Supporting Information

Real-time observation of conformational changes and translocation of endogenous cytochrome c within intact mitochondria

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Table of Contents

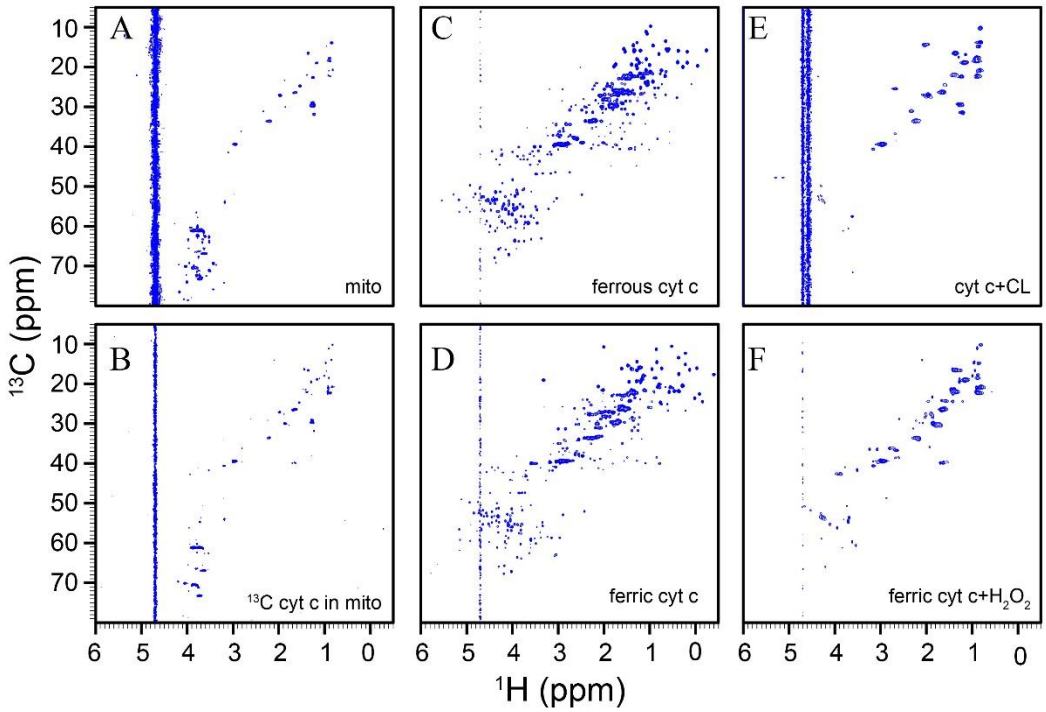

Figure S1. Full ^{1}H-^{13}C HSQC spectra of living mitochondria and cyt c under various conditions. A and B are spectra of living mitochondria without and with ^{13}C-labeled cyt c, respectively. C and D are spectra of ferrous and ferric cyt c at 0.1 mM concentration, respectively. E and F are spectra of 0.1 mM cyt c in the presence of 0.5 mM CL liposome and 0.5 mM H_2O_2, respectively.

