Supporting Information

Assembly of the Complexes of Oxidative Phosphorylation Triggers the Remodeling of Cardiolipin

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MATERIALS AND METHODS

Yeast strains and growth conditions

Saccharomyces cerevisiae strains, including wild-type (BY4741; *MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*), the tafazzin deletion strain (*taz1Δ; MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 taz1Δ::KanMX6*), the CL deacylase deletion strain (*cld1Δ; MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 cld1Δ::KanMX6*), the double deletion strain (*cld1Δtaz1 Δ; MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 cld1Δ::KanMX6 taz1Δ::KanMX6*), and their rho^0 derivatives, were previously described (1). Strains with knockouts of various mitochondrial proteins, including the wild-type parent strain BY4743, were obtained from the Yeast Knockout Collection of Dharmacon (http://dharmacon.gelifesciences.com). Yeast cells were grown at 30°C in YPD medium containing 10 g/L yeast extract, 20 g/L peptone, and 20 g/L dextrose, or in YPGE medium containing 10 g/L yeast extract, 20 g/L peptone, 3% glycerol, and 3% ethanol. For the culture of knockout strains from the Dharmacon Knockout Collection, the medium was supplemented with 0.2 g/L G418. Cells were harvested in mid-logarithmic or in stationary phase as indicated and stored frozen at –80 °C. In order to break the cell wall, the yeast cell pellets were digested in a medium containing 1.2 M glycerol, 100 mM sodium thioglycolate, 50 mM Tris-SO_4 (pH 7.5) and 1 g/L zymolyase for 15 min at room temperature.

Schneider cells and knockdown conditions

Drosophila melanogaster Schneider 2 (S2) cells were cultured at 23°C in Schneider's Drosophila medium (Gibco #21720-024) supplemented with 10% fetal bovine serum (Life Technologies #16000044), 100 IU/mL penicillin, 100 µg/mL streptomycin, and 292 µg/mL L-glutamine. In order to obtain knockdown reagents, double-stranded RNAs (dsRNA) were synthesized from DNA templates using the MEGASCRIPT T7 Transcription Kit from Ambion (AM1334) and purified with QIAGEN RNeasy Mini Kits. The template DNAs were obtained from the Drosophila RNAi Screening Center (DRSC) of Harvard Medical School and PCR amplified with the T7 promoter primer 5'-TAATACGACTCACTATAGGG-3'. For the knockdown experiments, S2 cells were suspended at a concentration of $4x10^6$/mL in serum-free Schneider's Drosophila medium. An aliquot of 0.1 mL ($4x10^5$ cells) was added per well of a 24-well plate and mixed with 9 µg dsRNA. After 2 hours at 23 °C, the baiting period was terminated by addition of 0.6 mL Schneider's Drosophila medium supplemented with 10% fetal bovine serum. The cells were then incubated for four days at 23-25 °C. Cells were harvested and processed for lipid analysis, Western blotting, and analysis of RNAi efficiency by quantitative RT-PCR.

Drosophila strains and genetics

Fly strains with tafazzin deletion (ΔTAZ, on the 2nd chromosome) were created by imprecise excision of P elements (2). The transgenic strain UAS-dPGC-1 (on the 3rd chromosome) was provided by David Walker, University of California at Los Angeles. The transgenic strain UASp-SIRT1 (on the 2nd chromosome) was obtained from the Bloomington Drosophila Stock Center (Stock No. 44216). The ubiquitous GAL4 driver line daughterless (da)-GAL4 (on the 3rd chromosome) was provided by Jessica Treisman, NYU School of Medicine. ΔTAZ and UASp-SIRT1 were recombined into the same 2nd chromosome to generate the ΔTAZ; UASp-SIRT1 strain. The strains ΔTAZ; da-GAL4 and ΔTAZ; UAS-dPGC-1 were generated by routine crosses. To overexpress *Drosophila* PGC-1 or SIRT1 in the ΔTAZ background, the strain ΔTAZ; da-GAL4 was crossed with the strain ΔTAZ; UAS-dPGC-1 or the strain ΔTAZ;

UASp-SIRT1, respectively. For muscle-specific knockdowns, females from *Dmef2*-Gal4 or *Mhc*-Gal4 (PMID 8893022) stocks were genetically crossed to male transgenic UAS-RNAi stocks from the Bloomington Drosophila Stock Center. The following transgenic UAS-RNAi stocks were used:
y[1] v[1]; P{y[+t7.7]=CaryP}attP2 (Bloomington, #36303, control),
y[1] v[1]; P{y[+t7.7]=CaryP}attP40 (Bloomington, #36304, control),
y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMS01590}attP2 (Bloomington, #36701, RNAi to NDUFV1),
y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMC05884}attP40 (Bloomington, #65010, RNAi to NDUFA6)
y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMS01584}attP2 (Bloomington, #36695, RNAi to NDUFA12)
y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HM05255}attP2/TM3, Sb[1] (Bloomington, #30511, RNAi to NDUFB7)
y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMS01560}attP2 (Bloomington, #36672, RNAi to NDUFB11)
y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HM05059}attP2 (Bloomington, #28573, RNAi to NDUFS2)
y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMC02929}attP40 (Bloomington, #44535, RNAi to NDUFS3)
y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMC03861}attP40 (Bloomington, #55180, RNAi to NDUFS5)
y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMC03497}attP40 (Bloomington, #53281, RNAi to SdhC)
y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMC05914}attP40 (Bloomington, #65040, RNAi to SdhD)
y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMS01057}attP2 (Bloomington, #34583, RNAi to Cytc1)
y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMC03242}attP2 (Bloomington, #51357, RNAi to UQCR-Q)
y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMC03394}attP2 (Bloomington, #51822, RNAi to UQCR-C1)
y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMJ22367}attP40 (Bloomington, #58282, RNAi to COX5A)
y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMS00815}attP2 (Bloomington, #33878, RNAi to COX6C)
y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMC05771}attP40 (Bloomington, #64898, RNAi to Cyt c)
y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMJ21900}attP40/CyO (Bloomington, #57853, RNAi to Cyt c)
y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF03150}attP2/TM3, Sb[1] (Bloomington, #28723, RNAi to ATPsynγ),
y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF02896}attP2 (Bloomington, #28059, RNAi to ATPsyn-α)
y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF02792}attP2 (Bloomington, #27712, RNAi to ATPsyn-β)
y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF02899}attP2 (Bloomington, #28062, RNAi to ATPsyn-b)
y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMC03581}attP40 (Bloomington, #53352, RNAi to ATP12)
y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMS01549}attP2 (Bloomington, #36661, RNAi to SesB),
y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMS02631}attP40 (Bloomington, #42938, RNAi to mRpS29),
y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF01564}attP2 (Bloomington, #31099, RNAi to TAZ),
y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF01748}attP2 (Bloomington, #31694, RNAi to TAZ),
y[1] v[1]; P{y[+t7.7] and v[+t1.8]=TRiP.HMC03231}attP40/CyO (Bloomington, #51484, RNAi to TAZ).

