Figures and figure supplements

MICOS coordinates with respiratory complexes and lipids to establish mitochondrial inner membrane architecture

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Figure 1. The MICOS complex is required for oxidative phosphorylation and normal mitochondrial ultrastructure and morphology. (A) Serial dilutions of the indicated yeast cells were plated on media containing glucose (left) and the non-fermentable carbon source, glycerol (right). (B) Mitochondrial morphology in the indicated strains was determined by imaging cells expressing the matrix marker mito-dsRed. Z-projections of confocal fluorescence images are shown, except for the right panel of ΔMICOS, which is a single plane. (C) Quantification of mitochondrial morphologies from cells imaged as in (B) were categorized. Approximately 100 cells from three independent experiments were quantified and data are represented as mean ± SEM. (D) Representative electron microscopy images are shown of chemically fixed yeast cells from the indicated strains. (E) Confocal fluorescence microscopy z-projections of cells from the indicated strains expressing mito-DsRed and the functional nucleoid marker Rim1-GFP are shown. The arrow marks Figure 1. continued on next page
aggregation of Rim1-GFP in a ΔMICOS cell. Scale bars: (B) 2 μm; (D) 500 nm; (E) 3 μm. See also Figure 1—figure supplement 1.

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Figure 1—figure supplement 1. Rim1 is a functional marker of nucleoids. (A) Serial dilutions of the wild type yeast and those expressing Rim1-GFP integrated at the trp1 locus plated on media containing glucose (left) and the non-fermentable carbon source, glycerol (right). (B) Z-projections of confocal fluorescence microscopy images of wild type rho+ (top) and rho0 (bottom) cells expressing Rim1-GFP and the mitochondrial matrix marker, mito-DsRed. Scale bar: 3 μm.

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Figure 2. Mic60 self-assembles independently and its inner membrane distribution is regulated by Mic19. (A) Z-projections of representative deconvolved fluorescence microscopy images are shown from wild type yeast cells expressing the indicated integrated GFP-tagged MICOS protein and mito-DsRed. The yellow box indicates the region of the cell shown in the inset below. The inset displays a single plane. (B) Representative images are shown as in (A) for ΔMICOS cells expressing the indicated GFP-tagged MICOS proteins re-integrated at their endogenous loci. The arrows mark sites of Mic60 localization. (C) Densitometric analysis of Representative images similar to those in (A) and (B) for cells expressing the indicated GFP-tagged MICOS proteins and mito-DsRed. Yellow boxes indicate the area analyzed. (D) Tandem affinity purification (TAP) and immunoblotting analysis of whole cell lysate from ΔMICOS cells re-integrating the indicated MICOS proteins. α-FLAG and α-GFP were used as controls. (E) Representative immunoblots from (D) show the detected proteins. (F) Graph showing the percentage of cells with Mic60 in the mitochondrial fraction. (G) Immunoprecipitation with α-FLAG antibody and immunoblotting with α-GFP antibody was carried out for ΔMICOS cells expressing the indicated GFP-tagged MICOS proteins re-integrated at their endogenous loci. The bars show the percentage of cells with Mic60 in the mitochondrial fraction. The data are representative of three independent experiments. Error bars indicate standard deviations.
foci. (C) The distribution of Mic60-EGFP in ΔMICOS cells was determined as in (B) with the indicated untagged MICOS protein re-integrated at their endogenous loci. (D) Representative Western blot analysis with the indicated antibodies are shown of whole cell lysates (left panel) and immunoprecipitation eluates (IPs; right panel) from ΔMICOS cells expressing either Mic60-FLAG or Mic60-EGFP at the MIC60 locus, and where indicated, expressing Mic60-EGFP from the ura3 locus using the MIC60 promoter (pMic60-EGFP). G6PDH antibody was used as a loading control. IPs were performed with the indicated antibodies. The asterisk marks a band consistent with the size of IgG heavy chain. (E) Western blot analysis with the indicated antibodies of total (T), supernatant (S), and insoluble (P) fractions of detergent-solubilized mitochondria isolated from wild type (left) or ΔMICOS (right) cells expressing Mic60-EGFP and centrifuged at 50,000 × g for 1 hr, a condition that pellets particles of 60S and greater. (F) A graph showing the percentage of cells with detectable Mic60 foci from ΔMICOS cells without and with Mic19 expression as shown in (B) and (C). Approximately 75 cells from three independent experiments were quantified and data are represented as mean ± SEM. (G) Table describing the number of total spectra and protein coverage for the indicated proteins (top) from purifications and mass spectrometry analysis using FLAG antibody from ΔMICOS cell lysate expressing the indicated combinations of Mic60 and Mic19 (left) expressed at their endogenous loci. Data shown are the mean of two independent experiments. Scale bars: (A–B) 3 μm; (C) 2 μm. See also Figure 2—figure supplement 1.

