EXTENDED EXPERIMENTAL PROCEDURES

Drosophila Strains and Genetics.

The following fly stocks were used: *y w; Dmef2-Gal4* and *w; mhc-Gal4* were the Gal4 transgenic lines used to express RNAi lines in muscles. w^{1118}/*mhc-Gal4* flies were used as wildtype (wt) controls. Other fly stocks used were y^1sc*v^1; P{TRiP.HMS00854}attP2 (Bloomington, #33911), y^1v^1; P{TRiP.HMS05059}attP2 (Bloomington, #28573), y^1v^1; P{TRiP.HMC02929}attP40 (Bloomington, #44535), y^1v^1; P{TRiP.HMC03554}attP40 (Bloomington, #53325), y^1sc*v^1; P{TRiP.HMC03861}attP40 (Bloomington, #55180), y^1sc*v^1; P{TRiP.HM05229}attP2 (Bloomington, #30487), y^1sc*v^1; P{TRiP.HMS01590}attP2 (Bloomington, #36701), y^1v^1; P{TRiP.HMC03429}attP40 (Bloomington, #51855), y^1sc*v^1; P{TRiP.HMC03653}attP40 (Bloomington, #52913), y^1sc*v^1; P{TRiP.GLC01699}attP2 (Bloomington, #50577),y^1sc*v^1; P{TRiP.HMC03662}attP40 (Bloomington, #52922), y^1sc*v^1; P{TRiP.HMS00798}attP2 (Bloomington, #32998), y^1sc*v^1; P{TRiP.HMS01584}attP2 (Bloomington, #36695), y^1sc*v^1; P{TRiP.HMC02678}attP2/ TM3, Sb^1 (Bloomington, #43279), y^1v^1; P{TRiP.HM05206}attP2 (Bloomington, #29528), y^1v^1; P{TRiP.HM22452}attP40 (Bloomington, #58322), y^1v^1; P{TRiP.GLC01422}attP2 (Bloomington, #43235), y^1v^1; P{TRiP.HMJ23156}attP40 (Bloomington, #61321), y^1sc*v^1; P{TRiP.HM05255}attP2/TM3, Sb^1 (Bloomington, #30511), y^1sc*v^1; P{TRiP.HMC03242}attP2 (Bloomington, #51357), y^1v^1; P{TRiP.HMJ22367}attP40 (Bloomington, #58282), y^1sc*v^1; P{TRiP.HMS00815}attP2 (Bloomington, #33878), y^1v^1; P{TRiP.JF02892}attP2 (Bloomington, #28056), y^1v^1; P{TRiP.JF02899}attP2 (Bloomington, #28062) and y^1sc*v^1; P{TRiP.HMS01560}attP2 (Bloomington, #36672). Transgenic RNAi stocks for disrupting *CG8680 (8680R-3), CG9172 (9172R-2), CG6463 (6463R-1), CG9350 (9350R-2), CG9762 (9762R-3), CG13240 (13240R-2), CG3283 (3283R-1) and CG3192 (3192R-3)* were from the National Institute of Genetics (NIG, Japan) *Drosophila* Stock Center. RNAi stocks for disrupting *CG12400 (v102590), CG7712 (v100616), CG12859 (v8786), CG4169 (v26405) and CG9306 (v23088)* were from the Vienna Drosophila Resource Center.

Mitochondria Purification.

Mitochondrial purification was performed essentially as described by Rera et al 2012 (Rera et al., 2011). Thoraxes were dissected and gently crushed with a pestle homogenizer in 500µl of pre-chilled mitochondrial isolation buffer containing 250 mM sucrose and 0.15 mM $MgCl_2$ in 10 mM Tris.HCl, pH 7.4, on ice. After two rounds of centrifugation at 500g for 5 minutes at 4°C to remove insoluble material, the supernatant was recovered and centrifuged at 5000g for 5 minutes at 4°C. The pellet which is enriched for mitochondria was washed twice in the mitochondrial isolation buffer and stored at -80°C until further processing.

Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE).

BN-PAGE was performed using NativePAGE gels from Life Technologies, following the manufacturer's instructions. Essentially, mitochondria were suspended in native PAGE sample buffer (Life Technologies) supplemented with 1% digitonin and protease inhibitors, and incubated on ice for 20 minutes. Following centrifugation at 20,000g for 30 minutes, the supernatant was recovered, mixed with the G-250 sample additive (Life Technologies) and Native PAGE Sample Buffer (Life Technologies), and loaded onto 3–12% pre-cast Bis–Tris Native PAGE gels (Life Technologies). The NativeMark Protein standard (Life Technologies), run together with the samples, was used to estimate the molecular weight of the protein complexes. Electrophoreses was performed using the Native PAGE Running buffer (as anode buffer, from Life technologies) and the Native PAGE Running buffer containing 0.4% Coomassie G-250 (cathode buffer). Gels were stained with the Novex Colloidal Blue staining kit (Life Technologies) to reveal the protein complexes.

Silver Staining.

Silver staining of native gels was performed with the SilverXpress staining kit from Life Technologies, following the manufacturer's protocol.

In-gel Complex I Activity.

Complex I activity in native gels was performed by incubating the native gels in 0.1 mg/ml NADH, 2.5 mg/ml Nitrotetrazolium Blue Chloride, 5 mM Tris-HCl (pH 7.4) overnight at room temperature.

Immunoblotting.

For immunoblotting of samples in native gels, protein complexes from native gels were transferred to PVDF membranes (BIO-RAD). For immunoblotting of samples in whole tissue lysates, thoraxes were homogenized in RIPA buffer (150 mM NaCl, 1% Triton X-100, 0.5% Sodium Deoxycholate, 0.1% SDS, 50mM Tris HCl, pH 8) supplemented with Halt protease inhibitors (Pierce), resolved on mini-PROTEAN TGX stain-free gels from BIO-RAD, and transferred to PVDF membranes. In both instances (native and non-native gels), the membrane was subsequently blocked in 5% (w/v) non-fat dry milk in Tris-buffered saline (TBS) for 30minutes, and incubated in the appropriate primary antibody dissolved in 2% BSA, 0.1% Tween 20 in TBS (TBST) overnight at 4°C. Following the overnight incubation, the blot was rinsed 4X10 minutes in 0.1%TBST, blocked for 30 minutes in 5% (w/v) non-fat dry milk in TBST and incubated for two hours with the appropriate HRP-conjugated secondary antibody dissolved in 2% BSA, 0.1% Tween 20 in TBS (TBST). After incubation in the secondary antibody, samples were

rinsed 4X10 minutes in 0.1%TBST. Immunoreactivity was detected by enhanced chemiluminescence (ECL) and analyzed by a ChemiDoc Gel imaging system from BIO-RAD. Antibodies used were anti-NDUFS3 (abcam, ab14711), anti-ND1 (abcam, ab74257), anti ATPsynß (Life technologies, A21351) anti-GFP (Life technologies, A6455) and anti-actin (EMD Millipore, MAB1501).

In-Gel Protein Digestion

The dried gel pieces were rehydrated and digested in 80 µL of 12.5 ng/µL Trypsin Gold/50 mM ammonium bicarbonate at 37°C overnight. Following the digestion, condensed evaporated water was collected from tube walls by brief centrifugation using benchtop microcentrifuge (Eppendorf, Hauppauge, NY). The gel pieces and digestion reaction were mixed with 50 µL 2.5% Trifluoroacetic acid (TFA) and rigorously mixed for 15 minutes. The solution with extracted peptides was transferred into a fresh tube, and the remaining peptides were extracted with 80µl of 70% Acetonitrile (ACN)/5% TFA mixture using by rigorously mixing for 15 minutes. The extracts were pooled and dried to completion (1.5–2 hours) in a SpeedVac. The dried peptides were reconstituted in 30 µl of 0.1% TFA by mixing for 5 minutes and stored on ice or at −20 °C prior to analysis.

