Supporting Information

Real-Time Monitoring ATP in Mitochondrion of Living Cells: a Specific Fluorescent Probe for ATP by Dual Recognition Sites

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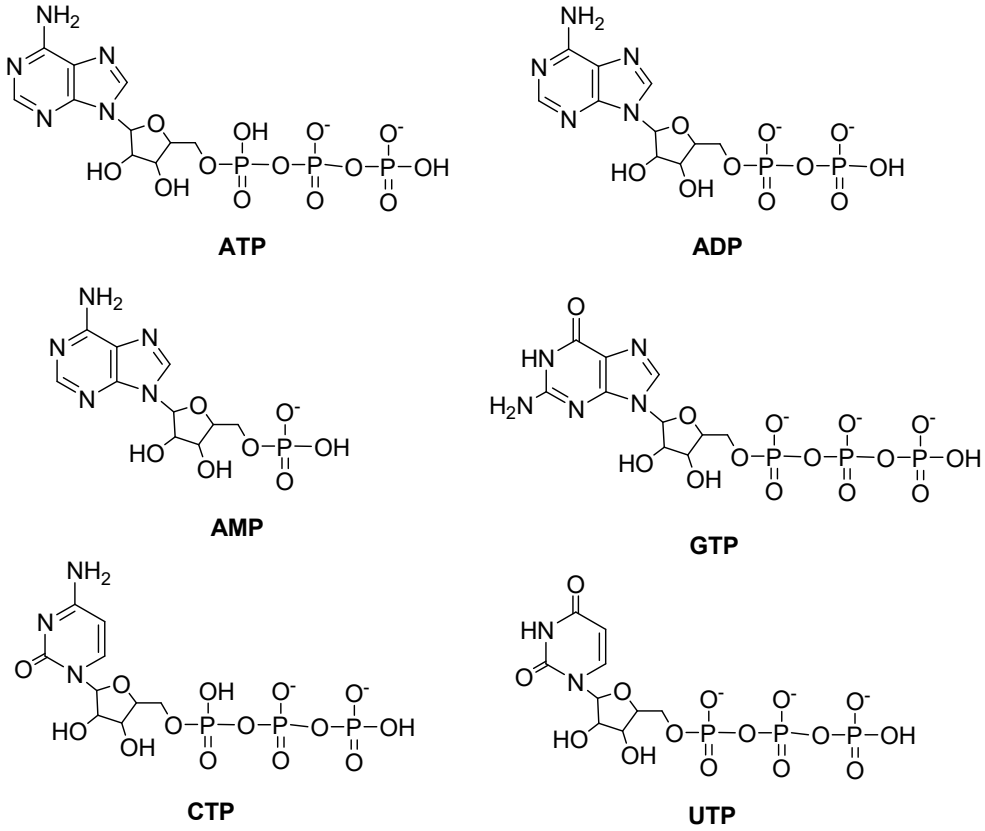

Figure S1. The chemical structures of organic phosphate anions.

