Supplementary Figure captions

Figure S1. Calibration curve for determining matrix and cytosolic pH from BCECF fluorescence ratio.
BCECF fluorescence was measured in the presence of 5 and 30 μM CCCP for isolated mitochondria and H9c2 cells, respectively, without the addition of malate. **(A)** Dots show experimental data. The solid line shows the theoretical curve of $(F_L/F_S) = (A + B \times 10^{(7\text{-}pH)})/(C + 10^{(7\text{-}pH)})$ and is fitted to the experimental data with least-square fitting, where A is 0.045, B is 0.37, and C is 0.042. The dichroic mirror used is U-MWBV (Olympus, Japan). **(B)** In H9c2 cells, the above-mentioned theoretical curve is fitted to the experimental data with least-square fitting, where A is 0.567, B is 2.40, and C is 0.097. The dichroic mirror used is U-MNIBA (Olympus, Japan).

Figure S2. Effects of TMRE concentration and illumination intensity on the transient depolarizations.
Frequencies of the transient depolarization were determined in the presence of 1 mM malate. **(A)** Effects of TMRE concentration on the transient depolarization. **(B)** Effects of excitation intensity on the transient depolarization. Excitation intensity was changed with the neutral density filters with 12, 25, and 50% transmittance.

Figure S3. Effects of ADP on respiration and antimycin A-induced ROS production.
(A) Effects of ADP on respiration in the absence of oligomycin. ADP was added to the mitochondrial suspension at 5 mM. **(B)** Effects of ADP on antimycin A (AA)-induced ROS production in the presence of oligomycin. Mitochondria were incubated with 1 μM antimycin A for 20 min at 25 °C. Values represent the mean ± SEM (n = 3). *$p< 0.05$ vs. control.

Figure S4. Effects of CCCP and NH_4^+ on H9c2 cells.
(A) Effects of CCCP concentration on Cell TMRE in H9c2 cells. **(B)** Effects of NH_4^+ on cytosolic pH in H9c2 cells. Values represent the mean ± SEM (n > 10). *$p<0.05$ vs 0 μM CCCP for A; control for B.

Figure S5. TMRE fluorescence of mitochondria in H9c2 cells.
The TMRE fluorescence image of H9c2 cells were obtained by using a 40× (Uapo40×/340, NA = 0.9; Olympus), with binning pixels 1 × 1, under computer control.

Figure S1

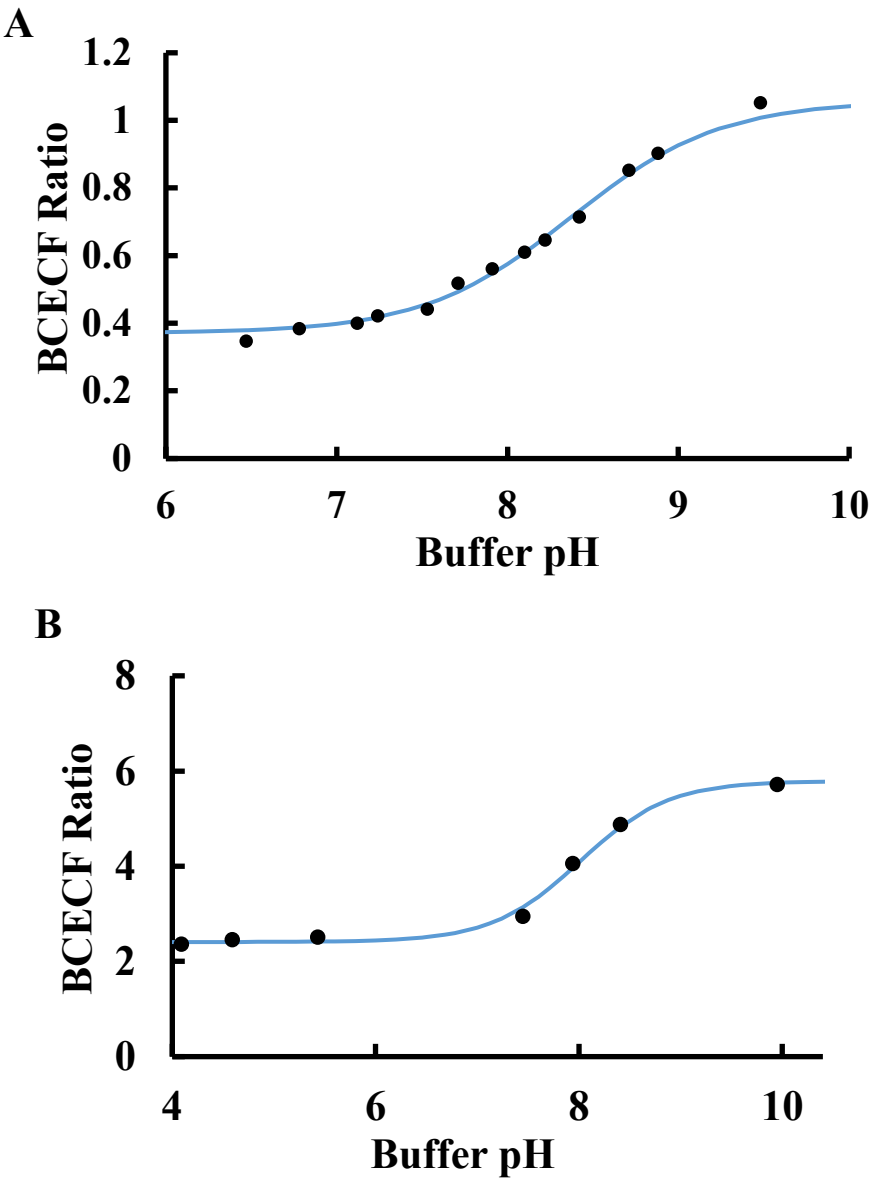
A
1.2
1
0.8
0.6
0.4
0.2
0
BCECF Ratio
6
7
8
9
10
Buffer pH
B
8
6
4
2
0
BCECF Ratio
4
6
8
10
Buffer pH