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Figure 2—figure supplement 1. Overexpression of Mic60 does not alter its focal localization in ΔMICOS cells. Confocal fluorescence microscopy z-projections of ΔMICOS cells expressing mito-DsRed and Mic60-EGFP expressed at its endogenous locus (left) or Mic60-FLAG from the MIC60 locus and pMic60-EGFP expressed from the ura3 locus (right). Arrows mark sites of focal Mic60. Scale bar: 3 μm.

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Figure 3. Mic10, Mic12, and Mic27 form an independent MICOS subcomplex. (A) Confocal fluorescence microscopy z-projections are shown from Δmic60 cells expressing the indicated GFP-tagged MICOS proteins expressed at their endogenous loci and the mitochondrial matrix marker mito-DsRed. The arrows shown mark the focal localization of Mic27 and Mic12. (B) Localization of Mic27-mCherry compared to the matrix marker mito-GFP was determined in the indicated MICOS deletion cells by confocal fluorescence microscopy as in (A). The arrows mark examples of Mic27 foci. (C) Images are shown as in (A) for ΔMICOS cells expressing Mic27-EGFP and the indicated untagged MICOS proteins expressed at their endogenous loci. The arrows mark Mic27 foci. (D) A graph displaying quantification of the percent of cells as in (C) with the indicated number of Mic27-EGFP foci per cell. Approximately 75 cells from three independent experiments were counted and data shown are represented as the mean ± SEM. (E) Images are shown as in (B) for the indicated yeast cells. (F) Quantification as in (D) for the cells shown as in (E). (G) A graph depicting the number of total spectral counts identified by mass spectrometry of the indicated MICOS proteins from lysates of Δmic60 cells relative to wild type cells from purifications of Mic27-EGFP with GFP antibody. Data shown are the mean and range of two independent experiments. Scale bars: (A, B) 2 μm; (C, D) 3 μm. See also Figure 3—source data 1.

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Figure 4. Mic27 assemblies mark cristae junctions. (A) Z-projections of confocal fluorescence microscopy images of (left) ΔMICOS cells expressing Mic60-EGFP at the MIC60 locus and mito-DsRed or (right) Δmic60 cells expressing Mic27-mCherry at its endogenous locus and mito-GFP. Cells are rho+ or rho0 where indicated. (B) Quantification of Figure 4. continued on next page.
Figure 4. Continued

Cells from (A) is shown indicating the number of cells with detectable Mic60 or Mic27 foci, as indicated. Approximately 75 cells from three independent experiments were counted and the data shown are represented as the mean ± SEM. Data from rho- cells for Mic60 and Mic27 are redisplayed from Figures 2F, 3F, respectively. (C) A graph depicting the number of total spectral counts identified by mass spectrometry of the indicated MICOS proteins from lysates of Δmic60 rho+ and rho0 cells relative to wild type cells from purifications of Mic27-EGFP with GFP antibody. Data shown are the mean and range of two independent experiments and data from rho+ cells are redisplayed from Figure 3G. (D) Single plane confocal fluorescence microscopy images in 0.4 μm steps through a Δmic60 cell expressing Mic27-EGFP and the Complex III marker Qcr2-mCherry at their endogenous loci and the mitochondrial matrix marker mito-TagBFP. Arrows indicate Mic27 foci identified and analyzed in (E). (E) Top row: individual channels and a merged image of a maximum z-projection image of the cell shown in (D). Bottom row: from left to right, a tracing of mitochondria, Qcr2 signal above the threshold detected by the ‘Moments’ algorithm of ImageJ, Mic27 foci identified in the z-projection, and a merged image indicating regions of the mitochondrial perimeter positive for Qcr2 signal and their position relative to Mic27 foci. (F) Quantification of the total percentage of mitochondrial perimeter considered positive for Qcr2 signal and the number of Mic27 foci localized to mitochondrial subregions positive for Qcr2 from cells in (E) and Figure 4—figure supplement 4. Scale bars: (A) 3 μm, (D–E) 1 μm. See also Figure 4—figure supplements 1–5 and Figure 4—source data 1.