LC-MS/MS Analysis

The concentrated peptide mix was reconstituted in a solution of 2 % ACN, 2 % Formic acid (FA) for MS analysis. Peptides were eluted from the column using a Dionex Ultimate 3000 Nano LC system with a 10 min gradient from 2% buffer B to 35 % buffer B (100 % ACN, 0.1 % FA). The gradient was switched from 35 % to 85 % buffer B over 1 min and held constant for 2 min. Finally, the gradient was changed from 85 % buffer B to 98 % buffer A (100% water, 0.1% FA) over 1 min, and then held constant at 98 % buffer A for 5 more minutes. The application of a 2.0 kV distal voltage electrosprayed the eluting peptides directly into the Thermo Fusion Tribrid mass spectrometer equipped with an EASY-Spray source (Thermo Scientific). Mass spectrometer-scanning functions and HPLC gradients were controlled by the Xcalibur data system (Thermo Finnigan, San Jose, CA).

Database Search And Interpretation Of MS/MS Data

Tandem mass spectra from raw files were searched against a *Drosophila* protein database using the Proteome Discoverer 1.4 software (Thermo Finnigan, San Jose, CA). The Proteome Discoverer application extracts relevant MS/MS spectra from the .raw file and determines the precursor charge state and the quality of the fragmentation spectrum. The Proteome Discoverer probability-based scoring system rates the relevance of the best matches found by the SEQUEST algorithm. The *Drosophila*

protein database was downloaded as FASTA-formatted sequences from Uniprot protein database (database released in May, 2015). The peptide mass search tolerance was set to 10ppm. A minimum sequence length of 7 amino acids residues was required. Only fully tryptic peptides were considered. To calculate confidence levels and false positive rates (FDR), Proteome Discoverer generates a decoy database containing reverse sequences of the non-decoy protein database and performs the search against this concatenated database (non-decoy + decoy). Scaffold (Proteome Software) was used to visualize searched results. The discriminant score was set at less than 1% FDR determined based on the number of accepted decoy database peptides to generate protein lists for this study. Spectral counts were used for estimation of relative protein abundance between samples.

Table S1: There are at least 42 orthologs of the 44 human complex I subunits in *Drosophila.* Related to Figure 1

Human Complex I Protein	Yarrowia lipolytica Complex I Protein	Escherichia coli Complex I Protein	Drosophila Ortholog (DIOPT Score)	Confirmed by Mass Spectrometry
NDUFS1	**NUAM**	**NuoG**	**CG2286 (11)**	+
NDUFS2	**NUCM**	**NuoD**	**CG1970* (11)** **CG11913 (6)**	+
NDUFS3	**NUGM**	**NuoC**	**CG12079 (10)**	+
NDUFS4	NUYM		CG12203 (10)	+
NDUFS5	NIPM		CG11455 (4)	+
NDUFS6	NUMM		CG8680 (11)	+
NDUFS7	**NUKM**	**NuoB**	**CG9172* (9)** **CG2014 (9)**	+
NDUFS8	**NUIM**	**NuoI**	**CG3944 (11)**	+
NDUFV1	**NUBM**	**NuoF**	**CG9140* (10)** **CG11423* (7)** **CG8102 (6)**	+ +
NDUFV2	**NUHM**	**NuoE**	**CG5703* (11)** **CG6485 (7)**	+
NDUFV3			CG11752 (1)	+
NDUFC1				
NDUFC2			CG12400 (8)	+
NDUFA1	NIMM		CG34439 (4)	+
NDUFA2	NI8M		CG15434 (11)	
NDUFA3	NI9M			
NDUFA5	NUFM		CG6463 (9)	+
NDUFA6	NB4M		CG7712 (11)	+
NDUFA7	NUZM		CG3621* (9) CG6914 (7)	+
NDUFA8	NUPM		CG3683 (10)	+
NDUFA9	NUEM		CG6020 (10)	+
NDUFA10			CG6343 (10)	+
NDUFA11	NUJM		CG9350 (7)	+
NDUFA12	N7BM		CG3214 (11)	+
NDUFA13	NB6M		CG3446 (7)	+
NDUFAB1	ACPM1 ACPM2		CG9160 (7)	+
NDUFB1			CG18624 (5)	+
NDUFB2			CG40002* (5) CG40472 (5)	+
NDUFB3	NB2M		CG10320 (8)	+
NDUFB4	NB5M		CG12859 (3)	+
NDUFB5			CG9762 (11)	+
NDUFB6			CG13240 (1)	+
NDUFB7	NB8M		CG5548 (11)	+
NDUFB8	NIAM		CG3192 (10)	+
NDUFB9	NI2M		CG9306 (11)	+
NDUFB10	NIDM		CG8844 (11)	+
NDUFB11	NESM		CG6008 (8)	+
ND1	**NU1M**	**NuoH**	**CG34092 (3)**	+
ND2	**NU2M**	**NuoN**	**CG34063 (6)**	
ND3	**NU3M**	**NuoA**	**CG34076 (7)**	
ND4	**NU4M**	**NuoM**	**CG34085 (3)**	+
ND4L	**NULM**	**NuoK**	**CG34086 (7)**	
ND5	**NU5M**	**NuoL**	**CG34083 (5)**	+
ND6	**NU6M**	**NuoJ**	**CG34089 (1)**	
	NUXM			
	NEBM			
	NUNM			
	NUUM			
	ST1			