Figure S2

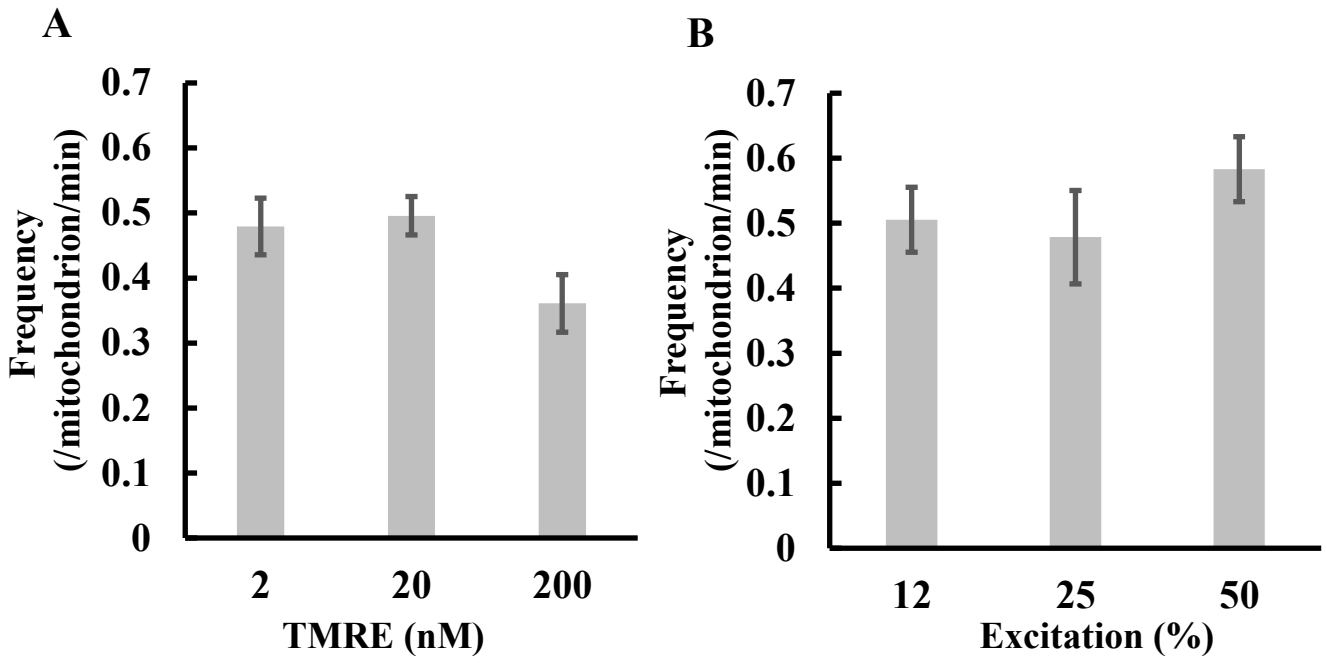
A
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Frequency (/mitochondrion/min)
2
20
200
TMRE (nM)
B
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Frequency (/mitochondrion/min)
12
25
50
Excitation (%)