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Figure 4—figure supplement 1. Steady-state Mic27 expression levels are maintained in Δmic60 rho0 cells compared to rho+ cells. Western blot analysis of whole cell lysates prepared from the indicated strains expressing Mic27-mCherry from its endogenous locus and detected with antibody against mCherry. G6PDH was used as a loading control.

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Figure 4—figure supplement 2. Individual respiratory complexes are not essential for Mic27 foci formation. (A) Z-projections of confocal fluorescence microscopy images of the indicated yeast cells expressing mito-GFP and Mic27-mCherry expressed from its endogenous locus. (B) Quantification of the percent of cells with detectable Mic27 foci in the indicated cells as in (A). Data shown are represented as the mean ± SEM from three independent experiments with approximately 75 cells counted per experiment. Data from Δmic60 cells are redisplayed from Figure 3F. Scale bars: 3 μm.
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Figure 4—figure supplement 3. Qcr2 and Atp2 are functional markers of Complex III and V, respectively, and localize to sub-mitochondrial regions in Δmic60 cells. (A) Serial dilutions of the yeast cells expressing the indicated markers expressed from their endogenous loci plated on media containing dextrose or ethanol/glycerol.
glucose (left) and the non-fermentable carbon source, glycerol (right). (B) Confocal fluorescence microscopy z-projections of wild type yeast cells expressing mito-GFP as well as Qcr2-mCherry (left) or Atp2-mCherry (right) expressed at their endogenous loci, respectively. (C) As in (B) for Δmic60 cells. (D–E) Left: individual channels and a merged image of Δmic60 cells expressing mito-TagBFP as well as both Qcr2-mCherry and Atp2-EGFP expressed at their endogenous loci. Right: single plane images in 0.4 μm steps of the cell shown on the left. Scale bars: (B, C) 3 μm; (D, E) 1 μm. DOI: 10.7554/eLife.07739.013
Δmic60
mito-TagBFP
Mic27-EGFP
Qcr2-mCherry

Figure 4—figure supplement 4. Mic27 assemblies localize adjacent to the cristae marker Qcr2. (A–N) Left: examples of z-projections of confocal fluorescence microscopy images of Δmic60 cells expressing mito-TagBFP as well as Mic27-EGFP and Qcr2-mCherry expressed from their endogenous
Figure 4—figure supplement 4. Continued

loci. Right: tracings of mitochondrial perimeter (blue), Qcr2-positive regions of the perimeter (red), and Mic27 foci (green) identified from image on left (as in Figure 4E). (O) Quantification of the mitochondrial perimeter, the Qcr2-positive perimeter, the number of Mic27 foci identified, and the number of Mic27 foci adjacent to Qcr2 signal from each example shown in (A–N) and Figure 4E. Scale bars: 1 μm.
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Figure 4—figure supplement 5. Mic27 assemblies localize adjacent to the cristae marker Atp2. (A–B) Left: individual channels and merged images of Δmic60 cells expressing mito-TagBFP as well as both Atp2-mCherry and Mic27-EGFP expressed at their endogenous loci. Right: single plane images in 0.4 μm steps of the cell shown on the left. Arrows indicate the position of Mic27 foci. Scale bars: 1 μm.
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Figure 5. Cardiolipin is required for stability of the Mic10/Mic12/Mic27 subcomplex. (A) Images are shown of confocal fluorescence microscopy z-projections of the indicated cells expressing Mic27-EGFP from its endogenous locus and mito-DsRed. (B) Quantification of the percent of cells with detectable Mic27 foci in the indicated cells. Data shown are represented as the mean ± SEM from three independent experiments with approximately 75 cells counted per experiment. (C) A graph depicting the number of total spectral counts identified by mass spectrometry of the indicated MICOS proteins from lysates of Δcrd1 and Δmic60 Δcrd1 cells relative to wild type cells from purifications of Mic27-EGFP with GFP antibody. (D) Serial dilutions of the indicated yeast cells plated on media.
containing glucose (left) and the non-fermentable carbon source, glycerol (right). (E) Quantification of mitochondrial morphologies from the indicated cells expressing mito-DsRed were categorized. Approximately 75–100 cells from three independent experiments were quantified and data are represented as mean ± SEM. Data for wild type and ΔMICOS cells are redisplayed from Figure 1C. Scale bars: 3 μm. See also Figure 5—figure supplement 1 and Figure 5—source data 1.

