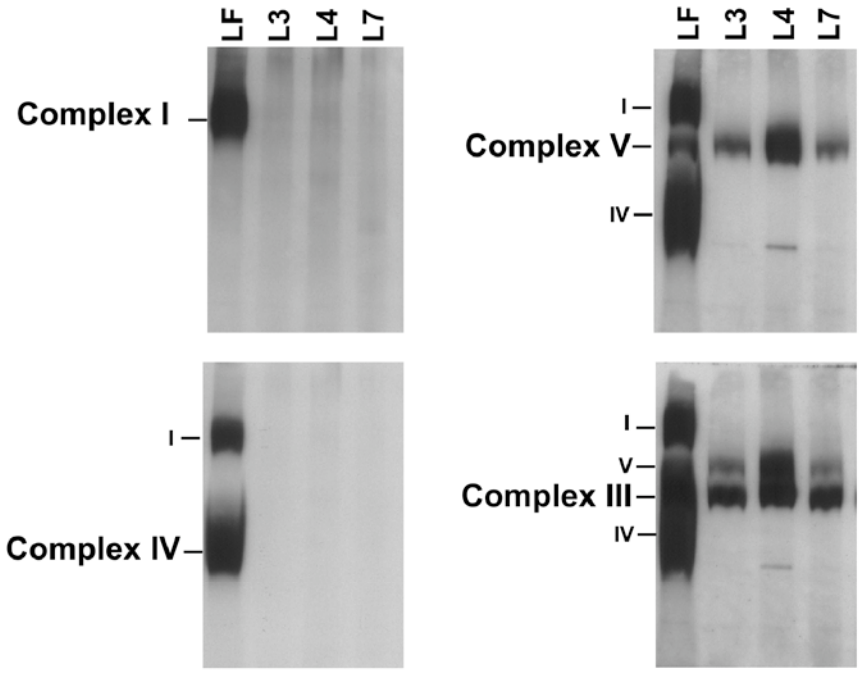


Fig. S1. Cyt c dKO cells lack OXPHOS complexes I and IV. Samples were resolved on a 4-13% blue-native PAGE gel. The proteins were transferred onto PVDF membrane and probed with antibodies raised against the different complex subunits, namely, Ndufa9 (complex I), Core2 (complex III), COXI (complex IV), and ATPase-≤ (complex V). Cyt c dKO cells (L3, L4, and L7) lack complexes I and IV.

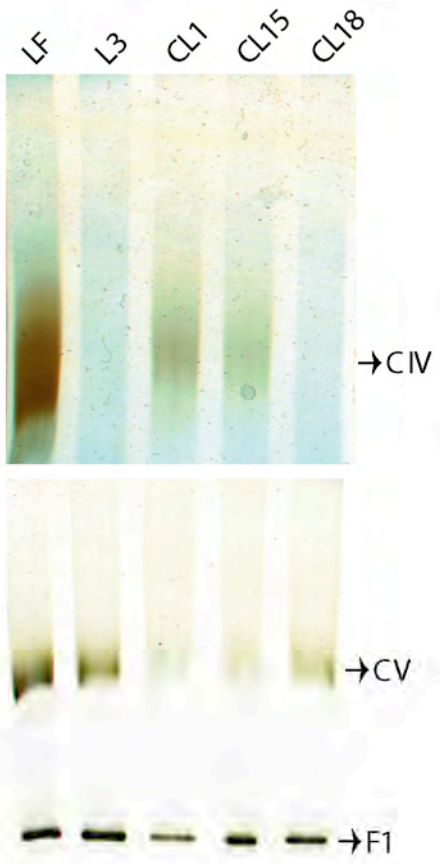


Fig. S2. Cyt c- dKO cells lack OXPHOS complex IV activity. Samples were resolved on a 4-13% blue-native PAGE gel and stained in-gel for the different complex activities as described in the methods. Cyt c dKO cells (L3) and those reintroduced with the W62S mutant cyt c cDNA (CL18) lack complex IV activity. CIV and CV are complexes IV and V, respectively. F1 is F1 subunit of complex V.