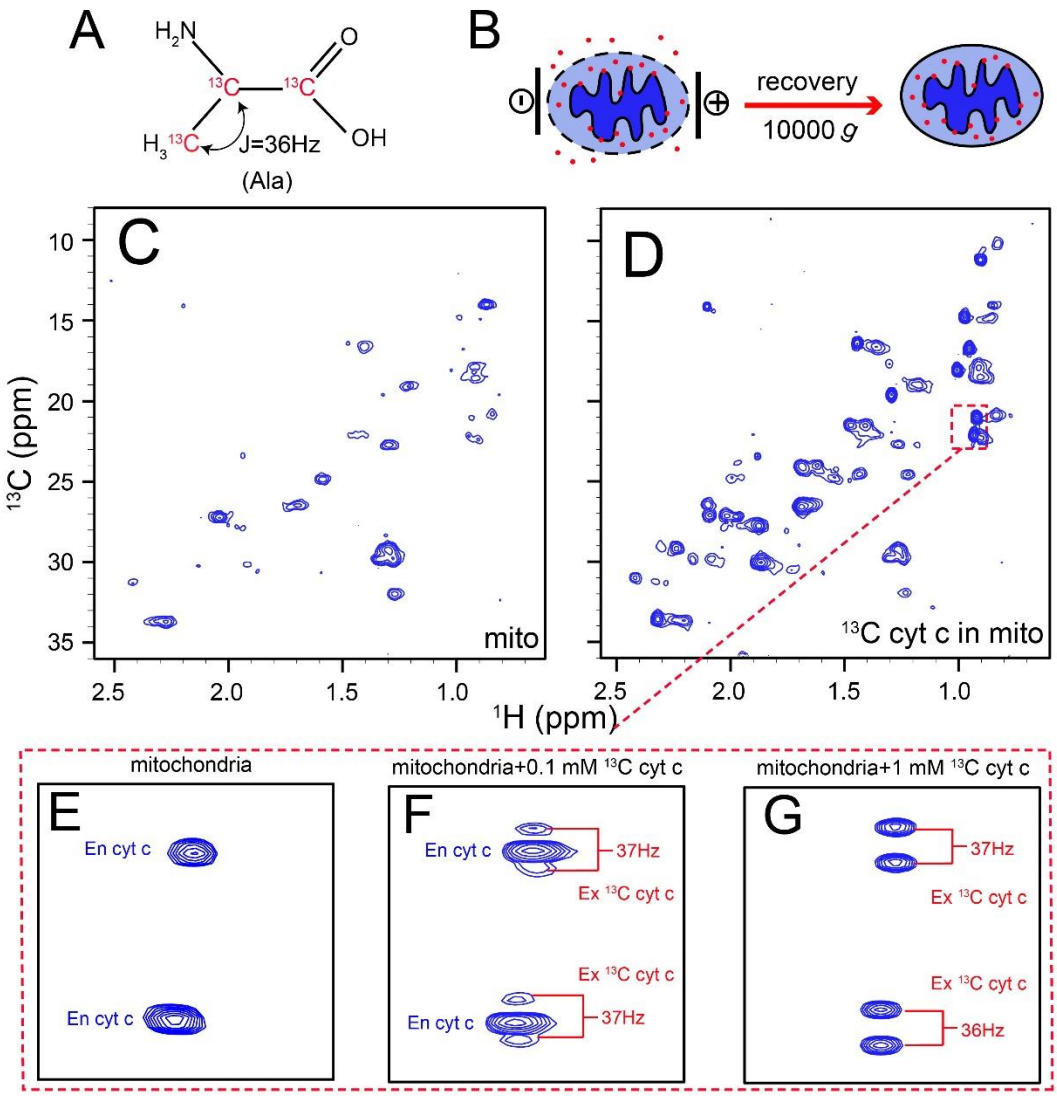

Figure S2. Homonuclear J-coupling split in the ^{1}H-^{13}C HSQC spectrum of ^{13}C labeled cyt c in living mitochondria. A. Diagram to illustrate the homonuclear $^{2}J_{C,C}$ coupling between adjacent ^{13}C atoms in alanine. **B.** Diagram of electroporation of exogenous cyt c to mitochondria. **C.** ^{1}H-^{13}C HSQC spectrum of mitochondria. **D.** ^{1}H-^{13}C HSQC spectrum of mitochondria with electroporation of ^{13}C labeled cyt c. **E-G** High-resolution and enlarged ^{1}H-^{13}C HSQC spectrum of mitochondria with electroporation of 0, 0.05 Mm, and 0.5 mM ^{13}C labeled cyt c.

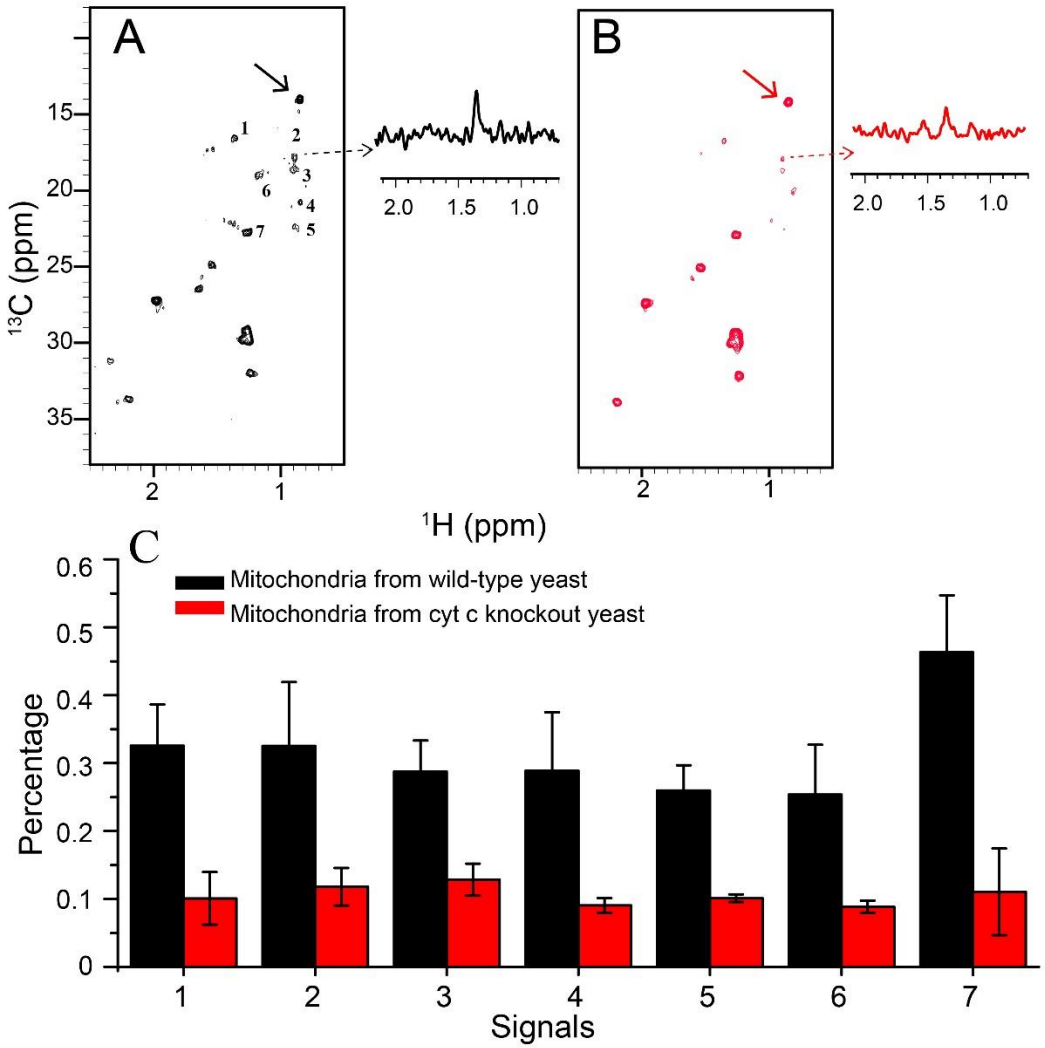

Figure S3. ^{1}H-^{13}C HSQC spectra of mitochondria extracted from wild-type and cyt c gene knockout yeast. A. ^{1}H-^{13}C HSQC spectra of mitochondria from wild-type yeast and the slice of one of signals of cyt c. **B.** ^{1}H-^{13}C HSQC spectra of mitochondria from cyt c knockout yeast and the slice of the same signal of cyt c. **C.** Comparison between signal intensities of cyt c in A and B. To eliminate the influence of concentration difference, a resonance of CL shown in both A and B (marked with an arrow) was chosen, and its intensities in A and B were used as inner references, respectively. Each column represents the ratio of one signal intensity of cyt c to the signal intensity of CL in the same spectrum. 7 signals of cyt c were compared, which were both shown in A and B and above the noise level (labeled in A).

