SUPPLEMENTARY INFORMATION

Figure legends

Fig. S1. Characterizations of spontaneous superoxide flashes in HeLa cells. a, Colocalization of ΔΨm indicator TMRM and the superoxide biosensor mt-cpYFP at the mitochondrion level (upper) and time courses of superoxide flash and concurrent ΔΨm depolarization. Scale bars: 5 μm. **b**, Swelling of flashing mitochondria. Paired traces of local change of mt-cpYFP signal (upper traces) and mitochondrial width (lower traces) scanned at 488 nm and 405 nm excitation. Notice the lack of change of cpYFP signal at 405 nm excitation during swelling and flashing. Scale bar: 5 μm. **c**, Effect of mPTP inhibitors, CsA (5 μM, n=30 cells) and BA (100 μM, n=31 cells), on superoxide flash activity.

Fig. S2. Characteristics of superoxide flashes in HeLa cells. Data shown are for superoxide flashes in the single or network mitochondria in untreated intact cells, or cells with CypD overexpression (OE) or knockdown (KD), or cells treated with selenite (10 μM, 12 hrs) in the presence of ROS scavengers, n=17-83 flashes.

Fig. S3. a, Proportions of punctiform, filiform, and network mitochondrial superoxide flashes. Selenite: 10 μM, staurosporine (STS): 0.2 μM, 12 hrs. Mock: cells transfected with vectors; OE: CypD overexpression; KD: CypD knockdown; Bax mutant: Bax C62⁄126S. n=15-62 flashes. **b**, Reversible activity of superoxide flash activity in response to brief selenite treatment (10 μM, 1 hr). n= 36-44 cells.

Fig. S4. Effects of CypD manipulation on H_2O_2-induced superoxide flashes and apoptosis. a, Superoxide flash frequency in CypD-manipulated cells in the absence or presence of H_2O_2 (120 μM, 6 hr). **b,** Global ROS level after H_2O_2 treatment (120 μM, 6 hr) was unaffected by CypD overexpression or knockdown. **c,** Overexpression of CypD exaggerated, and downregulation of CypD mitigated H_2O_2 (120 μM, 12 hr)-induced apoptosis. Data are mean ± s.e.m., n = 13-27 cells for frequency analysis. n = 10^4 cells from 4 experiments for apoptosis assay. *, p <0.05; **, p <0.01; ***, p <0.001 *versus* mock or scrambled controls. Mock, vector

control; CypD OE, overexpression of CypD; Scramble, scrambled CypD siRNA.

Fig. S5. Expression of Bcl-2 family proteins. a, Typical western blot showing Bcl-2 protein level in HeLa cells overexpressing Bcl-2-flag. **b**, Typical western blot showing Bax protein level in HeLa cells transfected with Bax-mCherry or Bax C62/126S-mCherry.

Movie legends

Movie 1. Spontaneous superoxide flashes in network mitochondria in a resting HeLa cell.

Movie 2. Synchronous mitochondrial swelling and superoxide flashes.

Movie 3. Superoxide flashes in punctiform mitochondria in a HeLa cell treated with selenite for 6 hours.