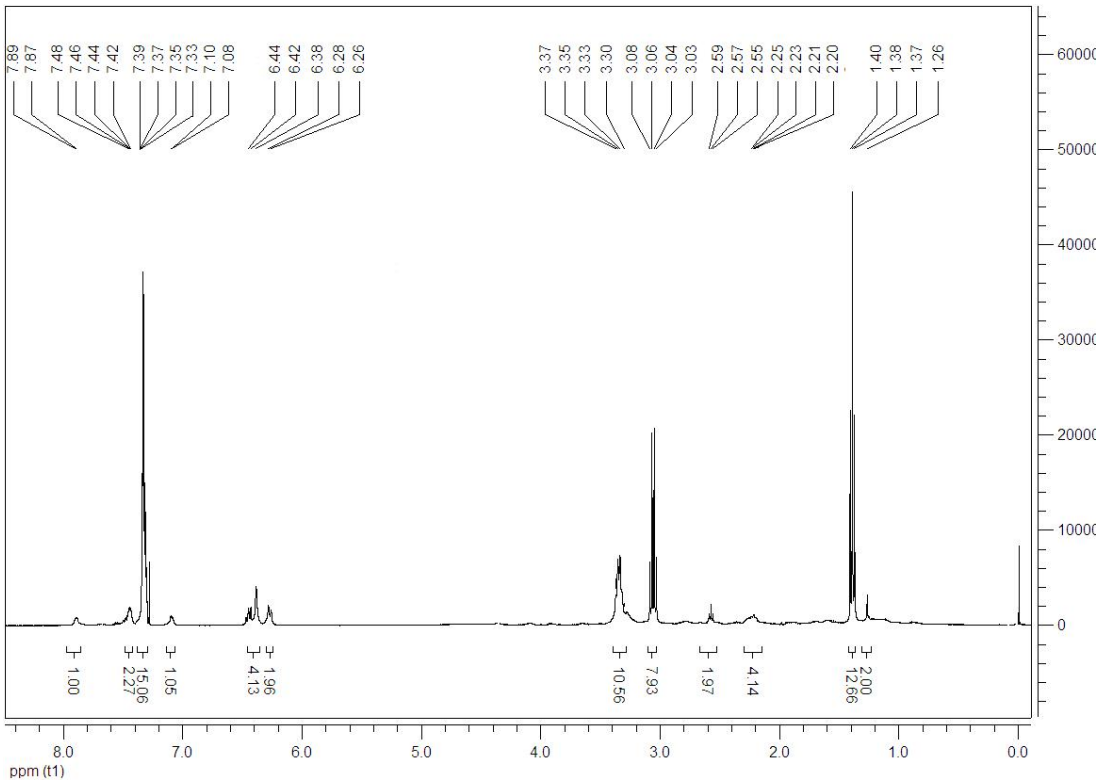

Figure S2. ^{1}H NMR spectra of Mito-Rh in $CDCl_3$.

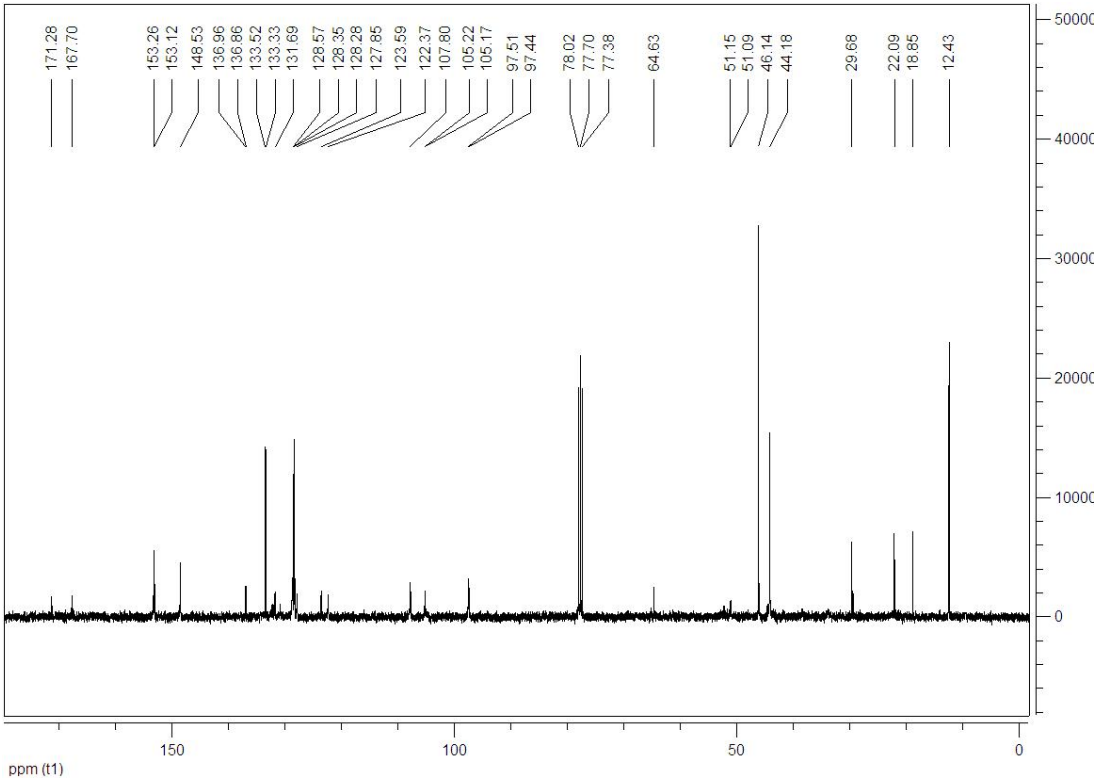

Figure S3. ^{13}C NMR spectra of Mito-Rh in $CDCl_3$.