Overexpression of ImpL2 was achieved by crossing UAS-ImpL2 (3) to *Dmef2*-Gal4. The flies were allowed to lay eggs for 48 hours, after which they were transferred to fresh vials of food and the larvae were allowed to develop at 25°C until they emerged as adults. Subsequently, flies were harvested within 1-3 days of hatching. All flies were raised on standard fly media. To isolate thoraces, flies were immobilized by carbon dioxide and dissected under the microscope. Dissected thoraces, in which the soft tissue consists almost entirely of indirect flight muscles, were stored at -80^{o}C.

Lipid analysis by mass spectrometry

Yeast spheroblasts, S2 cell pellets, or Drosophila thoraces (from 15 animals) were homogenized in 0.2 mL water and supplemented by a mixture of CL standards (Cardiolipin Mix I from Avanti Polar Lipids) containing 1.115 nmol CL 57:4 and 1.013 nmol CL 61:1. Homogenates were extracted with methanol/chloroform (4). The extracts were dried under nitrogen and re-dissolved in 0.1 mL chloroform/methanol (1:1). To screen mutants and knockdowns, the CL composition was analyzed by MALDI-TOF mass spectrometry (5). Briefly, the lipid extract was diluted 1:11 in 2-propanol/acetonitrile (3:2, v/v) and a 1-µL aliquot of that was mixed 1:1 with matrix solution containing 20 g/L 9-aminoacridine in 2-propanol/acetonitrile (3:2, v/v). One microliter of this mixture was spotted on a target plate. Measurements were performed with a MALDI Micro MX mass spectrometer (Waters) operated in reflectron mode. The pulse voltage was set to 2000 V, the detector voltage was set to 2200 V, and the TLF delay was set to 700 ns. The nitrogen laser (337 nm) was fired at a rate of 5 Hz and 10 laser shots were acquired per sub-spectrum. The instrument was operated in negative ion mode with a flight tube voltage of 12 kV, a reflectron voltage of 5.2 kV, and a negative anode voltage of 3.5 kV. We typically added 100 sub-spectra (representing 1000 laser shots) per sample in a mass range from *m/z*=400 to *m/z*=2000 via automatic acquisition. Spectra were only selected if their base-peak-intensity was within 10–95% of the saturation level. Data were analyzed with the MassLynx 4.1 software. To determine CL turnover or to identify CL molecular species, lipid extracts were analyzed by LC-MS/MS using the LTQ Orbitrap (Thermo Fisher Scientific) coupled to an Ascentis Express C8-reversed phase column (75x2.1 mm; 2.7 µm) from SUPELCO. Samples were injected by a 20-µL loop injector. A gradient was established by two conventional HPLC pumps run at a total flow rate of 0.1 mL/min. The gradient changed the solvent mixture 2-propanol/acetonitrile/water from 22.5/47.5/30 to 85.5/12.5/2 within 35 minutes under the continuous presence of 0.1% formic acid and 10 mM ammonium formate. The total run time was 90 minutes. The mass spectrometer was operated in negative ion mode using the instrument settings established by the automated tuning program. Data were acquired in centroid mode from *m/z*=300 to *m/z*=2000. The resolution was set to 60,000.

Measurement of lipid turnover with stable isotopes

The turnover of lipids was measured by isotopomer spectral analysis, which requires the incorporation of isotope-labeled metabolites. To incorporate labeled fatty acids into yeast, the YPGE medium was supplemented with 1 mM $^{2}H_{33}$-oleic acid and cells were cultured for 24 hours at 30°C. The labeled fatty acid was added as a 40 mM stock solution in 10% Brij58. To incorporate ^{13}C into Drosophila, adult flies were kept in vials that contained 0.2 mL of 1M $^{13}C_{6}$-glucose placed on a piece of filter paper (0.5 x 0.5 inch) as the only nutrient. Flies were maintained under these conditions for up to 2 weeks while the labeled glucose solution was replenished every 4 days. In most experiments, flies were incubated for 3 days only. After labeling thoraces were dissected, lipids were extracted, and analyzed by LC-MS/MS. The isotopomer distribution of a given species was measured in MS1 sub-spectra acquired over a 0.2-minute window centered at the peak retention time of that species. Data were extracted manually via the Quality Browser of XCalibur (Thermo Scientific). Fractional syntheses were estimated by isotopomer spectral analysis (6) as described in detail in a previous paper (7). Lipid turnover was also estimated by mass shifts as a result of ^{2}H incorporation. To this end, flies were incubated for 2 days on Instant Drosophila medium (Formula 4-24 from the Carolina Biological Supply Company) dissolved in $^{2}H_{2}O$ at a

concentration of 200 g/L. Under these conditions, ^{2}H entered lipids mainly thorough the de novo synthesis of palmitic acid. Again, lipids were extracted from fly thoraces and analyzed by LC-MS/MS. Mass shifts of CL species were calculated from MS1 sub-spectra.

BN-PAGE and Western blot analysis

BN-PAGE was performed using NativePAGE gels from Life Technologies, following the manufacturer's instructions. All other reagents used for BN-PAGE were also obtained from Life Technologies. Mitochondria were prepared from fly thorax homogenates (8) and suspended in NativePAGE sample buffer supplemented with 1% digitonin and protease inhibitors. They were incubated on ice for 20 minutes. Following centrifugation at 20,000 g for 30 minutes, the supernatant was recovered, mixed with the G-250 sample additive and NativePAGE sample Buffer, and loaded onto 3–12% pre-cast Bis–Tris NativePAGE gels. Electrophoreses was performed using the NativePAGE running buffer (anode buffer) and the NativePAGE running buffer containing 0.4% Coomassie G-250 (cathode buffer). Silver staining of native gels was performed with the SilverXpress staining kit from Life Technologies. For Western blot analysis, proteins were separated by SDS-PAGE, transferred to a PVDF membrane, and incubated with primary polyclonal tafazzin antibody (1 µg/mL) in Odyssey blocking buffer containing 0.01% Tween-20. Fluorescent LiCor GAM-IRDye680 secondary antibodies were used at a dilution of 1:15,000. Proteins were visualized and quantified using the LiCor scanner.