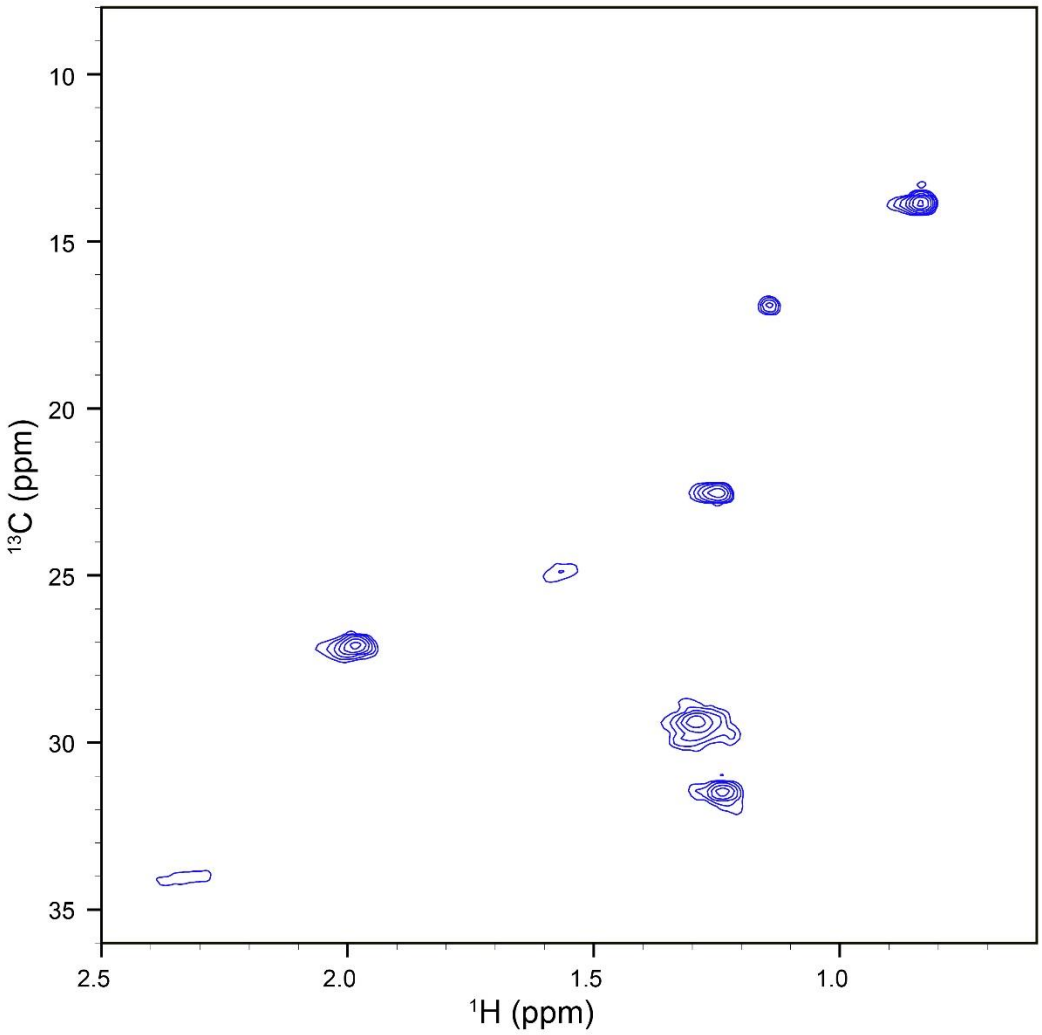

Figure S4. ^{1}H-^{13}C HSQC spectrum of 0.5 mM CL.

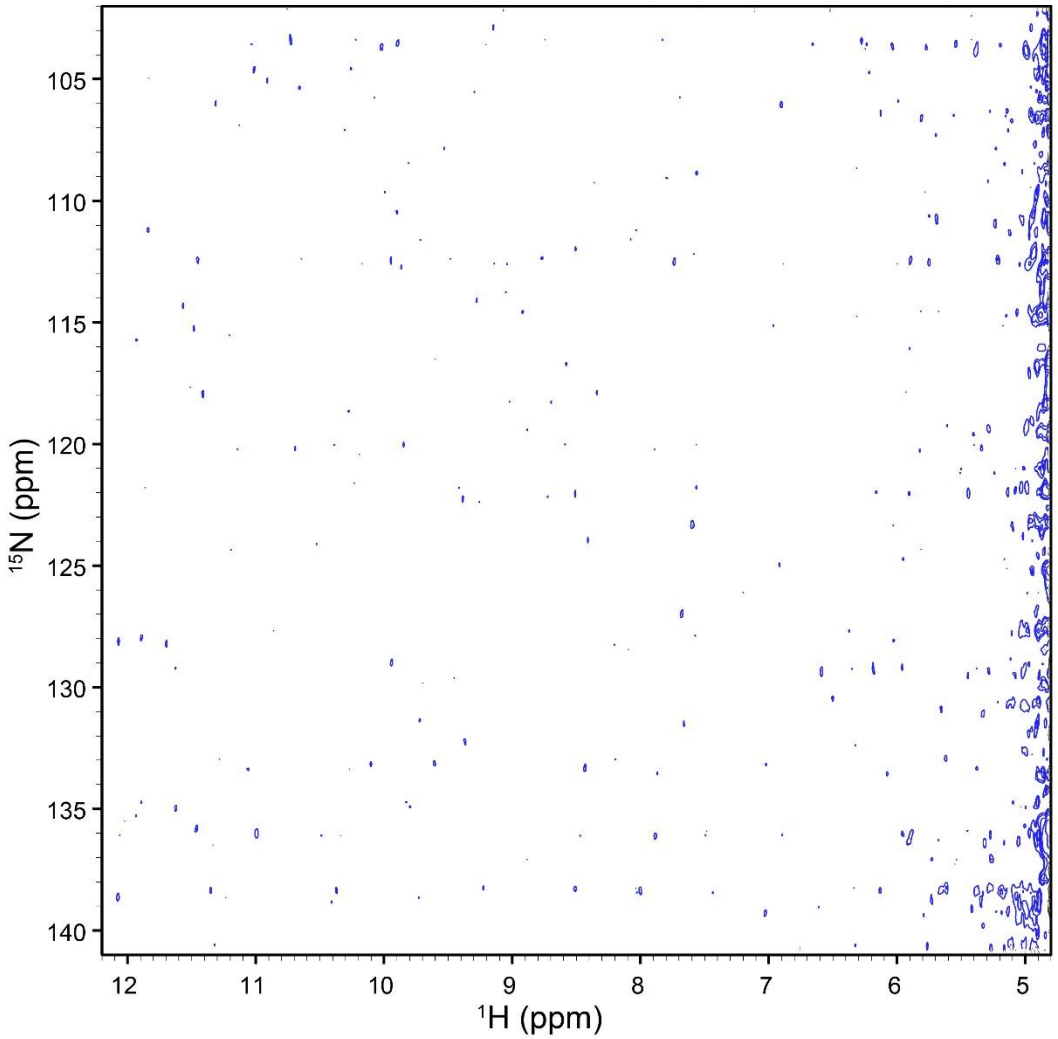

Figure S5 ^{1}H-^{15}N HSQC spectrum of the mitochondria with electroporation of ^{15}N labeled cyt c.