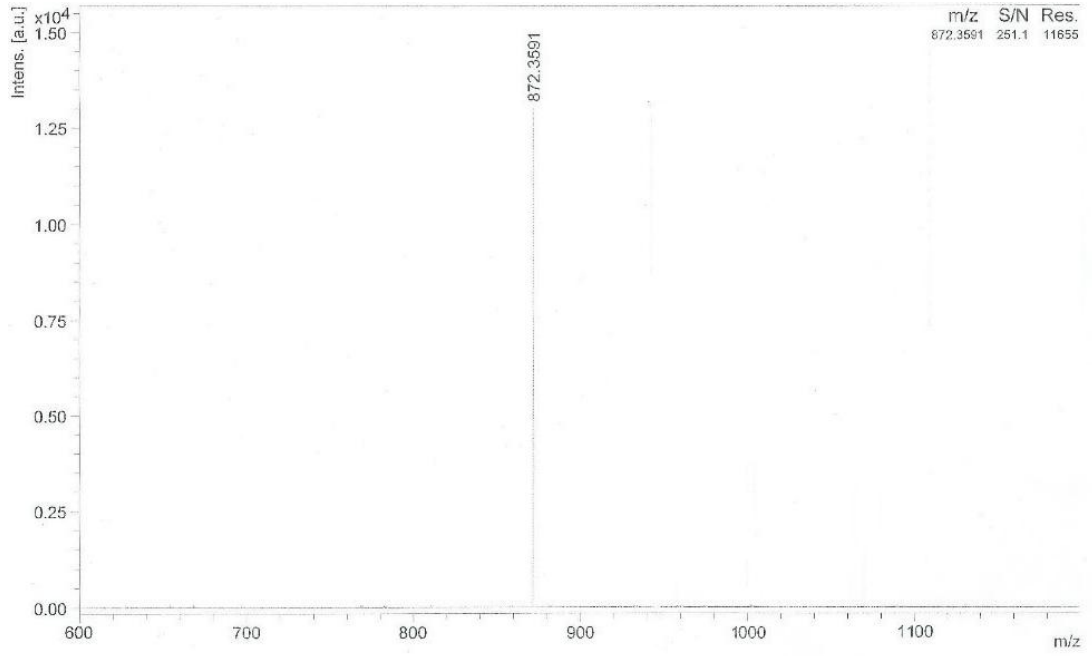

Figure S4. Mass spectra of Mito-Rh.

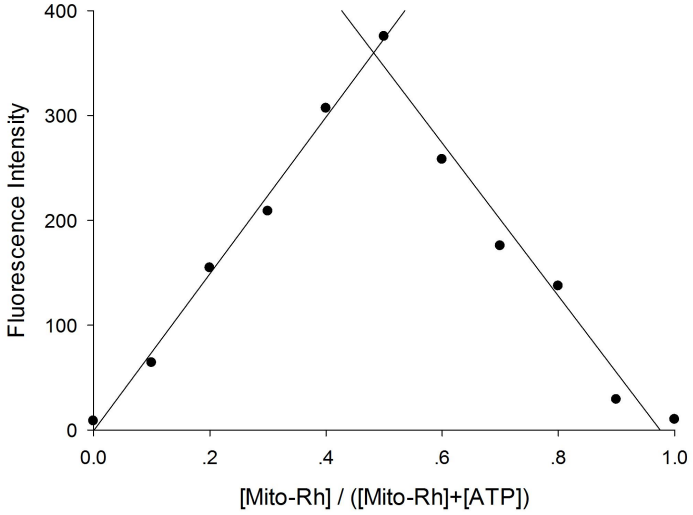

Figure S5. The Job plot of Mito-Rh and ATP using fluorescence intensity at 583 nm in PBS buffer solution of pH 7.4 with an excitation at 520 nm. The total concentration of [Mito-Rh] and [ATP] is 10 mM.

The binding constant (K) between the probe and ATP is determined by the Benesie-Hildebrand equation (1).

$$\frac{1}{F-F_0}=\frac{1}{K(F_{\max}-F_0)\times[ATP]}+\frac{1}{F_{\max}-F_0} \qquad (1)$$

Where F is the fluorescence intensity at 583 nm at any given ATP concentration, F_0 is the fluorescence intensity at 583 nm in the absence of ATP, and F_{max} is the maximal fluorescence intensity at 583 nm in the presence of ATP. Benesie-Hildebrand plot is shown in Figure S6. The K value between the probe and ATP is obtained from the slope of the straight line and is determined to be 215.6 M^{-1}.

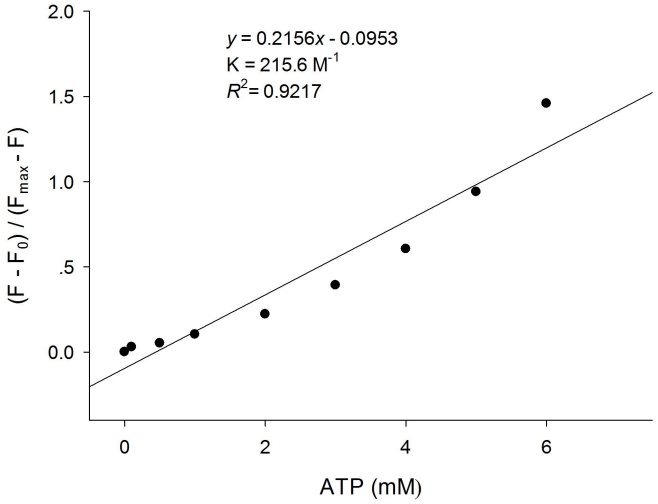

Figure S6. Benesie-Hildebrand plot of Mito-Rh-ATP complex in PBS buffered solution of pH 7.4.