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Figure 5—figure supplement 1. Interactions between cardiolipin synthesis and MICOS. (A) Western blot analysis of whole cell lysates prepared from the indicated strains expressing Mic27-EGFP from its endogenous locus and detected with antibody against GFP. G6PDH was used as a loading control. (B) Z-projections of confocal fluorescence microscopy images of yeast cells expressing mito-DsRed and Mic60-EGFP expressed from its endogenous locus in the indicated yeast cells. Arrows mark sites of Mic60 foci formation. (C) Serial dilutions of the indicated yeast cells plated on media containing glucose (left) and the non-fermentable carbon source, glycerol (right). Scale bars: 2 μm.

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Figure 6. Complex III and IV selectively contribute to the ΔMICOS phenotypes. (A) A Coomassie-stained Blue Native-PAGE gel of digitonin-solubilized mitochondria isolated from the indicated strains. Labels indicate approximate sizes of the indicated respiratory complexes and supercomplexes. (B–C) In-gel activity assays for samples run as in (A) for Complex IV activity (B) and ATP synthase activity (C). Molecular weight markers are shown.

Figure 6. continued on next page
Figure 6. Continued

on the left. (D) Top: Coomassie-stained Blue Native PAGE (BN-PAGE) gels of DDM-solubilized mitochondria isolated from the indicated strains. Labels indicate approximate sizes of the indicated respiratory complexes. Bottom: BN-PAGE analysis was followed by SDS-PAGE in a second dimension and silver staining analysis. Molecular weights markers are shown on the left. (E) Z-projections of confocal fluorescence microscopy images of the indicated yeast cells expressing mito-DsRed are shown. (F) Representative electron microscopy images are shown of chemically fixed ΔMICOS rho0 cells. (G) A graph displaying categorization of mitochondrial morphologies from the indicated cells imaged as in (E). Approximately 75–100 cells from three independent experiments were quantified and data are represented as mean ± SEM. Data for ΔMICOS cells are redisplayed from Figure 1C. Scale bars: (E) 3 μm; (F) 200 nm.

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Figure 7. Mic19 mediates an interaction between the Mic60 and Mic10/Mic12/ Mic27 subcomplexes. (A) A graph depicting the number of total spectral counts identified by mass spectrometry of the indicated MICOS proteins from lysates of Δmic19 cells relative to wild type cells from purifications of Mic60- or Mic27-EGFP with GFP antibody. Data shown are the mean and range of two independent experiments. (B) Images are shown of confocal fluorescence microscopy z-projections of Δmic19 rho⁺ (left) and rho⁰ (right) cells expressing Mic60-EGFP from the MIC60 locus and mito-DsRed. Figure 7. continued on next page
Figure 7. Continued

Arrows mark focal localization of Mic60. (C) Z-projections are shown of confocal fluorescence microscopy images of Δmic19 cells expressing mito-TagBFP as well as Mic60-EGFP and Mic27-mCherry expressed from their endogenous loci. Arrows indicate sites of Mic60 and Mic27 colocalization. (D) Single plane confocal fluorescence microscopy images in 0.4 μm steps through a Δmic19 cell expressing Mic60-EGFP and the Complex III marker Qcr2-mCherry at their endogenous loci and the mitochondrial matrix marker mito-TagBFP. Arrows indicate the position of Mic60 foci. (E) As in (D) for cells expressing Atp2-mCherry. (F) A schematic model of the organization and roles of MICOS and its constituent subcomplexes. The model also depicts the interrelationship between MICOS, cardiolipin, and respiratory complexes, and the coordination of these factors in generating mitochondrial inner membrane organization and cristae architecture. Scale bars: (B) 3 μm; (C) 2 μm; (D, E) 1 μm. See also Figure 7—figure supplements 1–2 and Figure 7—source data 1.

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Figure 7—figure supplement 1. Cardiolipin synthesis is not required for Mic60 foci formation in Δmic19 cells. Z-projections of confocal fluorescence microscopy images of yeast cells expressing mito-DsRed and Mic60-EGFP expressed from its endogenous locus in the indicated yeast cells. Arrows mark sites of Mic60 foci formation. Scale bars: 2 μm.

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Figure 7—figure supplement 2. Mic60 assemblies localize adjacent to the cristae markers Qcr2 and Atp2. (A–B) Left: individual channels and merged images of Δmic19 cells expressing mito-TagBFP as well as both Qcr2-mCherry and Mic60-EGFP.

Figure 7—figure supplement 2. continued on next page
and Mic60-EGFP expressed at their endogenous loci. Right: single plane images in 0.4 μm steps of the cell shown on the left. Arrows indicate the position of Mic60 foci. (C–D) As in (A–B) for cells expressing Atp2-mCherry. Right panel of (A) and (C) redisplayed from Figure 7D–E. Scale bars: 1 μm.
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