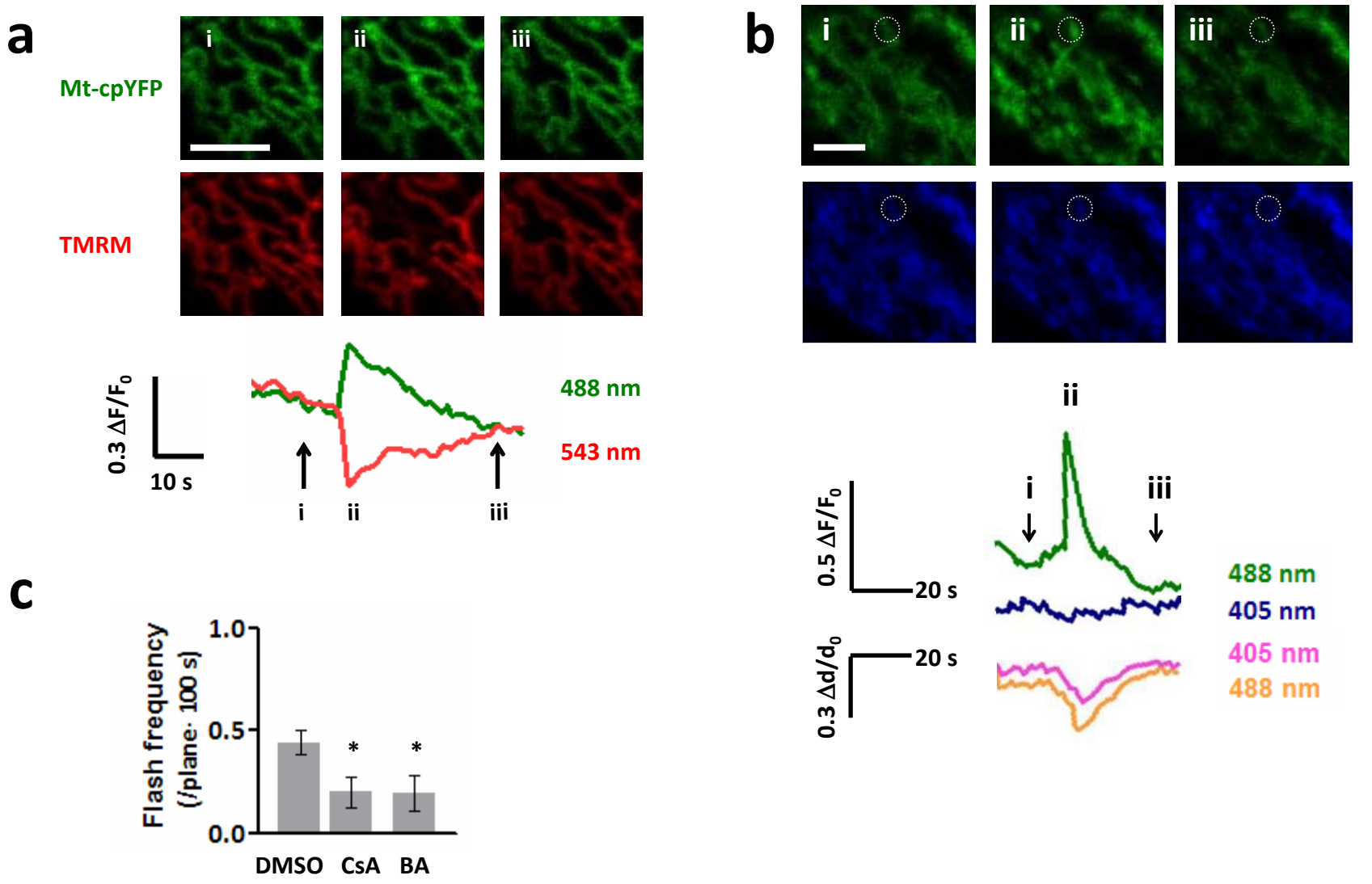
a
Mt-cpYFP
TMRM
i
ii
iii
0.3 ΔF/F0
10 s
488 nm
543 nm
b
0.5 ΔF/F0
20 s
0.3 Δd/d0
20 s
488 nm
405 nm
405 nm
488 nm
c
Flash frequency (/plane· 100 s)
1.0
0.5
0.0
DMSO
CsA
BA

Fig. S1

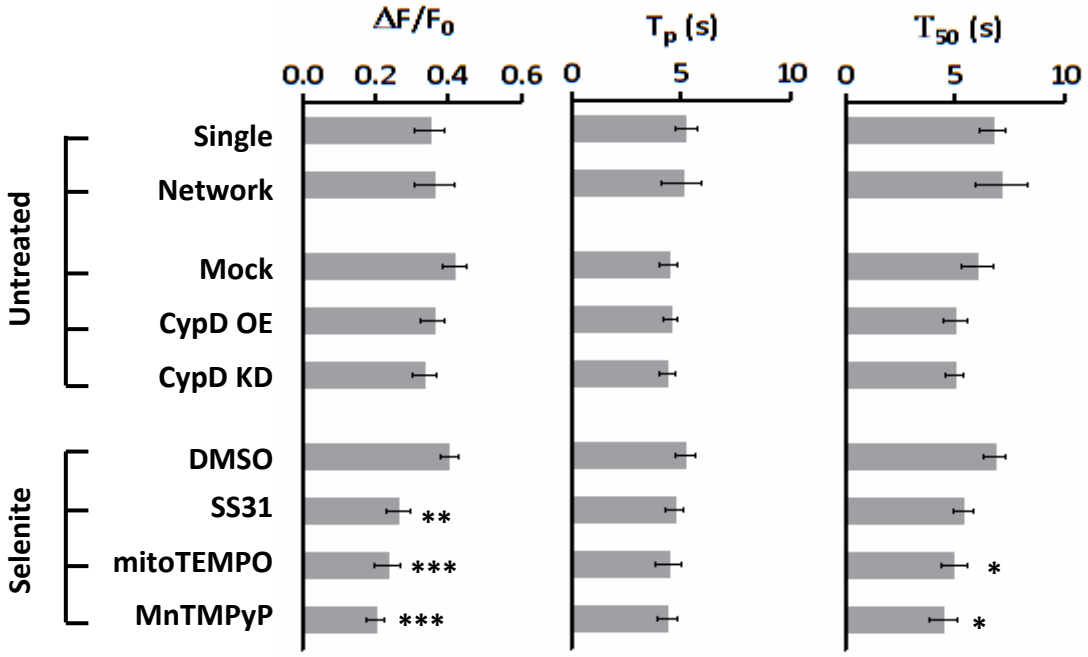
ΔF/F0
Tp (s)
T50 (s)
0.0
0.2
0.4
0.6
0
5
10
0
5
10
Untreated
Selenite
Single
Network
Mock
CypD OE
CypD KD
DMSO
SS31
mitoTEMPO
MnTMPyP
**
***
***
*
*