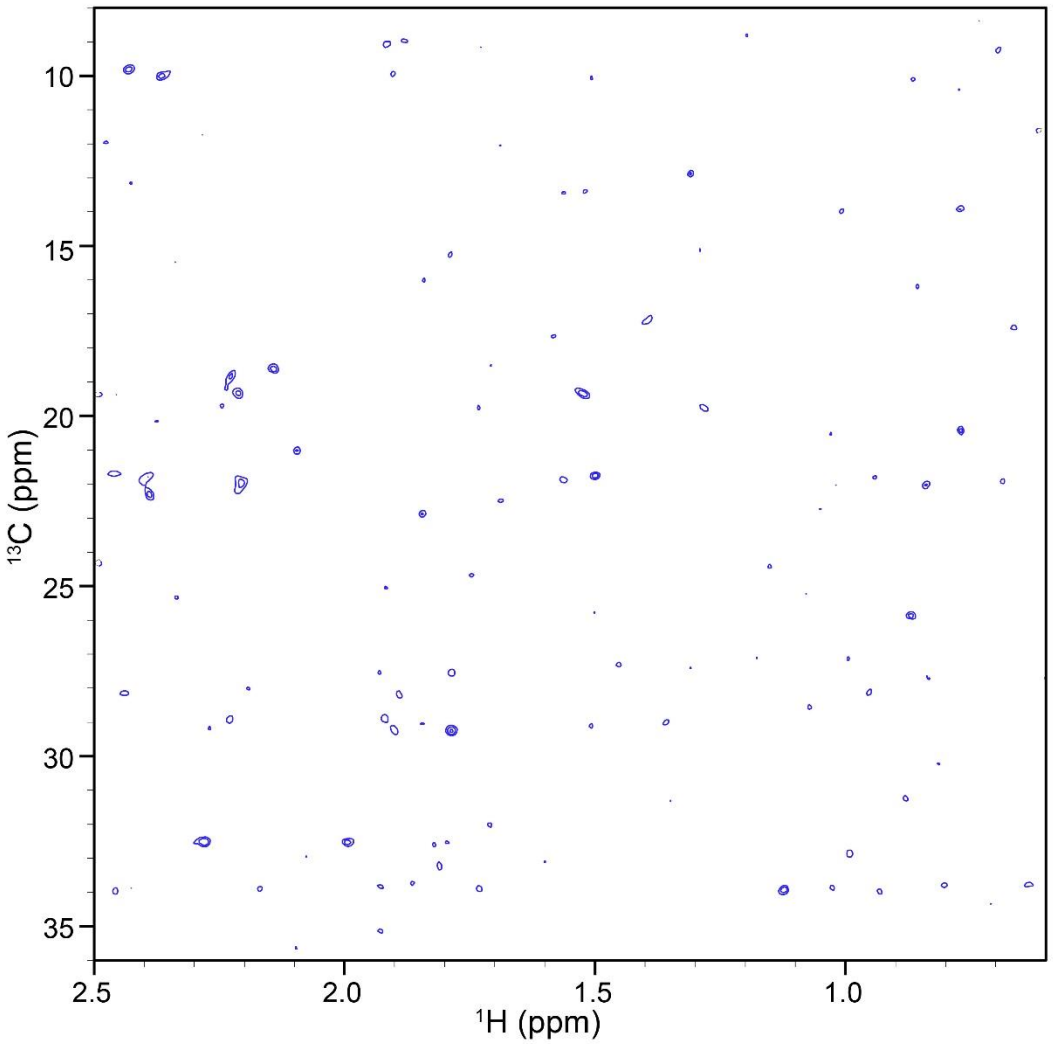

Figure S6. ^{1}H-^{13}C HSQC spectrum of the centrifuged supernatant from mitochondria after the NMR experiment.