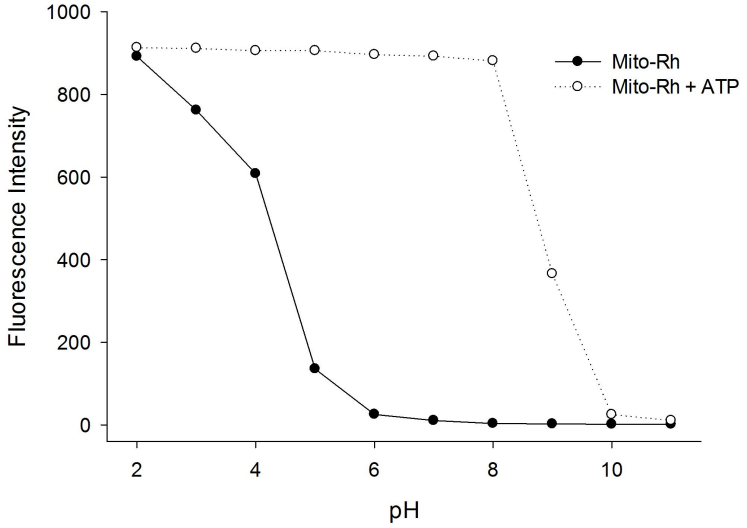

Figure S7. Effect of pH on the fluorescence intensity of Mito-Rh (10 μM) in the absence of ATP (solid line) and in the presence of ATP (10 mM, dotted line).

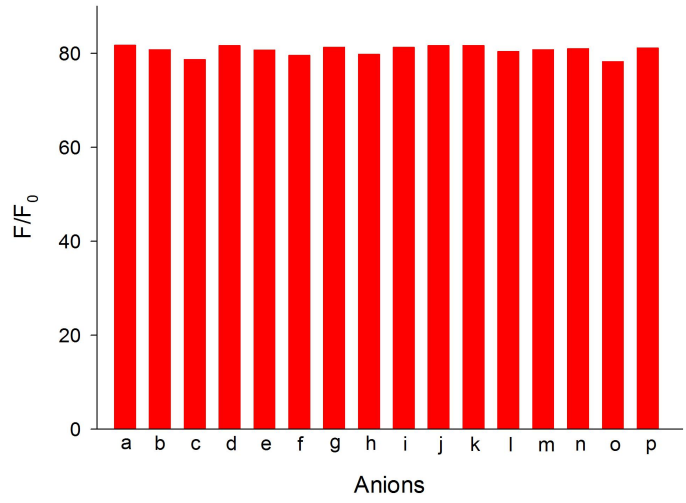

Figure S8. Fluorescence response of Mito-Rh (10 μM) for 10 mM of other anions at the present of ATP (10 mM) in the PBS buffer solution. Anions (from a to p): ATP, ADP, AMP, GTP, CTP, UTP, $P_3O_{10}^{5-}$, $P_2O_7^{4-}$, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}, Cl^-, SO_4^{2-}, NO_3^-, $CH_3CO_2^-$ and CO_3^{2-}.

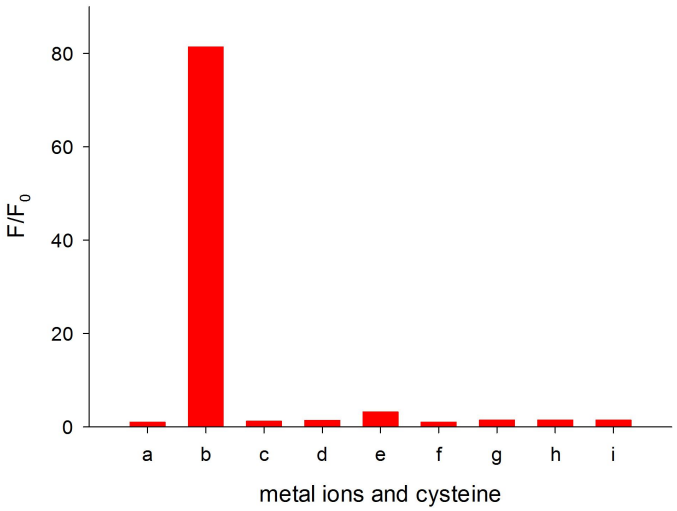

Figure S9. Fluorescence response of Mito-Rh (10 μM) for 10 mM of ATP or 10 mM of metal ions and cysteine in the PBS buffer solution. Metal ions and cysteine (from a to i): blank, ATP, cysteine, Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, Na^+ and K^+.