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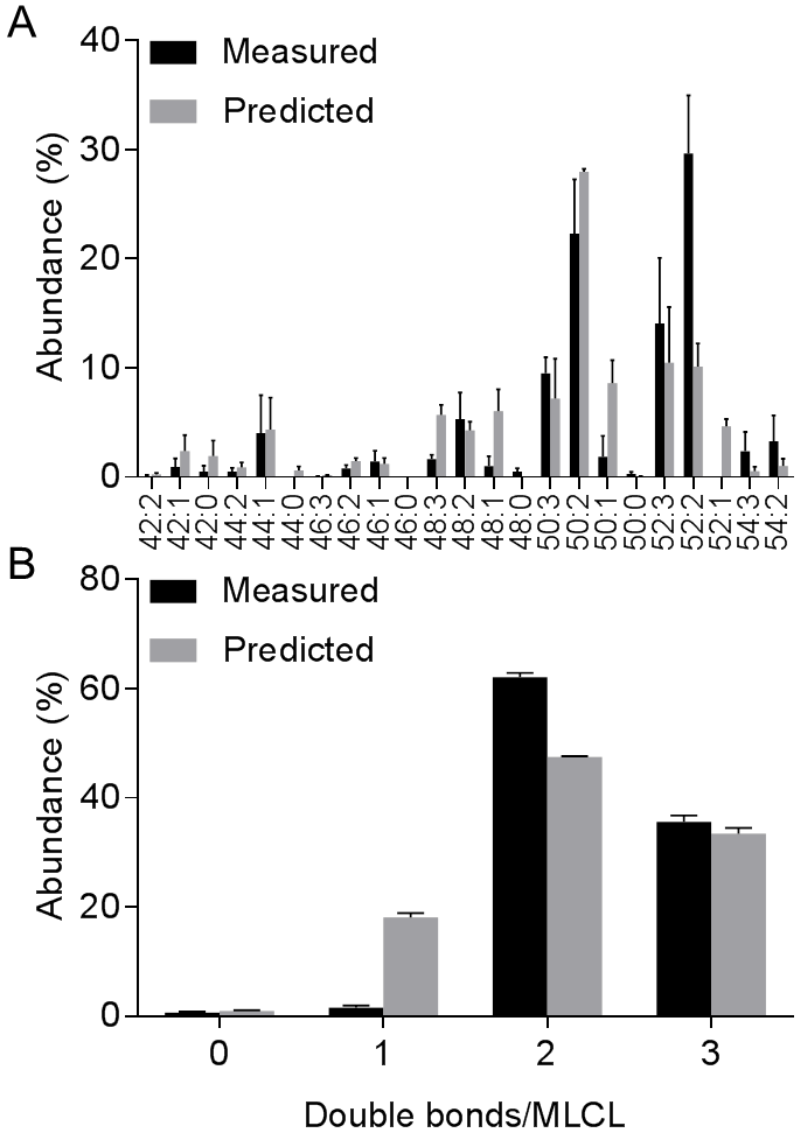


Figure S1. Cld1 selectively removes saturated acyl groups from CL. A-B: The *taz1Δ* yeast strain was grown in YPGE to the stationary phase. Lipids were analyzed by LC-MS. The graphs show the composition of MLCL measured by MS and the composition of MLCL predicted from the composition of CL assuming random cleavage of acyl groups. Data are mean values with ranges of duplicate measurements.

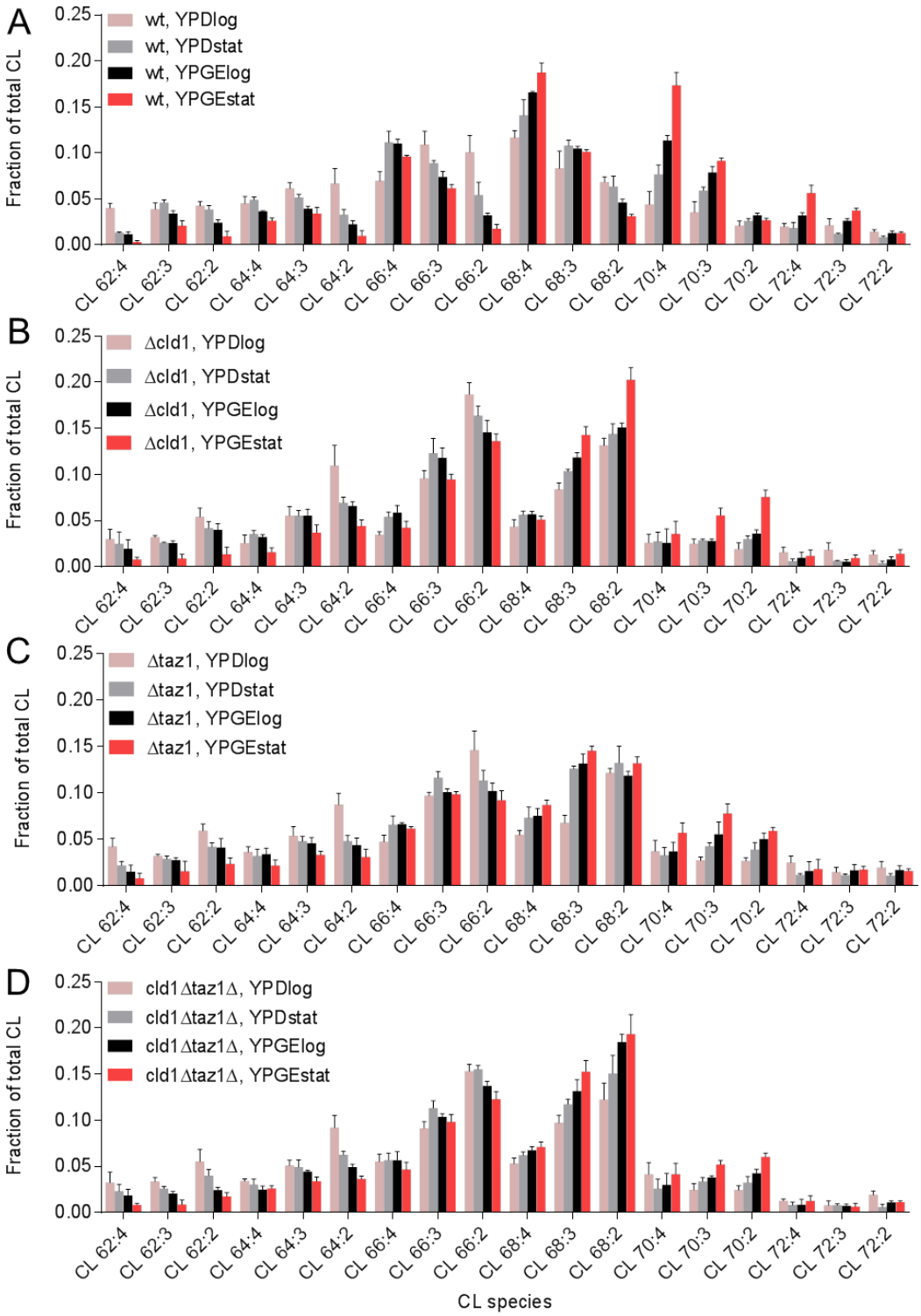
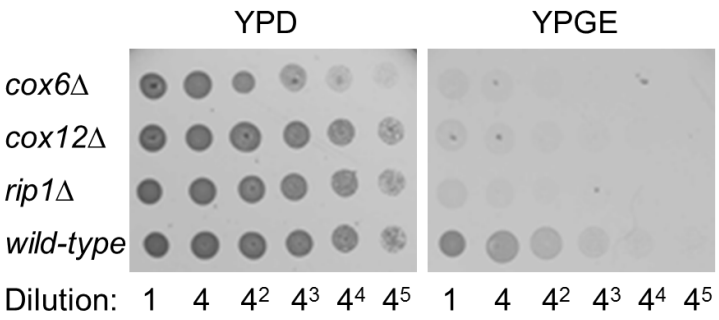


Figure S2. Culture conditions affect the molecular composition of CL in yeast. A-D: Yeast strains were grown in YPD or in YPGE. The cells were harvested either in the logarithmic (log) or in the stationary (stat) growth phase and lipids were analyzed by MS. Data are mean values with SEM (N=3).