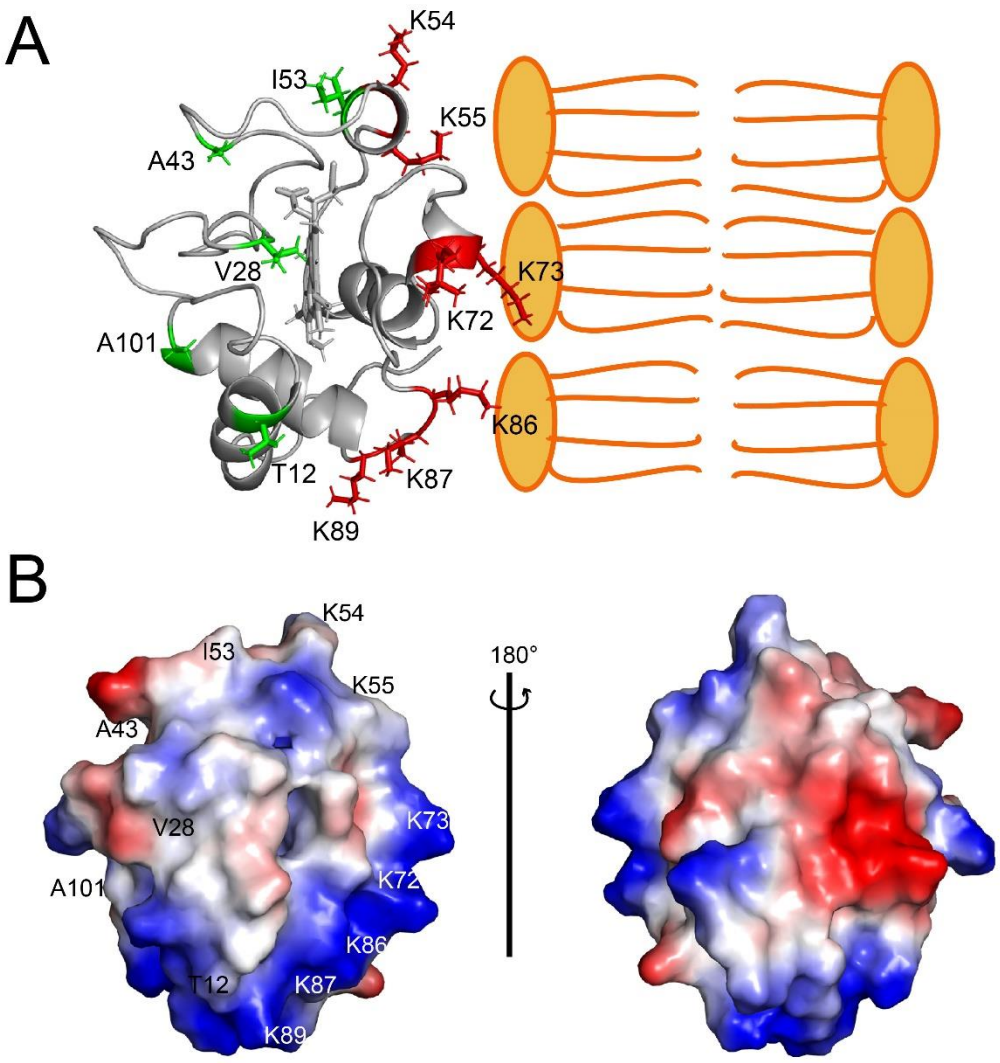

Figure S7 Schematic representation of cyt c binds to CL (PDB ID: 1YIC). A. The residues of cyt c in mitochondria observed by NMR, such as V28 and A43, are mainly located in the region away from the positive charged regains around K72, K73, K86, and K87 which are commonly assigned as constituents of the A site that involves CL binding, nearby lysines K54, K55, and K89 are also shown. **B.** The surface charge of cyt c.

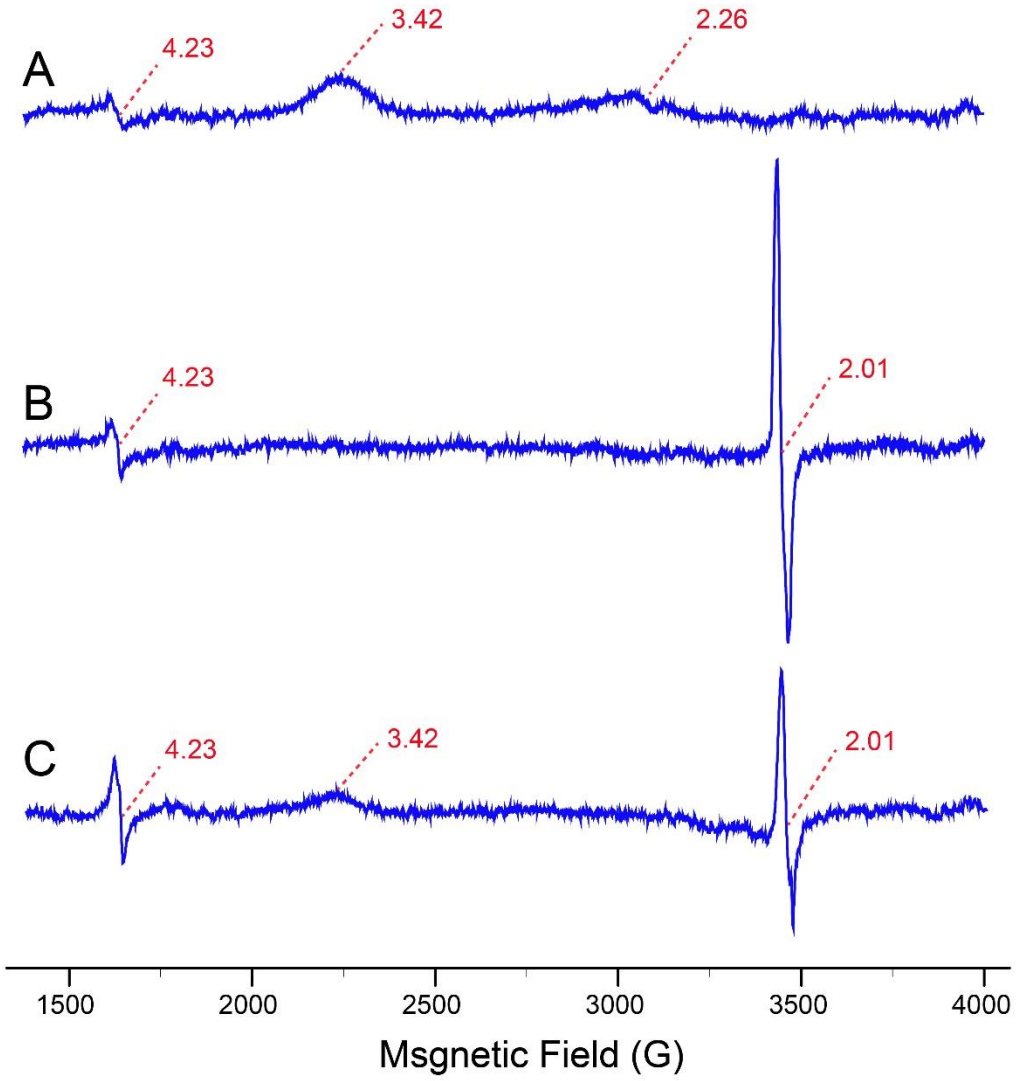

Figure S8. EPR spectra of cyt c and mitochondria. A. The EPR spectrum of 0.5 mM cyt c in the presence of 1 mM H_2O_2. **B.** The EPR spectrum of isolated mitochondria. **C.** The spectrum of mitochondria after electroporation of 0.5 mM cyt c. Signals from ferric iron of cyt c along with free radical (g=2.01) are detected within mitochondria.

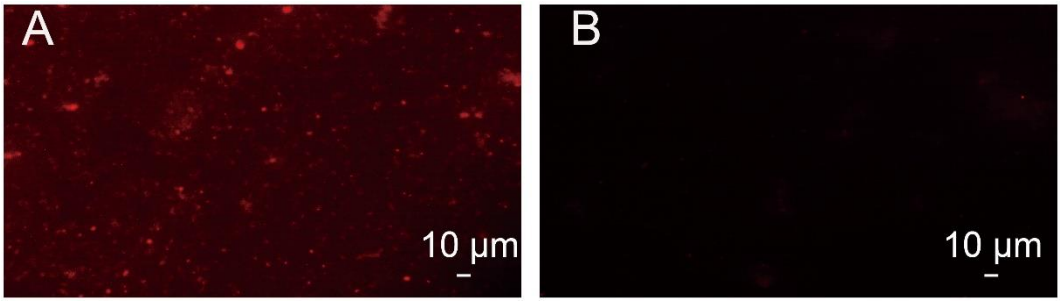

Figure S9 MitoTracker Red CMXRos stained mitochondria. A. Normal mitochondria. **B.** Mitochondria treated with digitonin.