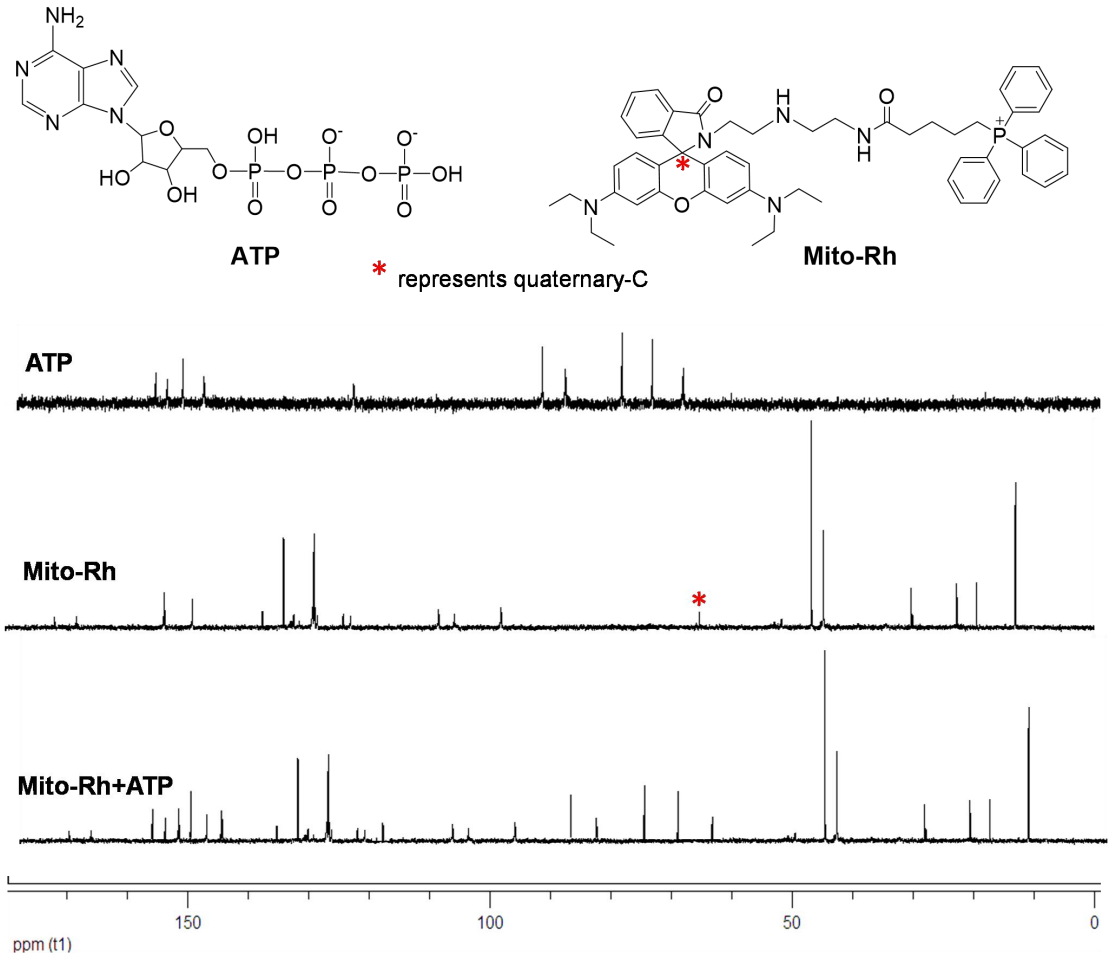

Figure S10. ^{13}C NMR spectra of ATP, Mito-Rh and Mito-Rh+ATP. NMR solvent: D_2O.