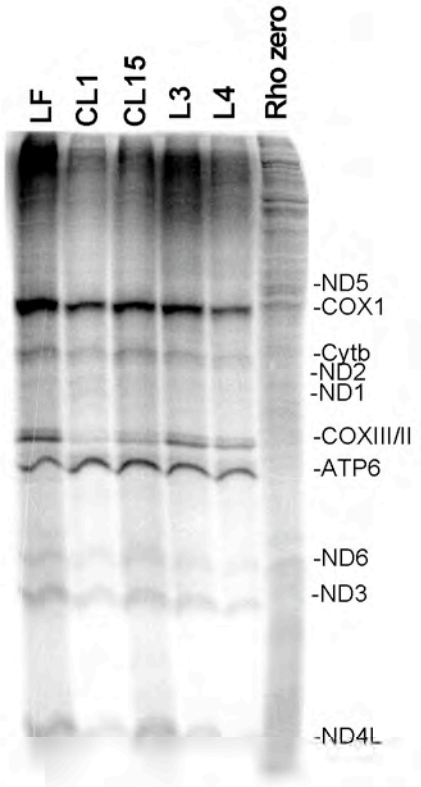
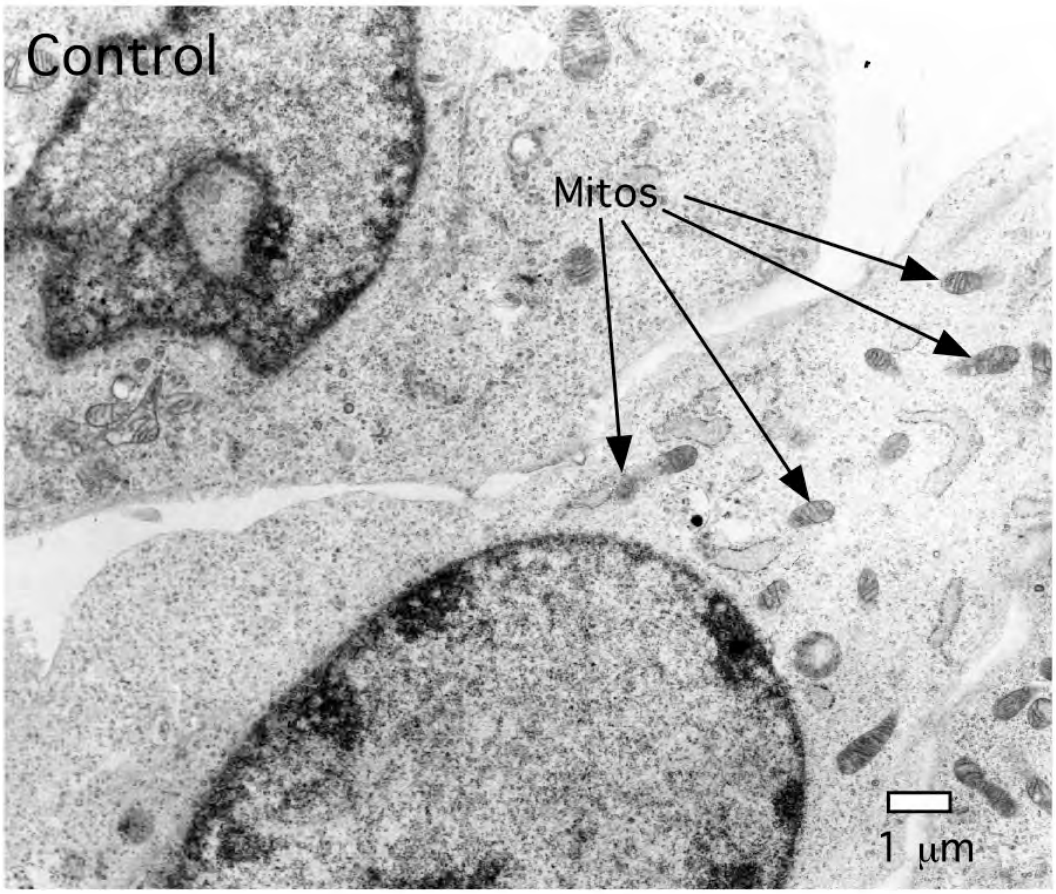


Fig.S3. Cyt c dKO cells synthesize the mitochondrial encoded OXPHOS subunits. Cells were pulse labeled with [35S]-methionine and cysteine, lysed and resolved on an SDS-PAGE. Except rho zero cells, which lack mtDNA, all the other cells efficiently synthesized the mitochondrial proteins.