Figure S3

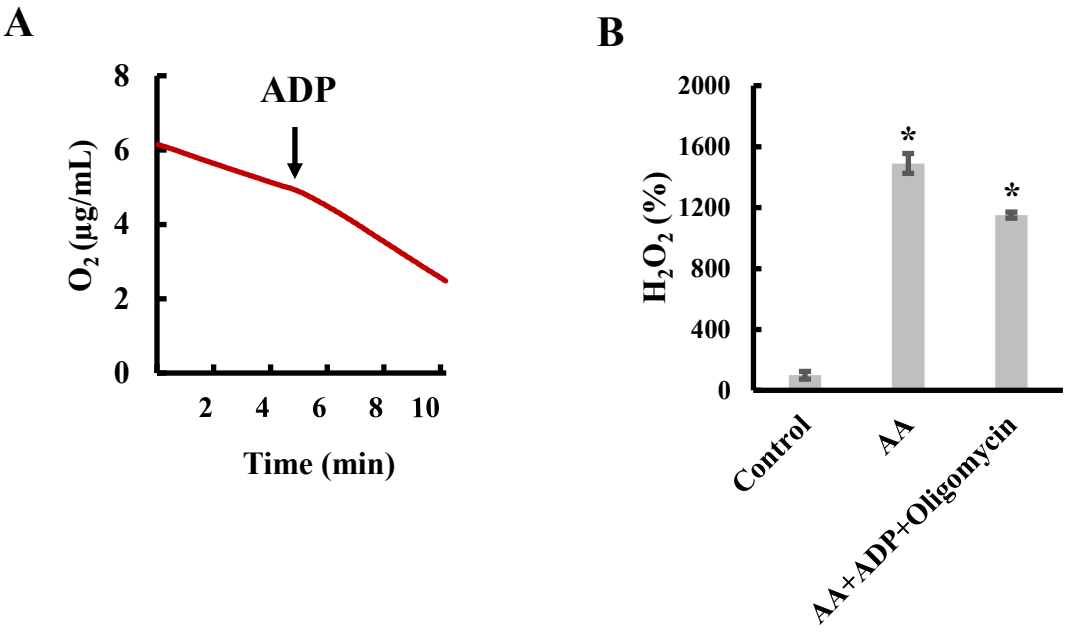
A
ADP
8
6
4
2
0
O_2 (μg/mL)
2
4
6
8
10
Time (min)
B
2000
1600
1200
800
400
0
H_2O_2 (%)
*
*
Control
AA
AA+ADP+Oligomycin

Figure S4

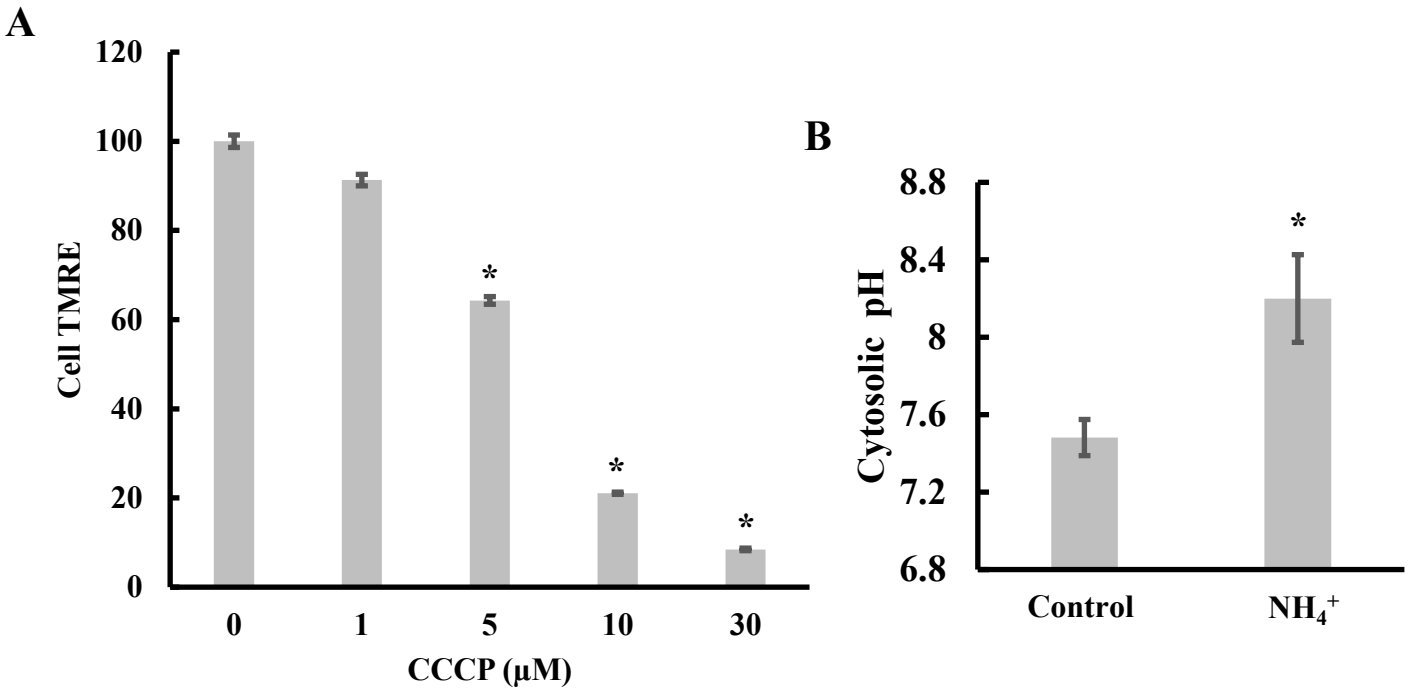
A
120
100
80
60
40
20
0
Cell TMRE
*
*
*
0
1
5
10
30
CCCP (μM)
B
8.8
8.4
8
7.6
7.2
6.8
Cytosolic pH
*
Control
NH4+

Figure S5