A



B

Strain	CL Δ2 (%)	CL Δ3 (%)	CL Δ4 (%)
cox6Δ	21.8±1.8	39.5±1.3	38.8±0.6
cox12Δ	7.9±0.7	34.2±0.6	57.9±1.2
rip1Δ	20.9±4.8	39.3±2.1	39.8±5.1
wild-type	11.0±4.1	37.0±1.5	52.0±3.3

Figure S3. Deletions of subunits *COX6*, *COX12*, and *RIP1* inhibit growth on YPGE but only deletions of COX6 and RIP1 alter the CL composition. A: Serial dilutions of overnight YPD cultures, adjusted to a uniform optical density of 3.9, were spotted (4 µL aliquots) on YPD or YPGE Agar plates and grown at 30°C. **B:** Yeast strains were grown in liquid YPD to stationary phase. CL was analyzed by MS. Data are mean values ± SEM (N=3).

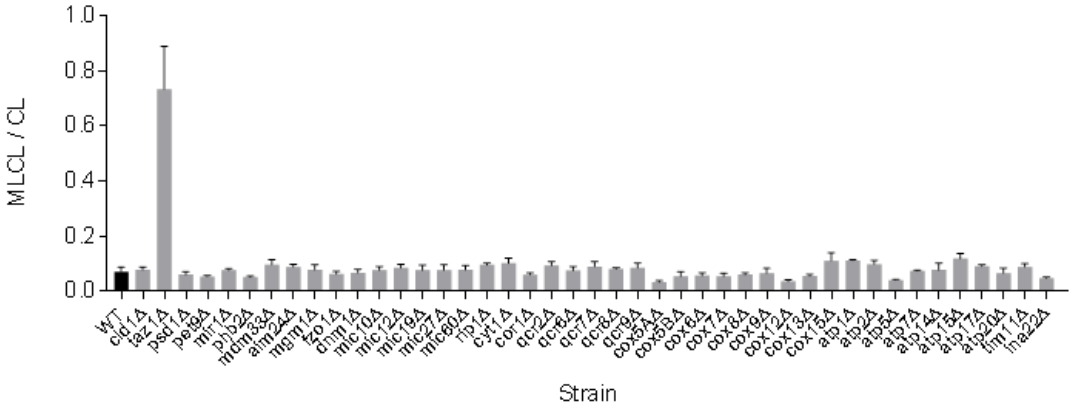


Figure S4. Deletion of OXPHOS proteins does not increase the MLCL/CL ratio. Yeast strains with the indicated deletions were grown in YPD to stationary phase and the MLCL/CL ratio was measured by MS. Data are mean values with SEM (N=3).

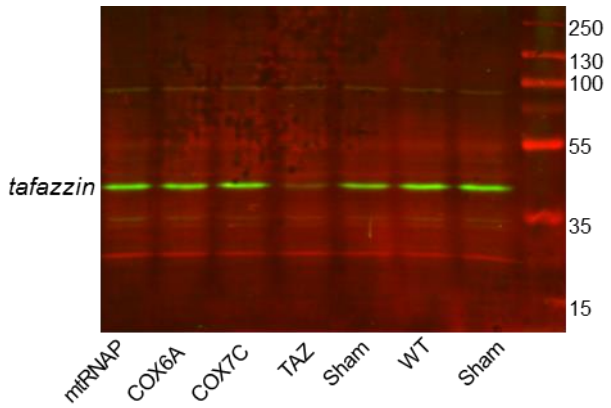


Figure S5. Knockdowns of mitochondrial RNA polymerase (mtRNAP) and subunits 6A and 7C of cytochrome oxidase (COX6A, COX7C) do not affect the expression of tafazzin (TAZ). Knockdowns of the indicated transcripts were performed with double-stranded RNA in S2 *Drosophila* cells. The abundance of tafazzin was measured by quantitative Western blot analysis with the LiCor system. The masses of marker proteins are given in kDa.

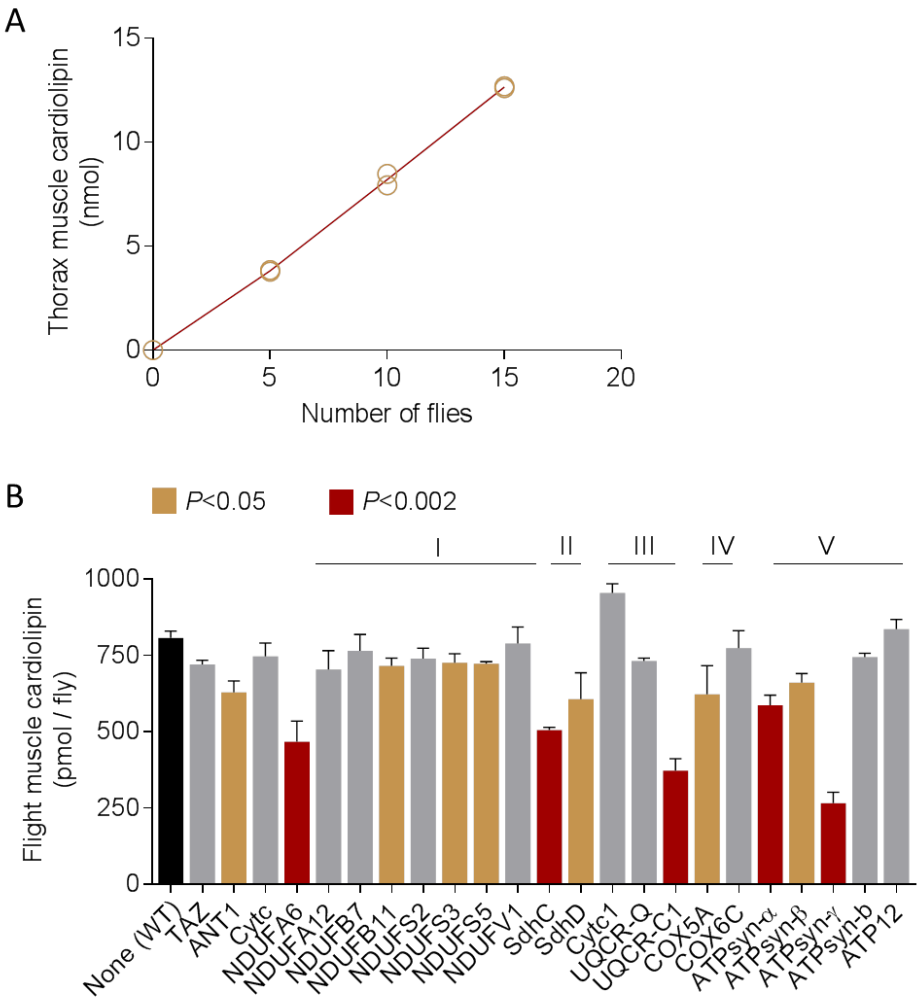


Figure S6. **Quantification of total flight muscle CL in *Drosophila*.** Fly thoraces were dissected under the microscope and stored at -80 C. Thoraces were homogenized in water, internal standards were added, and lipids were extracted. The extracts were analyzed by MS. **A:** Quantitative analysis of wild-type flies shows a linear relation between the number of dissected flies and the total amount of thorax CL. A single wild-type fly contained 809±41 pmol CL (mean ± SD, N=6) in the thorax. **B:** Muscle-specific knockdowns of several subunits of the OXPHOS system decreased the amount of CL in the thorax. Bar graphs are mean values ± SEM (N=4). Data were compared by t-test.