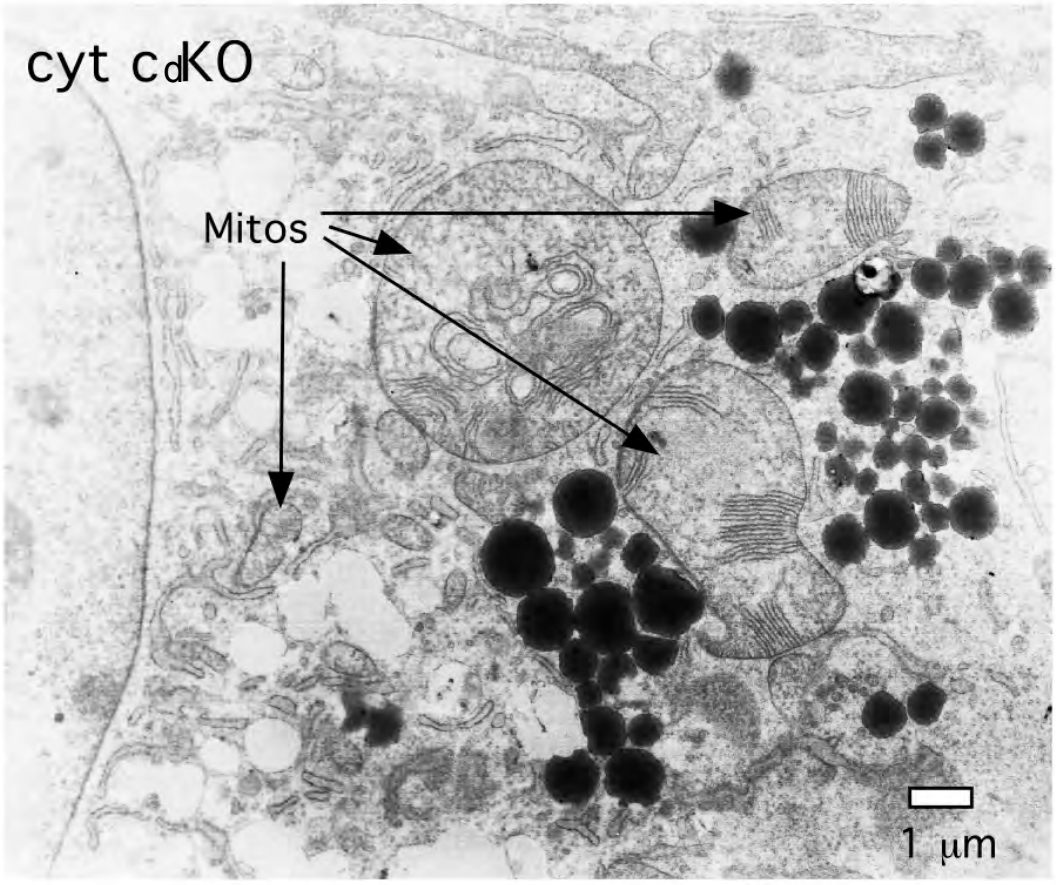


Fig. S4. Cyt c ablated cells have abnormal mitochondria. Cultured cells were fixed and processed for transmission electron microscopy. Arrows indicate the mitochondria (Mitos), which are enlarged and have abnormal cristae in the cyt c dKO cells.