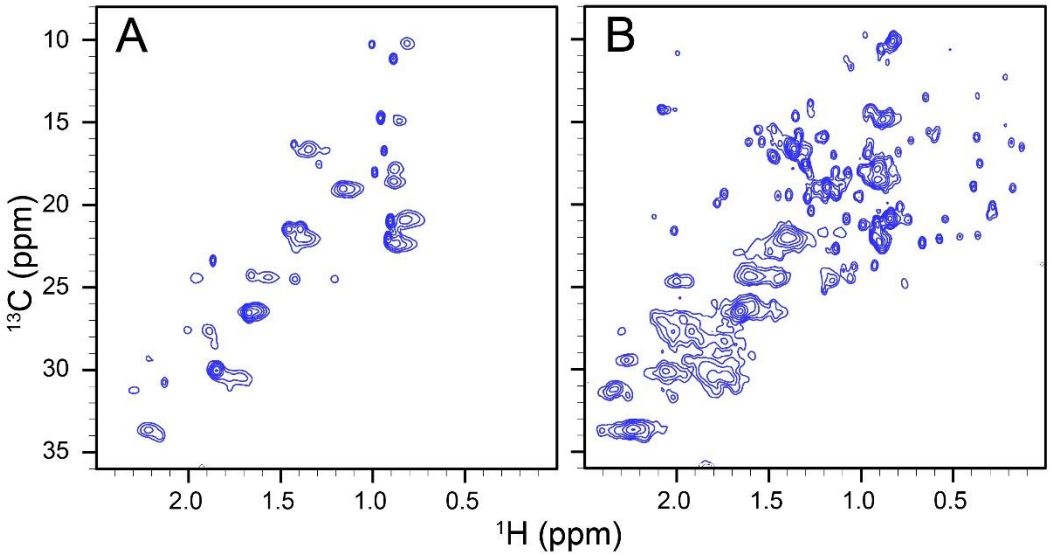

Figure S10. The ^{1}H-^{13}C HSQC spectra of cyt c under different environments. A. the spectrum of partially unfolded cyt c induced by H_2O_2 with the addition of 150 mM NaCl. **B.** the spectrum of partially unfolded cyt c induced by CL liposome with the addition of 150 mM NaCl.

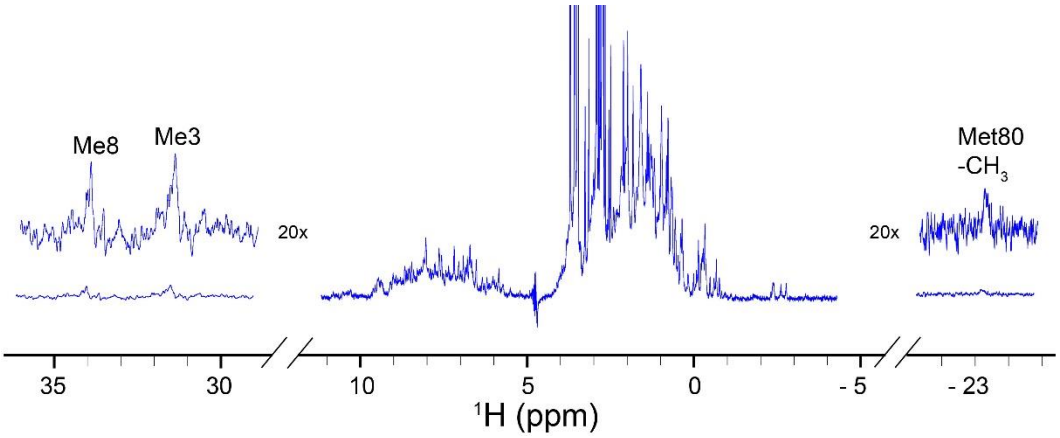

Figure S11. ^{1}H spectra of native cyt c extracted from inner mitochondrial membrane. Me8 and Me3 are two different methyl groups on the heme of cyt c. When cyt c is in an oxidized state, their NMR chemical shifts are specially around 30 - 35 ppm due to the paramagnetic effect of Fe^{3+}. Met80-CH_3 is the methyl group on the side chain of Met80 of cyt c. When cyt c is in an oxidized state, the NMR chemical shift of it is specially around -23 ppm due to the paramagnetic effect of Fe^{3+}. "20x" represents a signal amplification of 20 times.

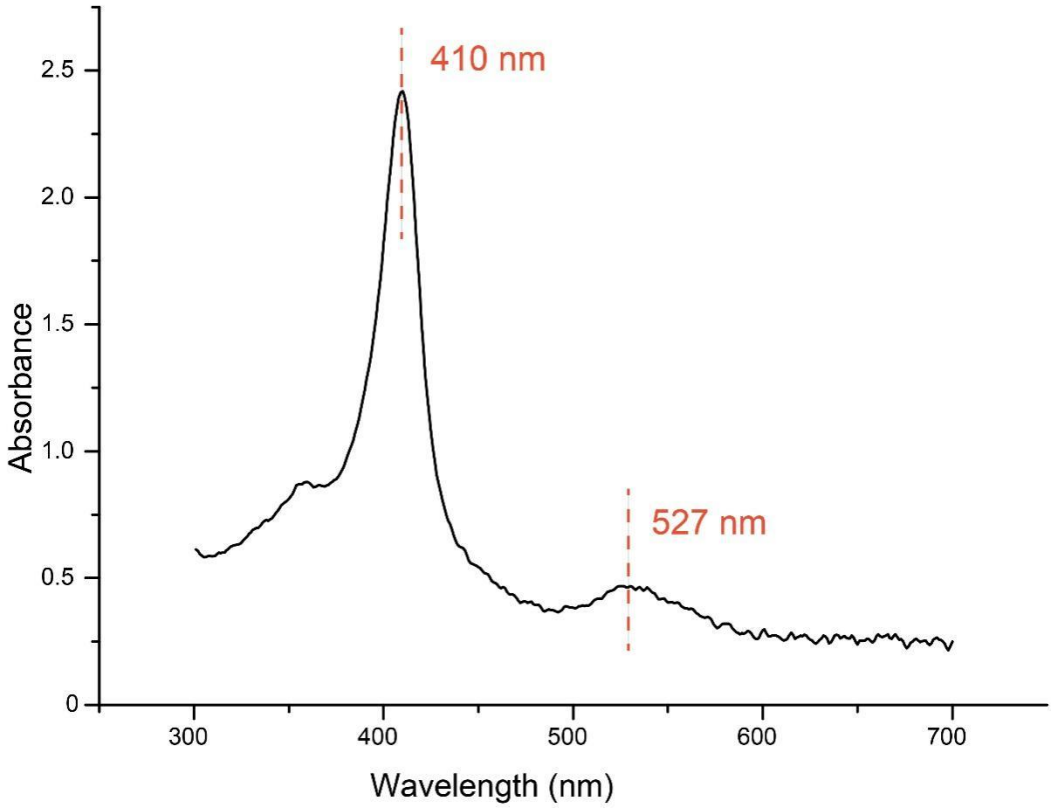

Figure S12. UV-visible spectrum of native cyt c extracted from inner mitochondrial membrane.