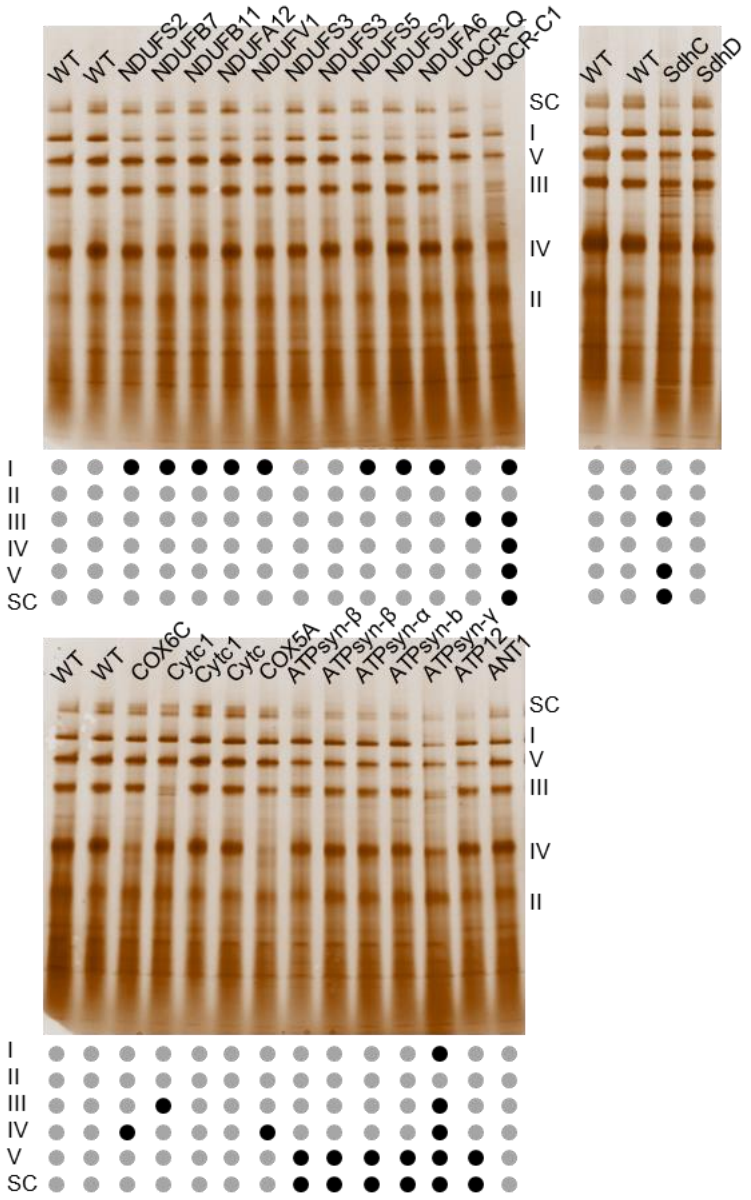


Figure S7. Effect of subunit Knockdowns on the assembly of OXPHOS complexes. Muscle-specific knockdowns of the indicated transcripts were achieved in adult Drosophila by RNAi expression using *Mhc*-Gal4. Fly thoraces were dissected and homogenized. Mitochondrial proteins were analyzed by BN-PAGE. The position of complexes I-V and the position of supercomplexes (SC, representing complex V dimers and respiratory supercomplexes) are indicated on the gels. Black dots mark complexes with reduced abundance.

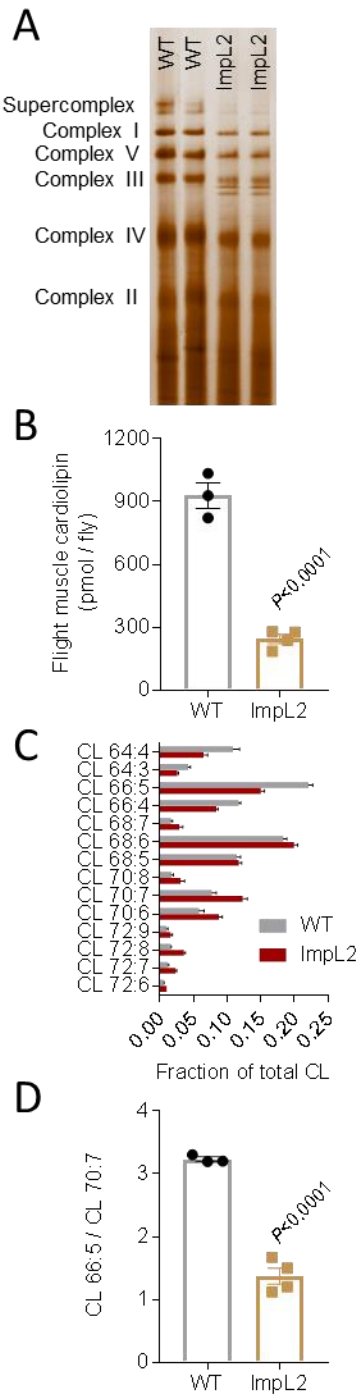


Figure S8. **Overexpression of ImpL2 reduces OXPHOS expression and alters CL composition.** Overexpression of ImpL2 was induced at the larval stage with Dmef2-Gal4. Thoraces were harvested from 3 day old adult flies. The effect on muscle OXPHOS proteins was analyzed by BN-PAGE and the effect on muscle CL was analyzed by MS. Data were compared by t-test.