Table S1: There are at least 42 orthologs of the 44 human complex I subunits in *Drosophila.* Related to Figure 1: *Shows which protein in a set of paralogs was confirmed by mass spectrometry. Core subunits are shown in bold font.

Figure S1: 1% Digitonin Is The Optimum Detergent Concentration For Resolving OXPHOS Complexes In *Drosophila* Thoraxes

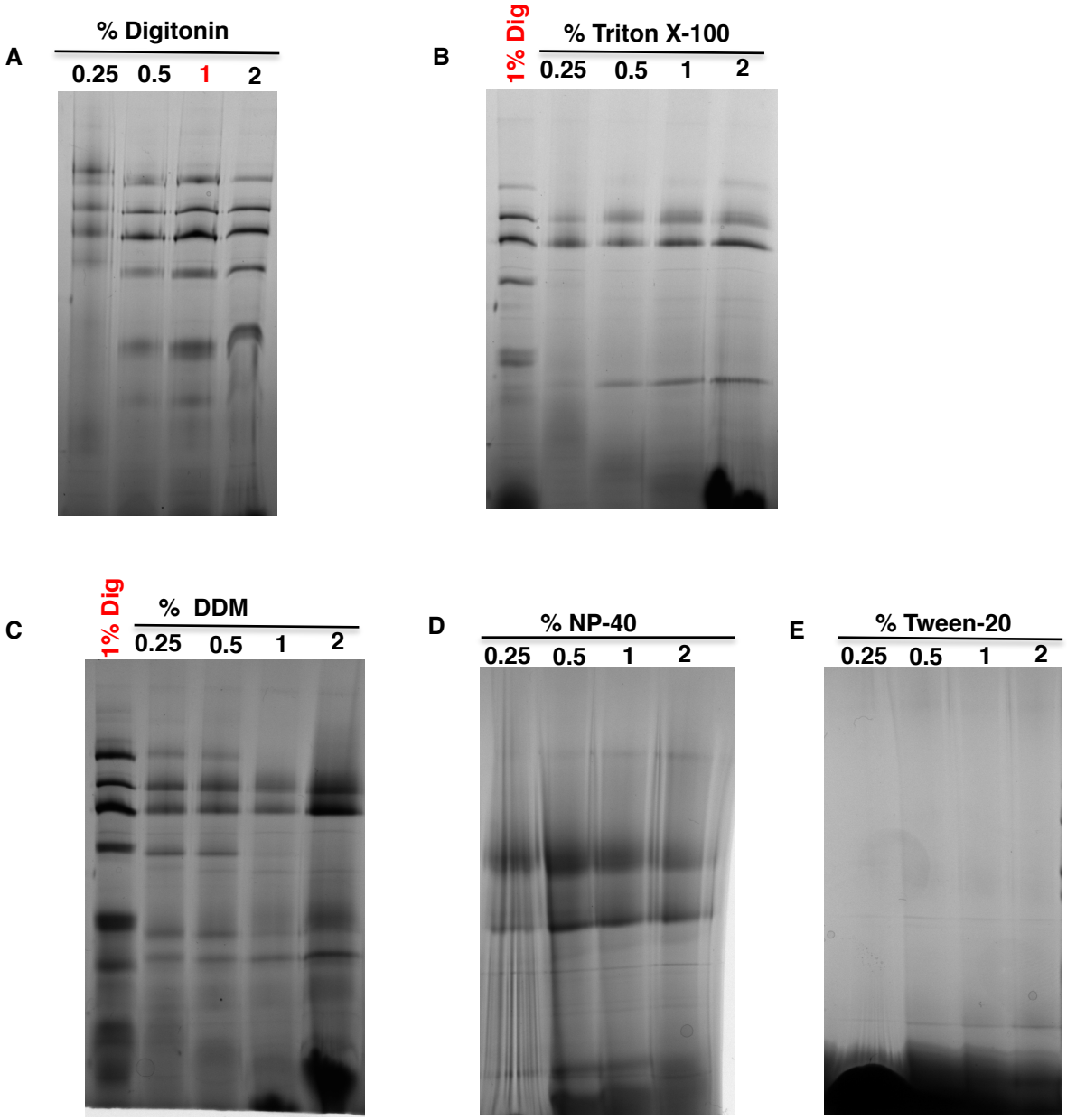

Figure S1: 1% Digitonin Is The Optimum Detergent Concentration For Resolving OXPHOS Complexes In *Drosophila* Thoraxes, Related to Figure 1. Mitochondrial protein complexes from wild-type thoraxes were solubilized in various concentrations of detergents as shown
(A) Digitonin at 0.25%, 0.5%, 1% and 2%
(B) 1% digitonin, and Triton X-100 concentrations of 0.25%, 0.5%, 1% and 2%
(C) 1% digitonin, and n-Dodecyl β-D-maltoside (DDM) concentrations of 0.25%, 0.5%, 1% and 2%
(D) NP-40 concentrations of 0.25%, 0.5%, 1% and 2%, and
(E) Tween-20 concentrations of 0.25%, 0.5%, 1% and 2%