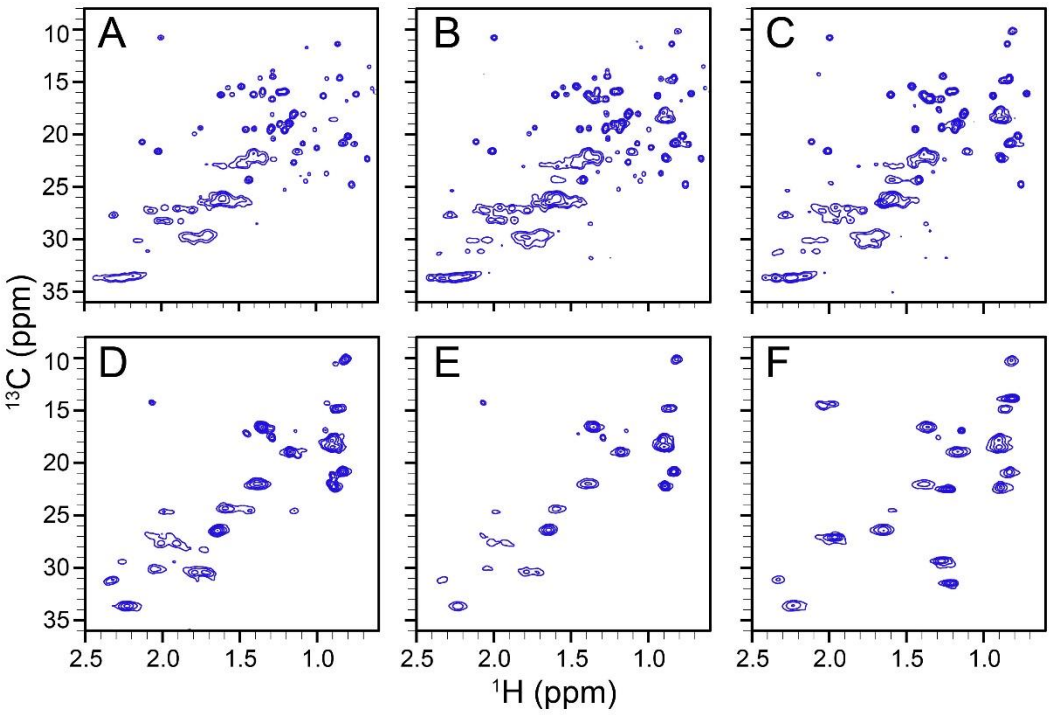

Figure S13 ^{1}H-^{13}C HSQC spectra of native ferric cyt c in addition to different concentrations of CL liposome. A. cyt c: CL=1:0. B. cyt c: CL=1:2. C. cyt c: CL=1:3. D. cyt c: CL=1:4. E. cyt c: CL=1:10. F. cyt c: CL=1:32.

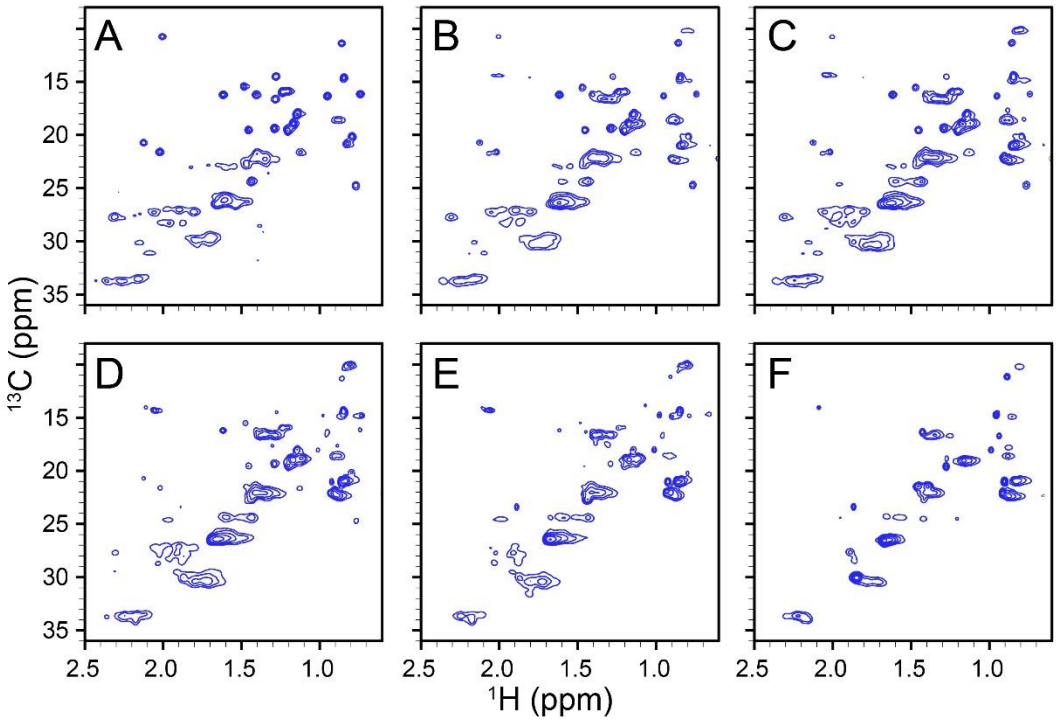

Figure S14 ^{1}H-^{13}C HSQC spectra of native ferric cyt c in addition to different concentrations of H_2O_2. A. cyt c: H_2O_2=1:0. B. cyt c: H_2O_2=1:1. C. cyt c: H_2O_2=1:2. D. cyt c: H_2O_2=1:3. E. cyt c: H_2O_2=1:4. F. cyt c: H_2O_2=1:5.