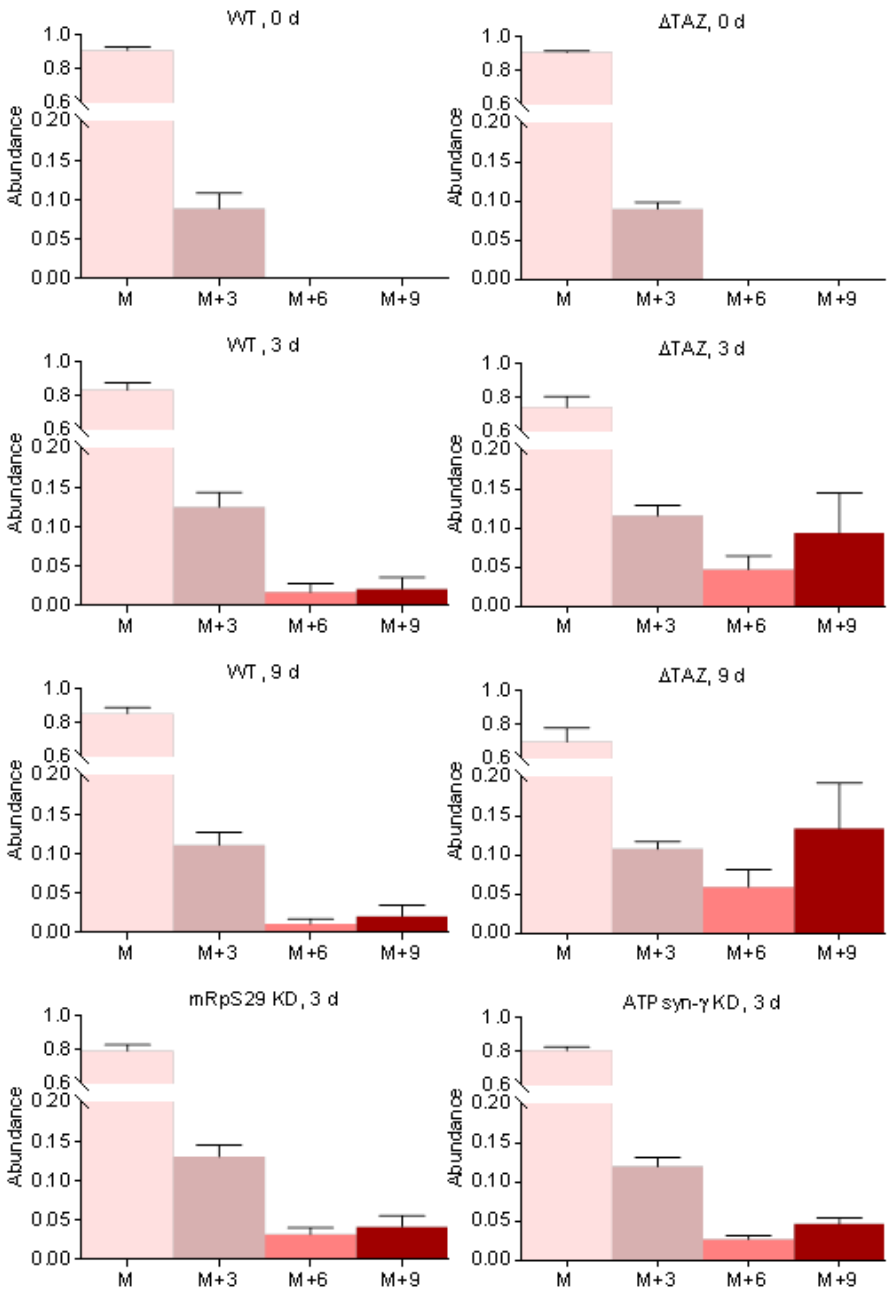


Figure S9. **Deletion of tafazzin (ΔTAZ) and knockdowns of mitochondrial ribosomal protein S29 (mRpS29) and the γ-subunit of ATP synthase (ATPsyn-γ) increase CL turnover compared to the wild-type (WT).** Different *Drosophila* strains were cultured in the presence of $^{13}C_6$-glucose for the indicated periods of time. Fly thoraces were isolated to analyze CL by MS. The abundances of 4 isotopomers were measured including the monoisotopic molecule (M), the molecule with 1 labeled glycerol group (M+3), the molecule with 2 labeled glycerol groups (M+6), and the molecule with 3 labeled glycerol groups (M+9). Data, representing multiple CL species, are mean values ± SD (N=14).

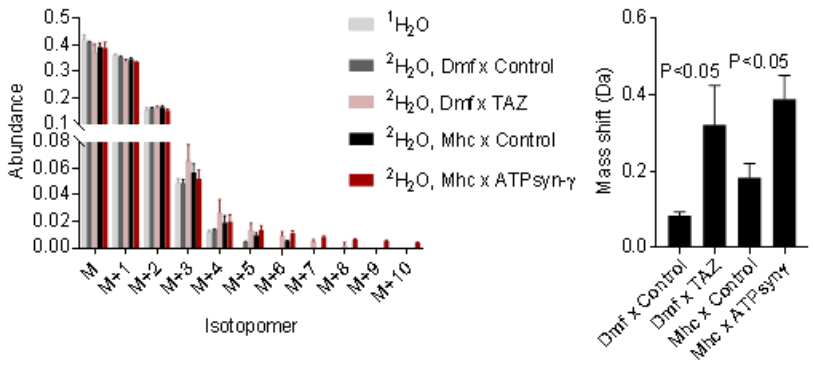


Figure S10. **Knockdown of tafazzin (TAZ) or the γ-subunit of the ATP synthase (ATPsyn-γ) increase CL turnover measured with $^{2}H_2O$.** Flies with the indicated knockdowns were cultured in the presence of $^{2}H_2O$ for 2 days and the isotopomer patterns of 5 CL species from flight muscle were analyzed to determine the increase in mean molecular mass. Data are mean values ± SEM. Data were compared by *t*-test.

Table 1. Proteins targeted in this study. The listed proteins were deleted from *S. cerevisiae* or knocked down in S2 cells or in flight muscles of *D. melanogaster*.

Organism	**Category/complex**	**Protein**	**Function**
S. cerevisiae	Phospholipid enzymes	CLD1	CL hydrolase
		TAZ1	Phospholipid transacylase
		PSD1	Phosphatidylserine decarboxylase
	Solute carriers	PET9	ADP-ATP carrier
		MIR1	Phosphate carrier
	Membrane organizers	PHB2	Prohibitin complex subunit
		MDM33	Inner membrane morphology/fission
		AIM24	Inner membrane morphology, MICOS stability
		MGM1	Mitochondrial fusion
		FZO1	Mitochondrial fusion
		DNM1	Mitochondrial fission
	MICOS complex	MIC10	MICOS complex subunit
		MIC12	MICOS complex subunit
		MIC19	MICOS complex subunit
		MIC12	MICOS complex subunit
		MIC60	MICOS complex subunit
	Complex III	RIP1	Catalytic/core subunit
		CYT1	Catalytic/core subunit
		COR1	Accessory subunit
		QCR2	Accessory subunit
		QCR6	Accessory subunit
		QCR7	Accessory subunit
		QCR8	Accessory subunit
		QCR9	Accessory subunit
	Complex IV	COX15	Required for heme A synthesis
		COX5A	Accessory subunit
		COX5B	Accessory subunit
		COX6	Accessory subunit
		COX7	Accessory subunit
		COX8	Accessory subunit
		COX9	Accessory subunit
		COX12	Accessory subunit
		COX13	Accessory subunit
	Complex V	ATP14	Subunit h of F_0 subcomplex
		ATP17	Subunit f of F_0 subcomplex
		ATP5	Subunit 5 of stator stalk
		ATP7	Subunit d of stator stalk
		ATP15	Subunit epsilon of central stalk
		INA22	Assembly factor of the stator stalk
		ATP1	Subunit alpha of F_1 subcomplex
		ATP2	Subunit beta of F_1 subcomplex
		ATP20	Subunit g, required for dimerization
		TIM11	Subunit e, required for dimerization