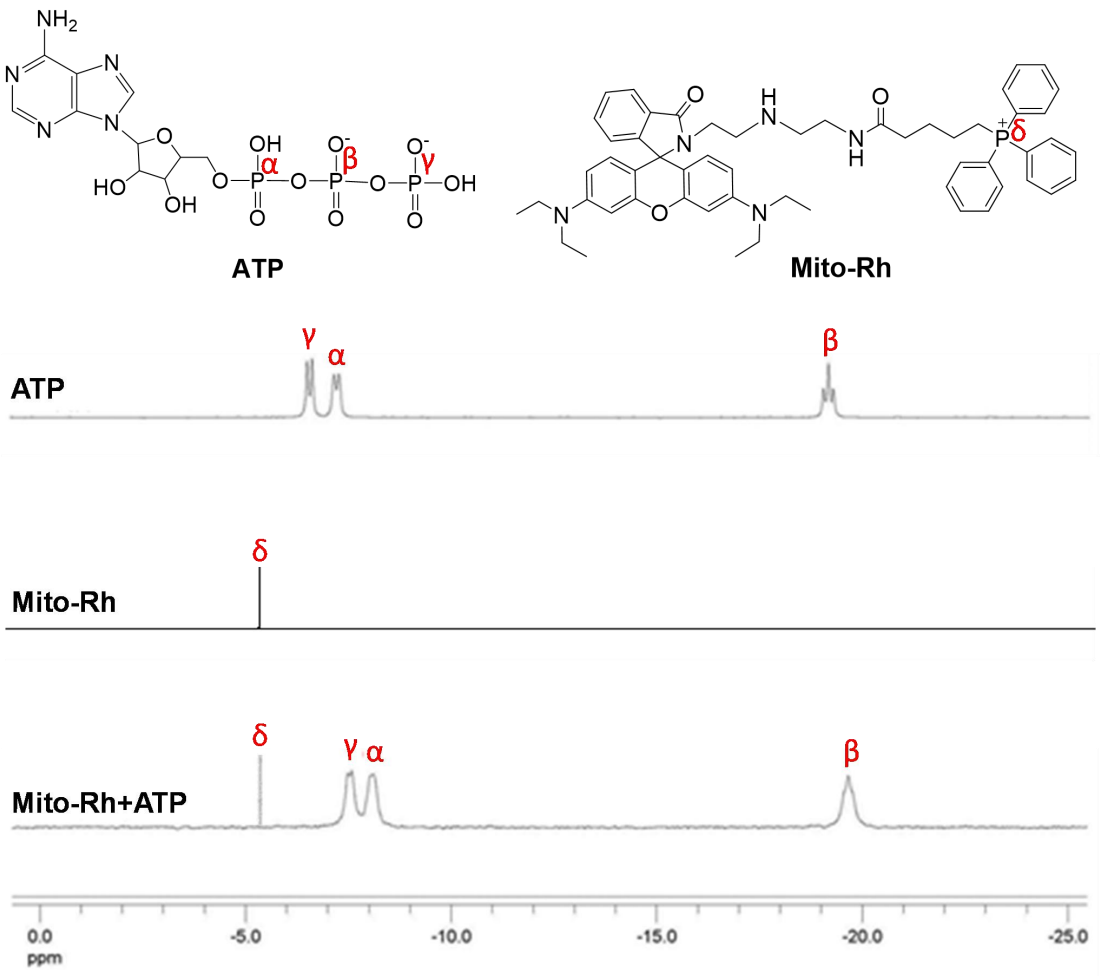

Figure S11. ^{31}P NMR spectra of ATP, Mito-Rh and Mito-Rh+ATP. NMR solvent: D_2O.

