Supporting Information for:

## Photo-regulated aptamer sensor for spatiotemporally controlled monitoring of ATP in mitochondria of living cells

Shanni Hong,<sup>a,b</sup> Xiaoting Zhang<sup>c</sup>, Ryan J. Lake,<sup>b,d</sup> Gregory T. Pawel,<sup>b,d</sup> Zijian Guo,<sup>c,\*</sup> Renjun Pei<sup>a,\*</sup> and Yi Lu<sup>b,d,\*</sup>

a. CAS Key Laboratory of Nano-Bio Interface, Suzhou Institute of Nano-Tech and Nano-Bionics,

Chinese Academy of Sciences, Suzhou, 215123, China. E-mail: rjpei2011@sinano.ac.cn.

b. Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, IL, 61801,

USA. E-mail: yi-lu@illinois.edu

c. State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing, 210093, China. E-mail: zguo@nju.edu.cn

d. DOE Center for Advanced Bioenergy and Bioproducts Innovation, University of Illinois at

Urbana-Champaign, Urbana, IL 61801, USA



Figure S1. Fluorescence quenching efficiency of different molar ratios of PC-Blocker-Q strand with 200 nM aptamer strand. Q represents PC-Blocker-Q strand, F represents aptamer strand.



Figure S2. Analysis by 12 % native PAGE. Lane 1: 200 nM PC-Apt; lane 2: 200 nM PC-Apt with light irradiation; lane 3: 200 nM PC-Apt with 5 mM ATP; lane 4: 200 nM PC-Apt with 5 mM ATP and light irradiation. Light irradiation time was 10 min.



Figure S3. Feasibility and stability of PC-Apt sensors in lysate of 5000 cells. Red Curve, fluorescence spectra of 200 nM PC-Apt probes mixed with 5 mM ATP in cell lysate solution without light irradiation. Blue curve, fluorescence spectra of 200 nM PC-Apt probes with 5 mM ATP incubated in cell lysate for 12 h without light irradiation. Black Curve, fluorescence spectra of 200 nM PC-Apt probes with 5 mM ATP incubated in cell lysate for 12 h without light irradiation. Black Curve, fluorescence spectra of 200 nM PC-Apt probes with 5 mM ATP incubated in cell lysate for 12 h followed by 20 min *hv* light irradiation. Ex: 530 nm, Em: 540 - 700 nm.



Figure S4. (A) Comparison of the fluorescence change between PC-Apt and PC-Apt/DQAsome complex with 365 nm light (*hv*) exposure when incubated with different concentrations of ATP. [ATP] = 0, 0.05, 0.1, 0.2, 0.5, 1, 2, 3, 4, 5 mM. Ex = 530 nm, Em = 565 nm. (B) Linear relationship between fluorescence and various concentrations of ATP of PC-Apt/DQAsome complex after 365 nm light exposure.



Figure S5. (A) Fluorescence images of mitochondrial membrane potential (JC-1 staining) of blank control (Blank) and the cells with the treatment of 20  $\mu$ g/ml DQAsomes (DQAsomes) or PC-Apt/DQAsome complex for 4 h. (B) Quantitative analysis of fluorescence images. MFI means the mean fluorescence intensity of three images. Error bars represent standard deviations from three experiments. Scale bar, 20  $\mu$ m.



Figure S6. Flow cytometry quantification of fluorescence of Hela cells with different treatments. NPC-Apt represents the negative control of the cell samples transfected with NPC-Apt/DQAsome. Untreated means untreated cell samples detect by PC-Apt/DQAsome. Oligomycin means the cell sample pretreated with 10  $\mu$ M Oligomycin and transfected with PC-Apt/DQAsome. CaCl<sub>2</sub> means the cell samples pretreated with 5 mM CaCl<sub>2</sub> and transfected with PC-Apt/DQAsome. Data are medians ± quartiles, n = 6.