D. melanogaster	Phospholipid enzyme	TAZ	Phospholipid transacylase
	Solute carriers	ANT1	ADP-ATP carrier
		ANT2	ADP-ATP carrier
	Membrane organizers	OPA1	Mitochondrial fusion
		DRP1	Mitochondrial fission
		FIS1	Mitochondrial fission
		FUNDC1	Mitophagy
		PINK1	Mitophagy
	MICOS complex	Mitofilin	MICOS complex subunit 60
	Mitochondrial genome expression	mtRNAP	Mitochondrial RNA polymerase
		mRpS29	Mitochondrial ribosomal protein
	Complex I	NDUFS1	Core subunit of the matrix arm
		NDUFS2	Core subunit of the matrix arm
		NDUFS3	Core subunit of the matrix arm
		NDUFA6	Accessory subunit of the matrix arm
		NDUFA12	Accessory subunit of the matrix arm
		NDUFB7	Accessory subunit of the membrane arm
		NDUFB11	Accessory subunit of the membrane arm
		NDUFS5	Accessory subunit of the membrane arm
		NDUFV1	N module of the matrix arm
	Complex II	SdhA	Core subunit (peripheral membrane protein)
		SdhB	Core subunit (peripheral membrane protein)
		SdhC	Core subunit (integral membrane protein)
		SdhD	Core subunit (integral membrane protein)
	Complex III	Cytc1	Core/catalytic subunit
		UQCR-C1	Core/catalytic subunit
		UQCR-Q	Accessory subunit
		Cytc	Substrate (peripheral membrane protein)
	Complex IV	COX5A	Subunit assembled early in complex formation
		COX6A	Subunit assembled late in complex formation
		COX6C	Subunit assembled late in complex formation
		COX7C	Subunit assembled late in complex formation
	Complex V	ATPsyn-c	Subunit c of F_0 subcomplex
		ATPsyn-α	Subunit alpha of F_1 subcomplex
		ATPsyn-β	Subunit beta of F_1 subcomplex
		ATPsyn-γ	Subunit gamma of F_1 subcomplex
		ATPsyn-b	Subunit b of stator stalk
		ATP12	Assembly factor

Table 2. CL content of yeast. Strains were grown in YPD and harvested either in mid-logarithmic or in stationary phase. The cellular content of CL is expressed in percent of the content in wild-type yeast at stationary phase. Data are mean values ± SEM. The number of independent measurements is given in parenthesis. Asterisks marks a statistically significant difference to the wild-type content at stationary phase (*t*-test, $P<0.05$).

Strain	CL content (%)	
	Mid-logarithmic phase	Stationary phase
wild-type	27.5±3.3 (6)*	100±16 (6)
rho0	4.8±0.8 (5)*	21.3±2.5 (5)*
cld1Δ	18.7±1.3 (3)*	90.8±9.2 (3)
taz1Δ	24.7±7.4 (3)*	46.6±4.4 (3)*
psd1Δ	ND	100.4±16.8 (3)
pet9Δ	ND	83.8±11.4 (3)
mir1Δ	ND	77.3±13.3 (3)
phb2Δ	ND	121.2±18.4 (3)
mdm33Δ	ND	75.7±16.0 (3)
mgm1Δ	ND	27.4±5.3 (3)*
fzo1Δ	ND	36.0±7.1 (3)*
dnm1Δ	ND	85.4±31.5 (3)
rip1Δ	50.0±13.7 (3)	68.5±6.6 (3)
cyt1Δ	47.1±13.3 (3)	59.5±2.8 (3)
cor1Δ	49.4±10.6 (3)	105.8±3.4 (3)
qcr2Δ	35.1±13.1 (3)*	50.5±7.1 (3)
qcr6Δ	48.2±8.3 (3)	109.1±14.7 (3)
qcr7Δ	41.8±10.9 (3)	70.6±14.6 (3)
qcr8Δ	42.1±10.5 (3)	68.5±14.4 (3)
qcr9Δ	56.6±14.2 (3)	96.0±19.4 (3)
cox5AΔ	38.4±2.4 (6)*	134.7±8.2 (6)
cox5BΔ	34.9±2.2 (6)*	132.7±5.9 (6)
cox6Δ	13.6±0.9 (6)*	52.7±6.6 (6)*
cox7Δ	38.6±1.6 (6)*	145.4±24.4 (6)
cox8Δ	45.5±4.6 (6)*	133.5±6.1 (6)
cox9Δ	10.7±1.2 (6)*	36.6±2.2 (6)*
cox12Δ	29.1±2.8 (6)*	120.9±20.7 (6)
cox13Δ	40.0±2.7 (6)*	114.9±4.5 (6)
cox15Δ	25.3±5.5 (6)*	45.5±2.6 (6)*
atp1Δ	37.6±15.7 (3)*	62.2±7.3 (3)
atp2Δ	38.4±12.1 (3)*	68.1±3.8 (3)
atp5Δ	29.7±1.7 (3)*	60.3±8.8 (3)
atp7Δ	52.4±16.9 (3)	115.6±17.0 (3)
atp14Δ	29.5±12.8 (3)*	39.9±7.8 (3)*
atp15Δ	25.5±4.8 (3)*	28.4±2.9 (3)*
atp17Δ	30.5±6.5 (3)*	50.8±18.4 (3)
ina22Δ	43.9±5.1 (3)	137.5±17.2 (3)

Table 3. RNAi efficiencies in S2 Drosophila cell cultures. Transcripts of the indicated genes were knocked down by addition of double-stranded RNA. The knockdown efficiencies were measured by quantitative RT-PCR. Data are mean values ± SD (N=4).

Gene	Knockdown efficiency (%)
TAZ	67±13
ANT2	63±18
ND30	63±12
ND75	95±6
SdhA	44±15
SdhB	88±15
UQCR-C1	75±30
COX6A	82±19
COX7C	68±26
ATPsyn-c	70±16
mtRNAP	76±10
Mitofilin	62±4
OPA1	52±6
DRP1	62±7
FIS1	60±11
FUNDC1	50±32
PINK1	94±8

Table 4. Cardiolipin composition in Drosophila flight muscles of various knockdown strains.

Lipids were extracted from fly thoraces and analyzed by MALDI-TOF MS. The molecular species of the CL groups were identified by LC-MS/MS using an LTQ-Orbitrap. Data are mean values with SD of 4 independent measurements.