Figure S2: Strong Expression of Dmef2-Gal4 During Development

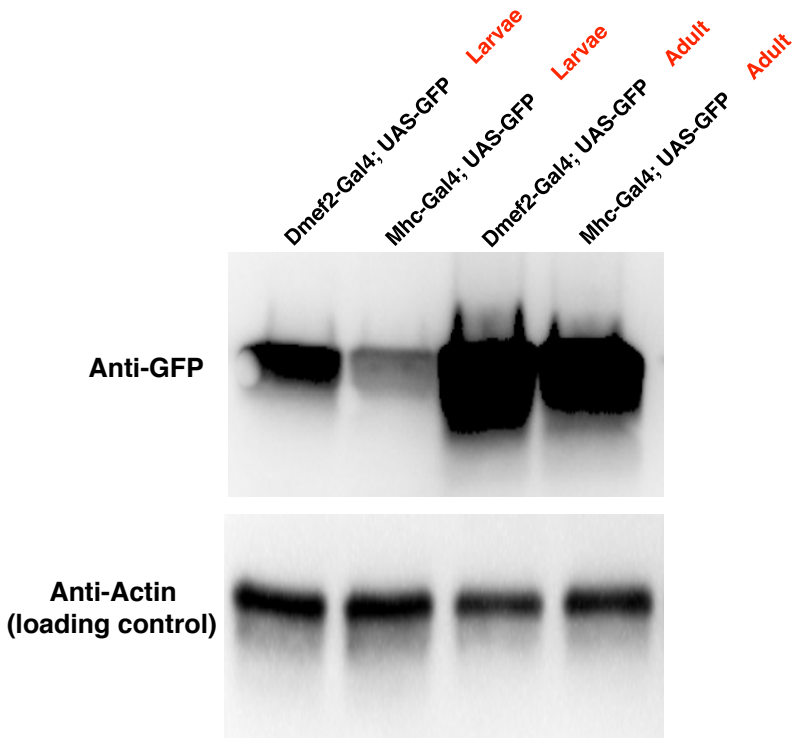

Figure S2: Strong Expression Of Dmef2-Gal4 During Development, Related to Figure 2
Western blot showing extent of GFP expression in *Dmef2-Gal4; UAS-GFP* and *mhc-Gal4; UAS-GFP* larval somatic and adult thoracic muscles respectively. Expression of β-actin serves as a loading control. Note that the *mhc-Gal4* driver has a weaker expression during development relative to the *Dmef2-Gal4* driver.

Figure S3: Detection of Smaller Subcomplexes of CV

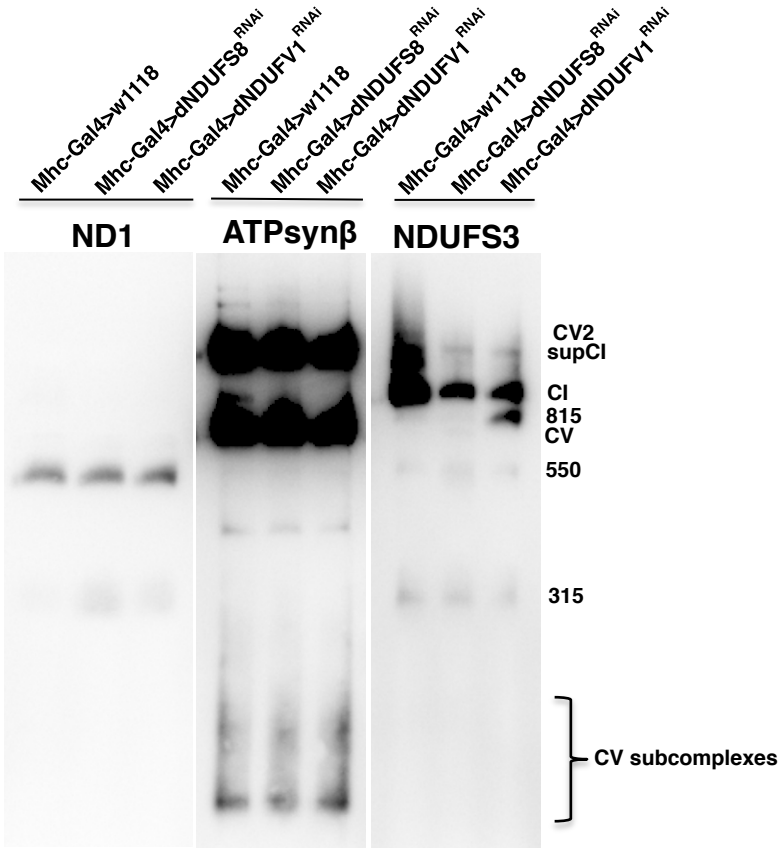

Figure S3: Detection Of Smaller Subcomplexes Of CV, Related to Figure 3

Immunoblots of samples obtained from wildtype, mhc>dNDUFS8^{RNAi} and mhc>dNDUFV1^{RNAi} thoraxes of flies aged for 24 hours after eclosure to detect CI and CV assembly intermediates. In the left and right panels, anti-ND1 and anti-NDUFS3 antibodies detect the CI holoenzyme and supercomplex, and the ~315 kDa, ~550 kDa and ~815 kDa CI assembly intermediates; but no assembly intermediates less than about 300 kDa are detected by these antibodies. However, in the middle panel, anti-ATPsynβ detects the CV monomer and dimer as well as several assembly intermediates some of which are smaller than 300 kDa.