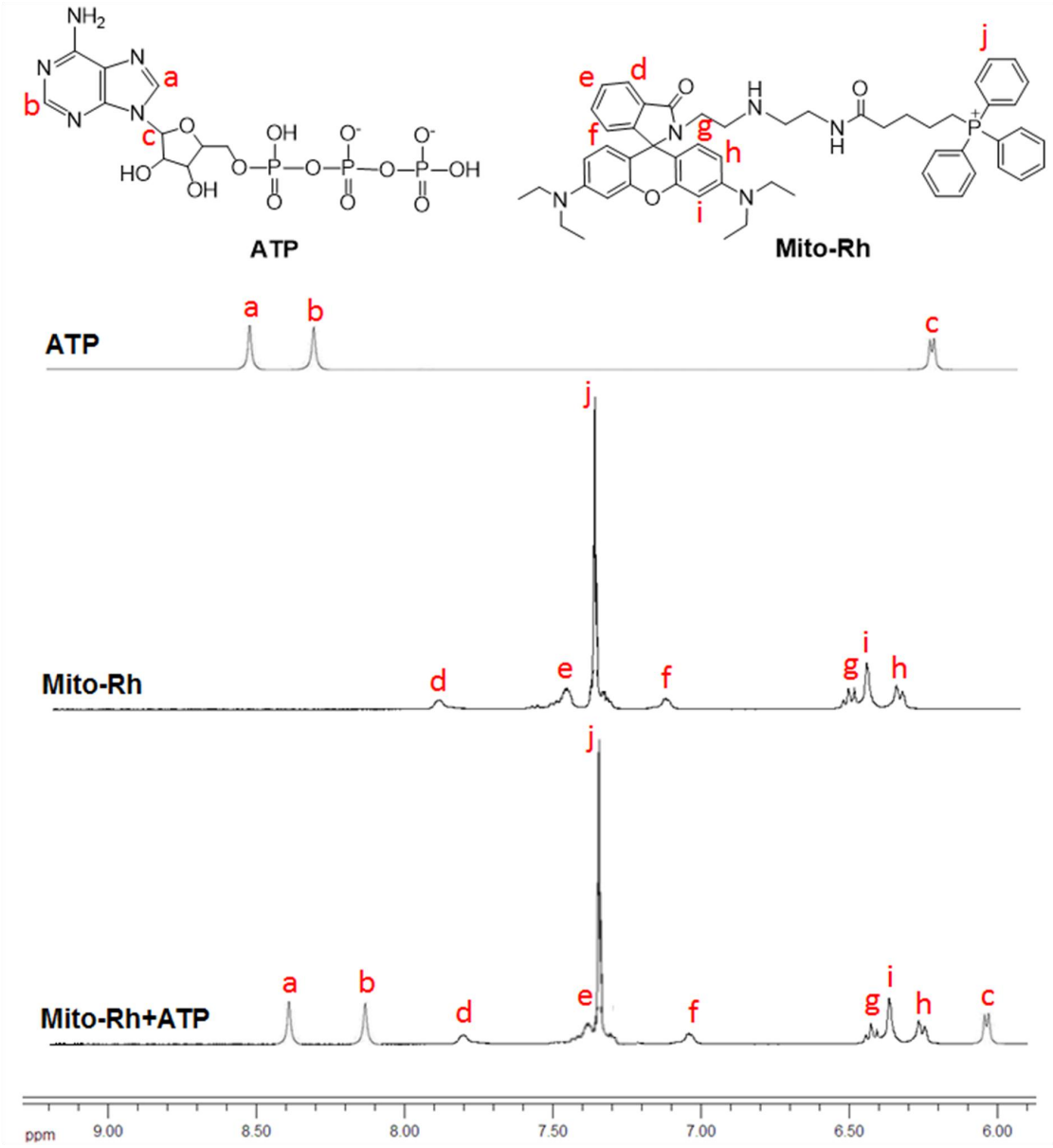

Figure S12. Partial ^{1}H NMR spectra of ATP, Mito-Rh and Mito-Rh+ATP. NMR solvent: D_2O.

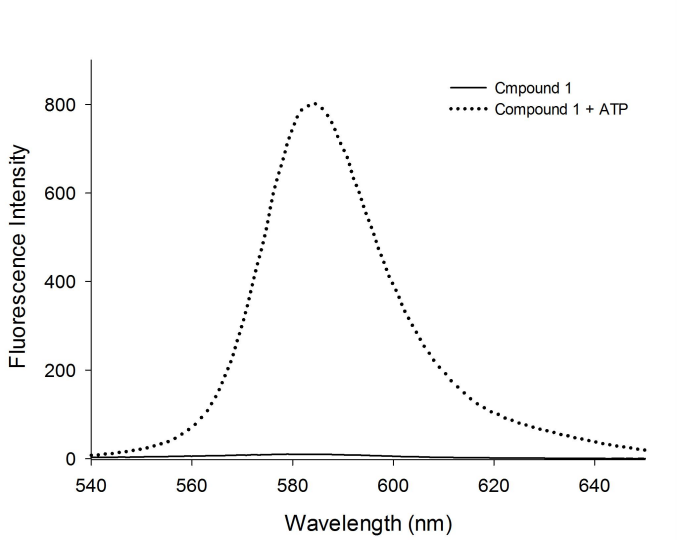

Figure S13. Fluorescence spectra of compound 1 (10 μM) in the absence of ATP (solid line) and in the presence of ATP (10 mM, dotted line).

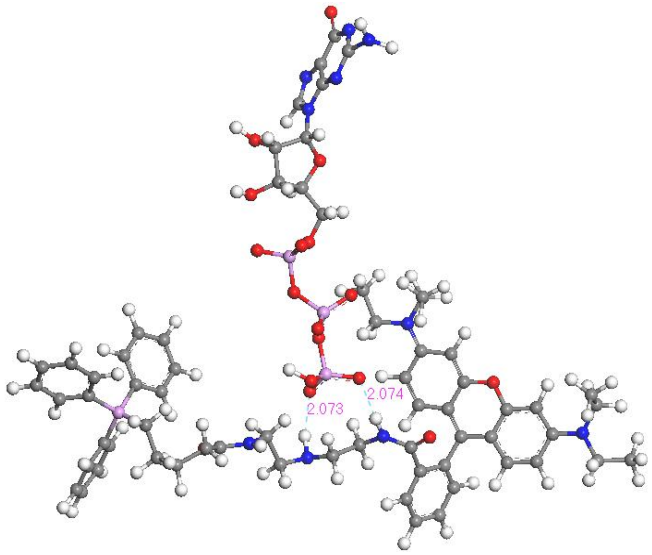

Figure S14. The optimized structures of Mito-Rh reacting with GTP. The hydrogen bond interaction is denoted by blue dotted lines. Carbon, hydrogen, oxygen, nitrogen and phosphorus atoms are colored with grey, white, red, blue and pink, respectively.