		Distribution (%)																							
Knockdown target:		None (WT)		TAZ		ANT		Cytc		NDUFA6		NDUFA12		NDUFB7		NDUFB11		NDUFS2		NDUFS3		NDUFS5		NDUFV1	
CL group	Major species	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CL 64:4	16:1/16:1/16:1/16:1	4.45	0.42	3.93	0.70	4.15	0.51	3.36	0.51	4.52	0.45	4.35	0.53	3.51	0.52	4.39	0.38	3.68	0.33	3.40	0.29	4.34	0.08	4.73	0.07
CL 66:6	16:1/16:1/16:1/18:3	2.63	0.57	2.13	0.37	2.34	0.65	1.90	0.20	2.43	0.57	2.03	0.28	1.67	0.22	2.03	0.20	1.76	0.19	1.91	0.21	2.20	0.17	2.53	0.14
CL 66:5	16:1/16:1/16:1/18:2	13.55	1.18	8.97	0.66	8.04	0.82	9.86	0.40	13.94	1.06	10.30	0.31	8.89	0.45	11.59	0.42	9.52	0.27	9.54	0.98	10.10	0.27	11.80	0.09
CL 68:7	16:1/16:1/18:2/18:3	5.40	0.99	5.33	0.20	5.38	1.85	4.91	0.38	4.99	1.18	4.90	0.72	4.05	0.29	4.62	0.27	4.20	0.33	4.74	0.51	5.40	0.25	6.06	0.18
CL 68:6	16:1/16:1/18:2/18:2	24.50	1.12	20.43	0.44	18.59	1.15	22.95	0.59	25.30	0.56	21.75	0.47	20.76	0.62	23.38	0.96	21.55	0.74	21.45	1.01	21.52	0.39	22.76	0.40
CL 70:8	16:1/18:2/18:2/18:3	6.80	0.73	8.46	0.40	8.20	1.80	7.37	0.62	6.76	1.40	6.94	0.47	7.01	0.60	6.89	0.46	7.00	0.52	7.43	0.45	7.66	0.17	8.09	0.25
CL 70:7	16:1/18:2/18:2/18:2	25.83	1.36	27.55	1.13	27.75	1.83	28.56	0.64	25.25	2.84	27.86	0.53	29.41	1.24	27.81	0.79	29.13	0.77	28.71	0.78	27.41	0.10	25.03	0.19
CL 72:9	18:2/18:2/18:2/18:3	4.50	0.56	6.78	0.25	7.25	1.17	5.69	0.56	4.89	0.95	5.46	0.64	6.37	0.84	4.64	0.33	5.86	0.75	5.77	0.40	5.64	0.34	6.06	0.71
CL 72:8	18:2/18:2/18:2/18:2	12.34	1.63	16.41	1.01	18.31	1.94	15.40	0.16	11.90	1.79	16.40	0.32	18.33	0.55	14.67	0.41	17.31	0.56	17.05	1.83	15.73	0.28	12.95	0.10

		Distribution (%)																							
Knockdown target:		SdhC		SdhD		Cyt c1		UQCR-Q		UQCR-C1		COX5A		COX6C		ATPα		ATPβ		ATPγ		ATPb		ATP12	
CL group	Major species	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CL 64:4	16:1/16:1/16:1/16:1	1.34	0.07	1.85	0.22	4.42	0.44	4.76	0.06	8.82	0.99	3.20	0.35	3.87	0.08	4.15	0.23	3.35	0.60	6.45	3.05	3.25	0.13	4.88	0.39
CL 66:6	16:1/16:1/16:1/18:3	1.24	0.13	1.68	0.40	2.22	0.23	1.85	0.13	3.01	0.13	1.70	0.31	1.94	0.16	1.86	0.20	1.78	0.18	2.60	0.45	2.20	0.94	2.19	0.34
CL 66:5	16:1/16:1/16:1/18:2	5.40	0.19	6.77	1.47	9.99	0.59	11.55	0.02	11.14	1.28	7.96	0.47	9.73	0.34	6.08	0.17	5.80	0.75	10.25	1.48	7.56	0.21	10.48	0.24
CL 68:7	16:1/16:1/18:2/18:3	4.57	0.30	4.69	0.88	4.56	0.12	4.38	0.05	5.46	0.88	4.00	0.30	4.35	0.29	4.43	0.64	3.95	0.66	4.57	1.46	5.23	1.78	4.74	0.78
CL 68:6	16:1/16:1/18:2/18:2	15.36	0.03	17.49	2.00	20.91	0.85	23.54	0.08	17.58	0.69	19.04	0.22	21.24	0.45	13.82	0.83	13.72	1.18	15.23	2.18	17.78	1.43	20.72	1.03
CL 70:8	16:1/18:2/18:2/18:3	9.09	0.33	8.78	0.21	7.62	0.11	6.25	0.04	8.63	1.48	7.57	0.31	7.32	0.32	9.15	0.26	8.70	0.96	7.34	1.53	9.24	2.41	7.59	0.94
CL 70:7	16:1/18:2/18:2/18:2	28.63	0.32	28.42	0.66	27.15	0.67	28.01	0.31	22.49	0.57	29.49	1.21	28.58	0.72	26.65	0.48	26.82	0.74	23.09	1.48	27.87	2.94	26.20	1.76
CL 72:9	18:2/18:2/18:2/18:3	9.83	0.13	8.93	1.38	6.99	0.35	4.49	0.07	7.84	0.42	7.18	0.60	6.06	0.81	10.53	1.55	10.67	1.18	8.58	0.55	8.12	1.73	7.19	1.11
CL 72:8	18:2/18:2/18:2/18:2	24.54	0.71	21.38	3.17	16.14	0.79	15.17	0.05	15.04	0.27	19.85	1.05	16.92	0.35	23.33	0.52	25.21	2.67	21.88	2.58	18.76	2.18	16.01	0.73

Table 5. Rate constants and half-lives of CL species from Drosophila flight muscle. The fractional syntheses of individual CL species were measured at 12 different time points (1-9 days). Rate constants were estimated by non-linear regression. Half-life times were calculated from the rate constants.

Drosophila strain	CL species	Turnover rate constant ± SE (10^{-3} day^{-1})	Half life time (days)
WT	CL 66:5	1.2 ± 0.2	567
	CL 68:6	1.4 ± 0.2	492
	CL 68:5	3.8 ± 0.6	182
	CL 70:7	1.8 ± 0.3	376
	CL 70:6	3.6 ± 0.5	194
	CL 72:8	3.8 ± 0.5	181
ΔTAZ	CL 66:5	24.9 ± 2.2	28
	CL 66:4	40.4 ± 4.0	17
	CL 68:6	28.9 ± 1.3	24
	CL 68:5	41.1 ± 3.2	17
	CL 68:4	66.9 ± 6.1	10
	CL 70:7	26.0 ± 1.0	27
	CL 70:6	28.5 ± 1.1	24

Table 6. Number of lipid molecules associated with each OXPHOS complex. The numbers were calculated from the molecular mass of the complexes, assuming a lipid-to-protein mass ratio of 22:78 and an average lipid mass of 750 Da.

Complex	Mass (10^6 Da)	Number of lipids
I	1.0	376
II	0.1	38
III_2	0.5	188
IV	0.2	75
V	0.6	226