Figure S4: Destabilization of Complex I Is Not Specifically Linked To Stress

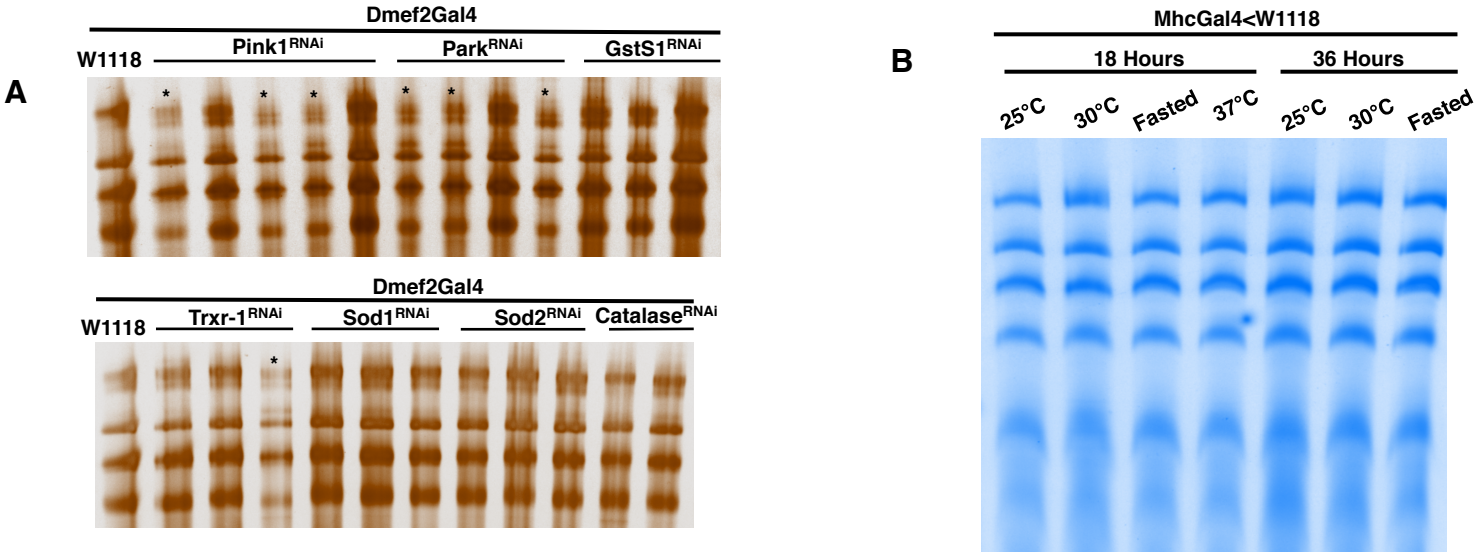

Figure S4: Destabilisation Of CI Is Not Specifically Linked To Stress, Related to Figure 2
(A) Silver-stained gels containing OXPHOS complexes isolated from wildtype, Dmef2Gal4>PINK1^{RNAi}, Dmef2Gal4>Park^{RNAi}, Dmef2Gal4>GSTS1^{RNAi}, Dmef2Gal4>Trxr-1^{RNAi}, Dmef2Gal4>Sod1^{RNAi}, Dmef2Gal4>Sod2^{RNAi} and Dmef2Gal4>catalase^{RNAi} thoraxes of flies aged for 72 hours after eclosure to determine the integrity of the OXPHOS complexes. Lanes marked with an asterisk denote instances where assembly of several OXPHOS complexes were impaired.
(B) BN-PAGE showing mitochondrial protein complexes from mhc>w1118 (wild-type) thoraxes of flies aged for 24 hours; and starved or maintained at 25C, 30C or 37C for 18 or 36 hours. Note that there were no overt alterations in assembly of the OXPHOS complexes.