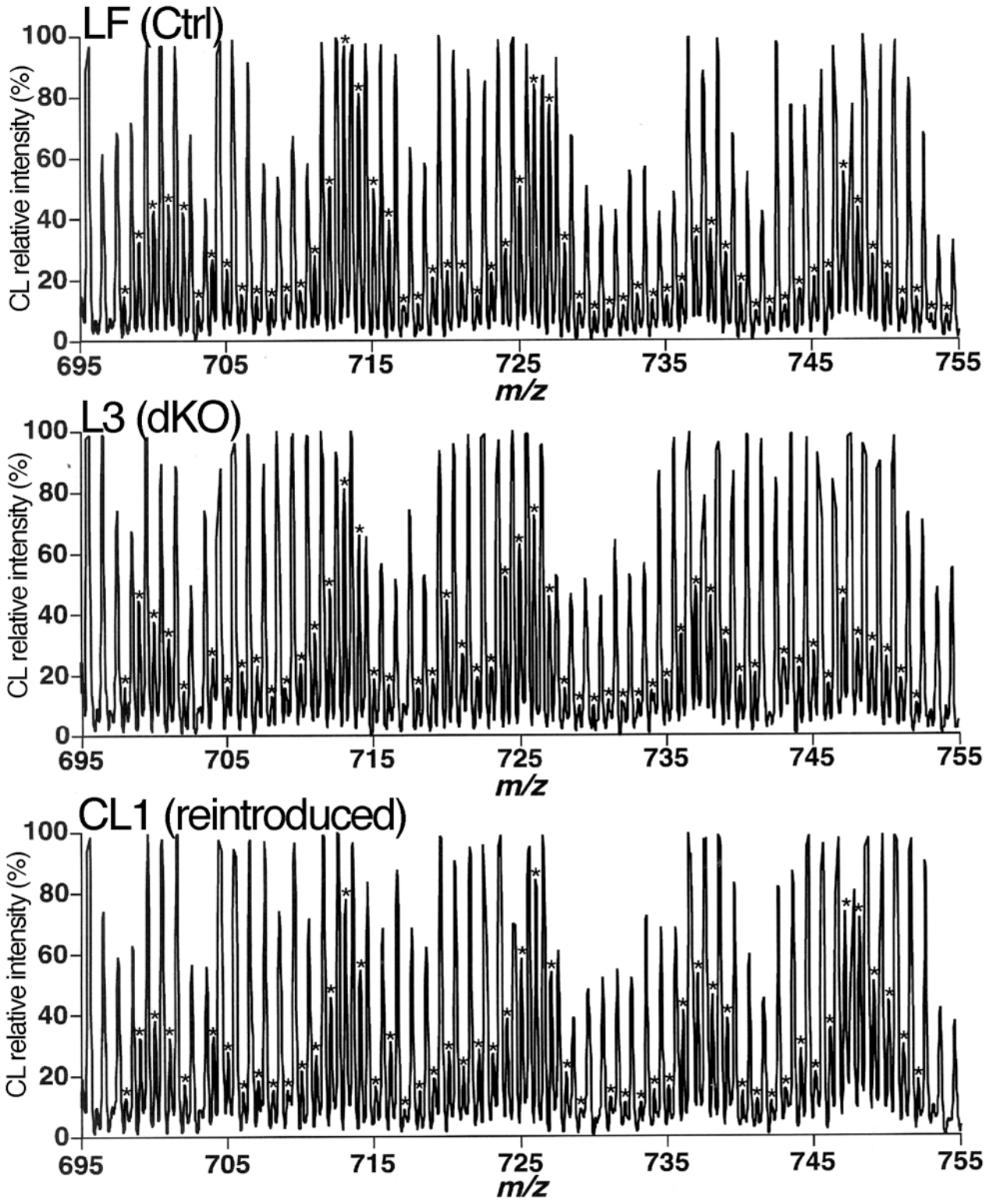


Fig. S5A. Cardiolipin composition of mitochondria lacking cyt c. Expanded negative-ion ESI mass spectra of cell lipid extracts. Lipid extracts of LF, L3, and CL1 were prepared by a modified Bligh and Dyer procedure and electrospray ionization mass spectra were acquired in the negative-ion mode by using a triple quadrupole mass spectrometer. The asterisks indicate the identified cardiolipin plus-one isotopologues which were characteristic of the doubly charged cardiolipin molecular species and were utilized to quantify individual cardiolipin molecular species based upon ion intensity. Each spectrum is displayed after being normalized to the internal standard for quantitation of cardiolipin molecular species.