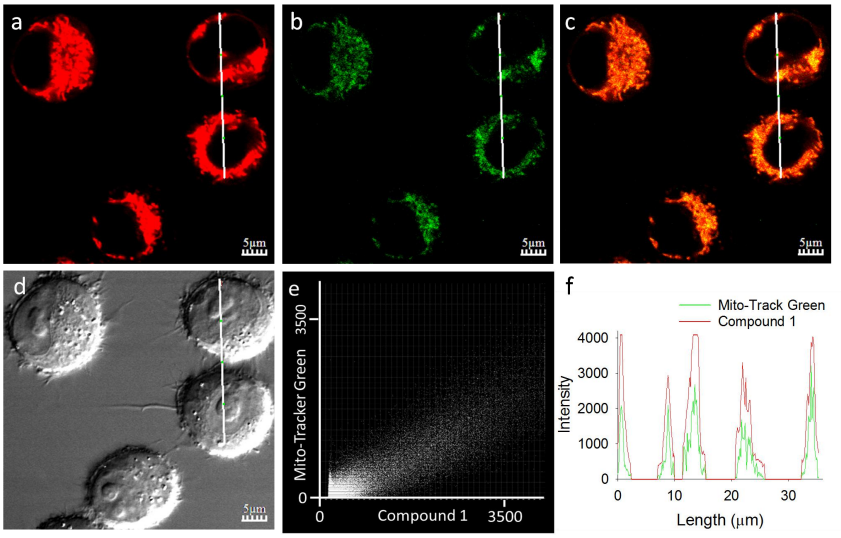

Figure S15. Colocalization imaging of HeLa cells staining with compound **1** and Mito-Tracker Green. (a) The cells were stained with compound **1** (1 μM) on red channel. (b) The cells were stained with Mito-Tracker Green (1 μM) on green channel. (c) Merged image; (d) Bright-field image. (e) Intensity scatter plot of compound **1** and Mito-Tracker Green. (f) Fluorescence intensity profile of regions of interest (white line in a and b). Scale bar, 5 μm.

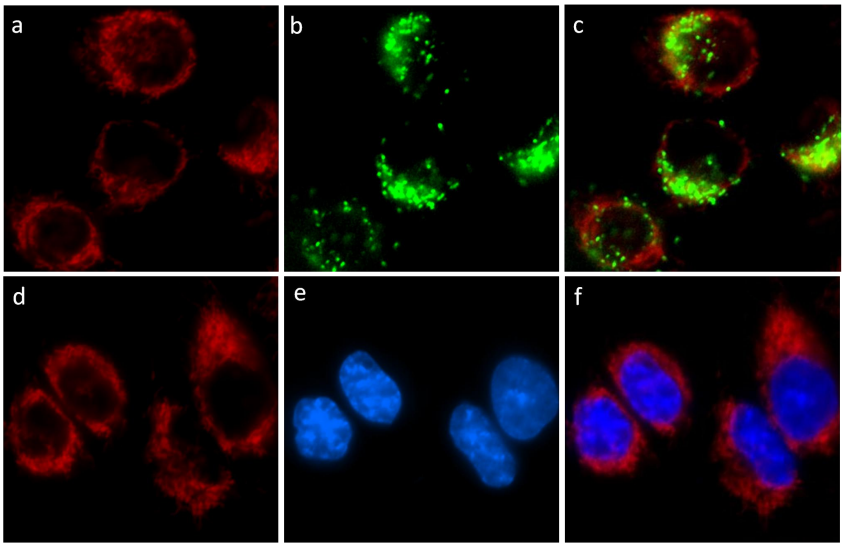

Figure S16. Fluorescence images of HeLa cells treated with Mito-Rh and Lyso-Tracker Green or DAPI. (a) The cells were stained with Mito-Rh (1 μM) on red channel. (b) The cells were stained with Lyso-Tracker Green (1 μM) on green channel. (c) Merged image of (a) and (b). (d) The cells were stained with Mito-Rh (1 μM) on red channel. (e) The cells were stained with DAPI (1 μM) on blue channel. (f) Merged image of (d) and (e).

Quantification of mitochondrial membrane potential alterations ($\Delta\Psi_m$) in HeLa cells before and after treatment with CCCP was studied using JC-1 dye. JC-1 forms aggregates with red fluorescence in healthy cells with intact mitochondria, but it shows monomeric green fluorescence in early apoptotic cells. And thus $\Delta\Psi_m$ can be displayed by the ratio of red fluorescence and green fluorescence. HeLa cells stained with JC-1 were treated before and after the addition CCCP (10 μM) and fluorescence red / green ratio changed from 4.2 to 0.6 (Figure S17). The results indicate that CCCP can decrease mitochondrial membrane potential.

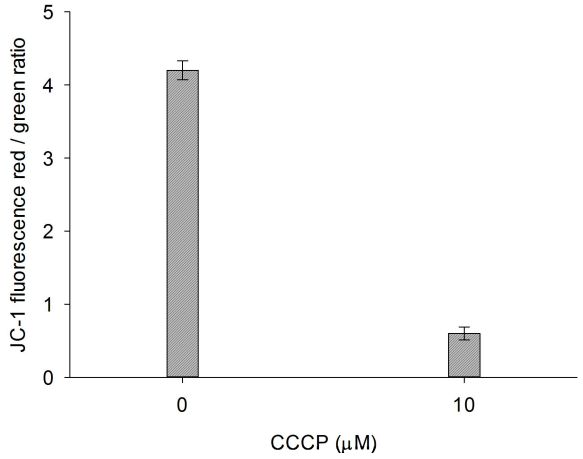

Figure S17. Quantification of mitochondrial membrane potential alterations ($\Delta\Psi_m$) in HeLa cells before and after treatment with CCCP (10 μM) was studied using JC-1 dye. Error bars represent the standard deviations of 6 trials.