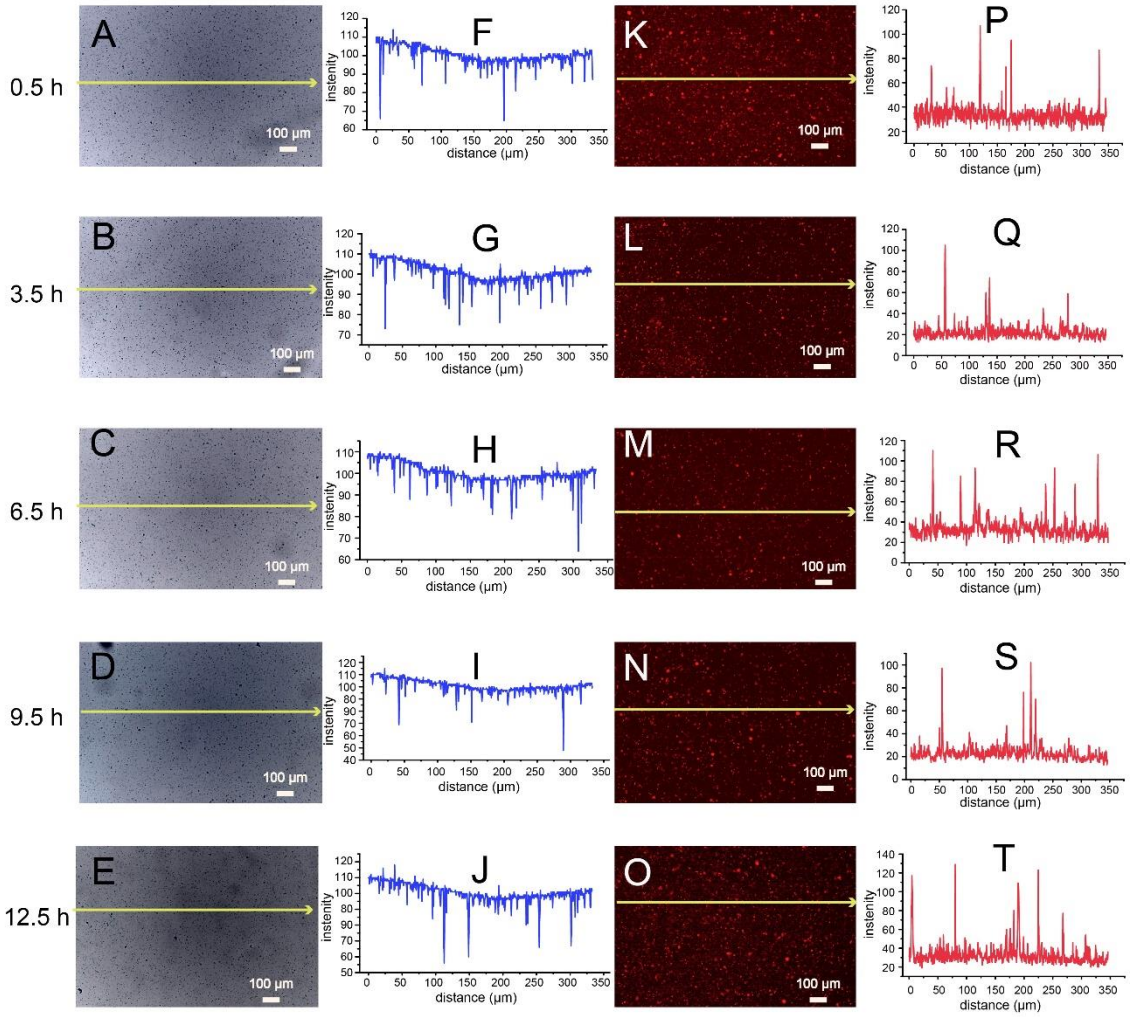

Figure S15. Jenners green B and MitoTracker Red CMXRos stained mitochondria at different time points. A - E are images of mitochondria stained with Janus Green B at different time points. F - J are profile intensity curves corresponding to the staining points along the yellow lines from left to right in A - E, respectively. In the plots, and the downward depth of each peak represents the degree of mitochondrial staining on this profile, the intensity of each peak (y-axis) is the absorbance of Jenners green B, which reflects the degree of mitochondrial staining on this profile. The lower reading of the intensity, the stronger staining. K - O are fluorescence images of mitochondria stained with MitoTracker Red CMXRos at different time points. P - T are profile intensity curves corresponding to the staining points along the yellow lines from left to right in K - O, respectively. In these plots, the intensity of each peak (y-axis) is the fluorescence of MitoTracker Red CMXRos dye, which represents the degree of mitochondrial staining on this profile, the higher reading of the intensity, the stronger staining. The position of the yellow line is random.

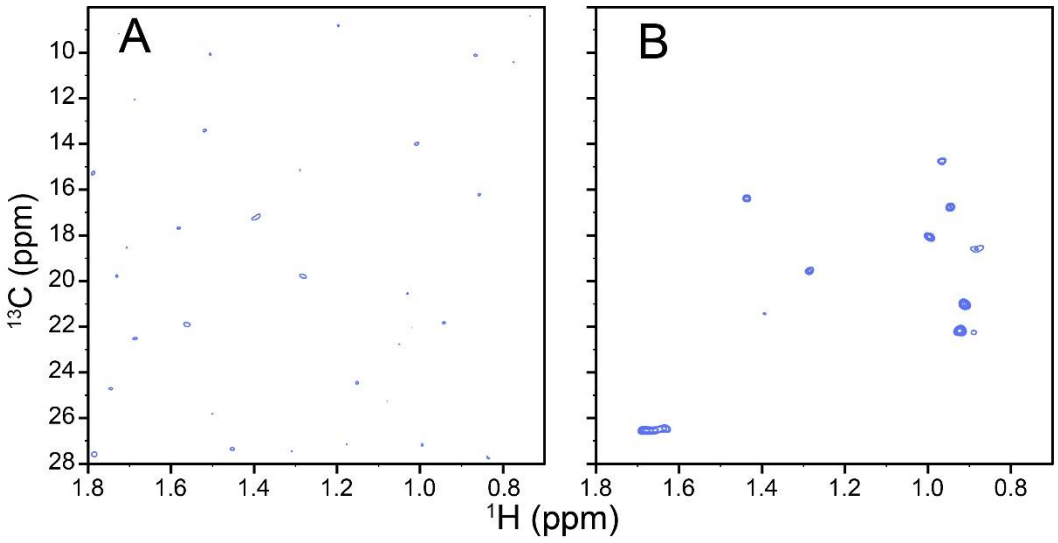

Figure S16 ^{1}H-^{13}C HSQC spectra of the centrifuged supernatant from mitochondria. A. The mitochondria were left at room temperature for 2 hours; B. The mitochondria were left at room temperature over 12 hours.