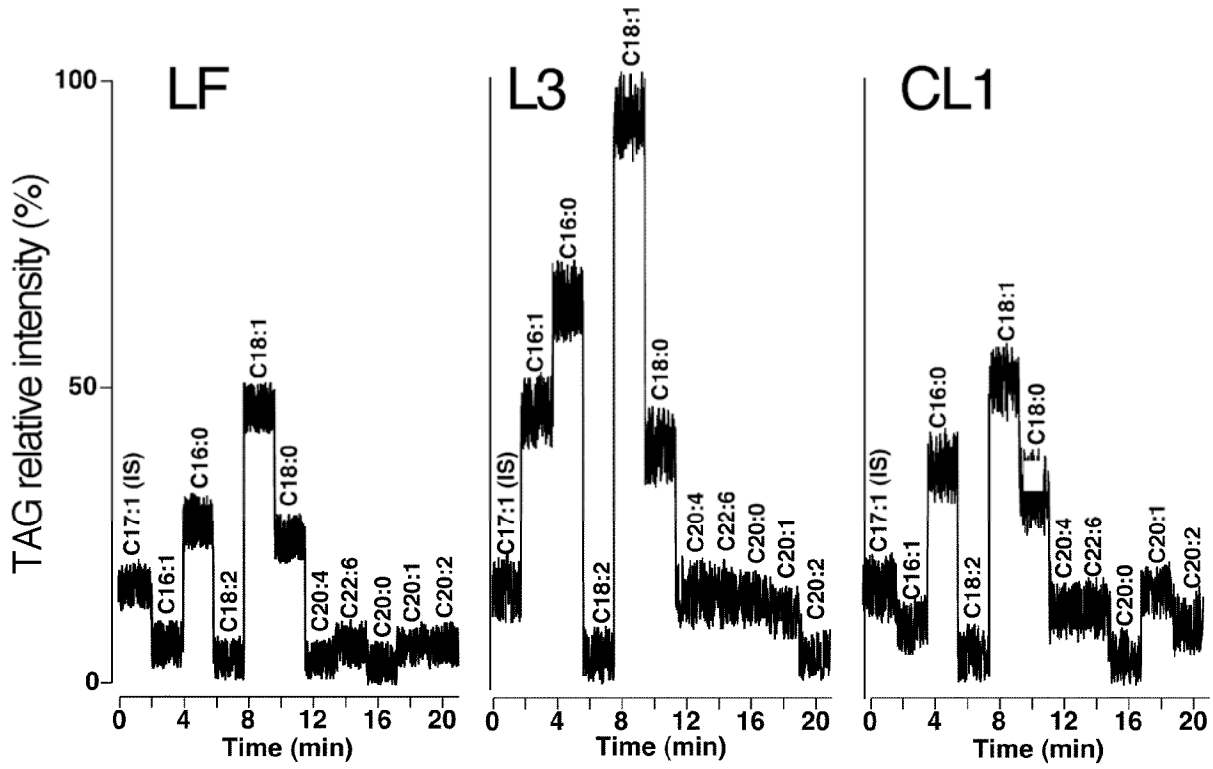


Fig. S5B. Determination of triacykglecerols (TAG). Total ion current chromatograms by stepwise scanning of naturally-occurring fatty acids neutrally lost from TAG molecular species in cell lipid extracts. Lipid extracts of LF, L3, and CL1 were prepared by a modified Bligh and Dyer procedure and were analyzed in the positive-ion mode after infusion of the diluted lipid extract in the presence of a small amount of LiOH at a flow rate of 4 ㎕/min. The stepwise neutral loss (NL) scanning was performed by a sequential and customized program operating under Xcalibur software. Each segment of individual NL scanning was taken for 2 min in the profile mode. For tandem mass spectrometry in the NL mode, both the first and third quadrupoles were coordinately scanned with a mass difference (i.e., neutral loss) corresponding to the neutral loss of a non-esterified fatty acid as indicated from triacylglycerol molecular species, while collisional activation was performed in the second quadrupole. The chromatograms were displayed after being normalized to the total ion current of C17:1 FA, the internal standard for quantitation of individual triacylglycerol molecular species.