, F1 β and Su e and subunits of the MICOS-complex: Mic60, Mic10, Mic26, and Mic27, using western blot analysis.