Fig. S2

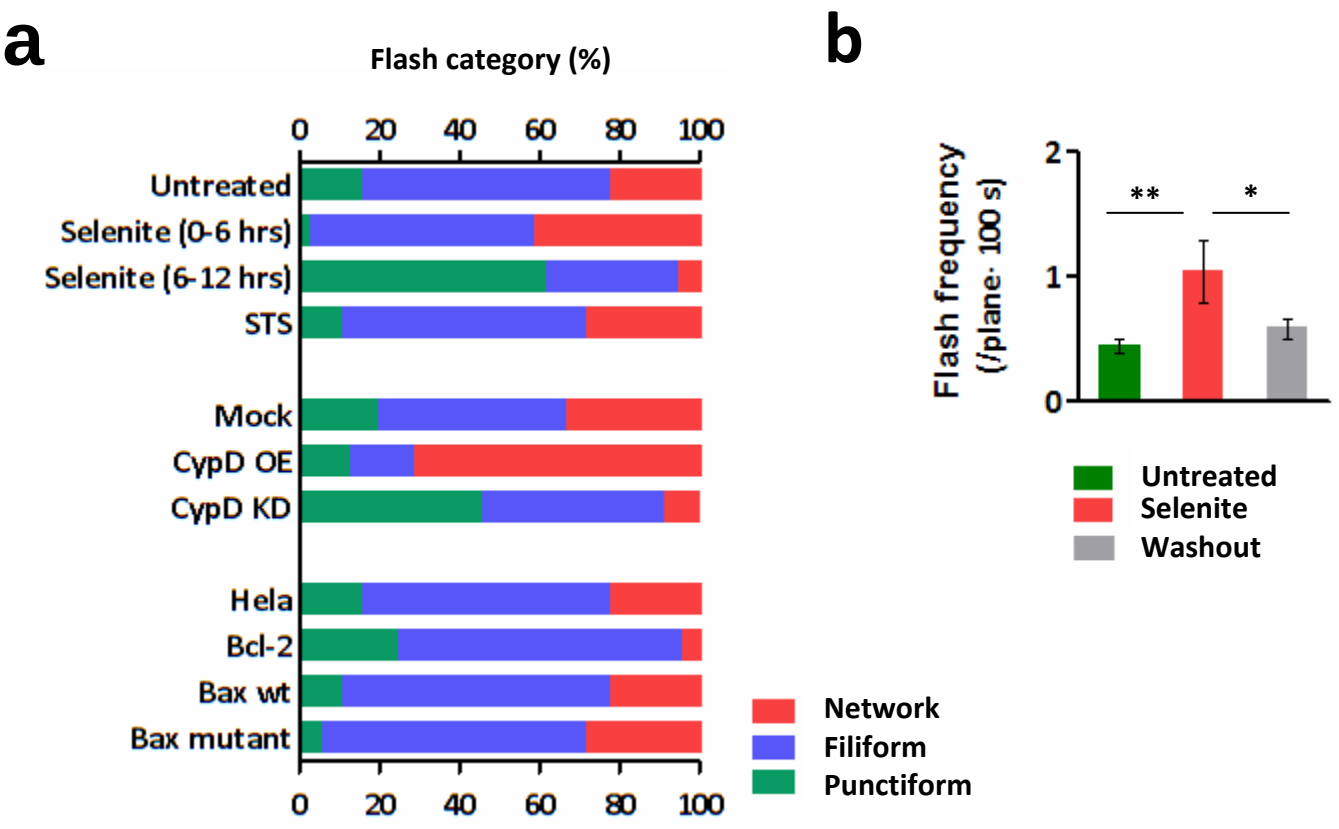
a
Flash category (%)
0
20
40
60
80
100
Untreated
Selenite (0-6 hrs)
Selenite (6-12 hrs)
STS
Mock
CypD OE
CypD KD
Hela
Bcl-2
Bax wt
Bax mutant
Network
Filiform
Punctiform
b
Flash frequency (/plane· 100 s)
2
1
0
**
*
Untreated
Selenite
Washout

Fig. S3

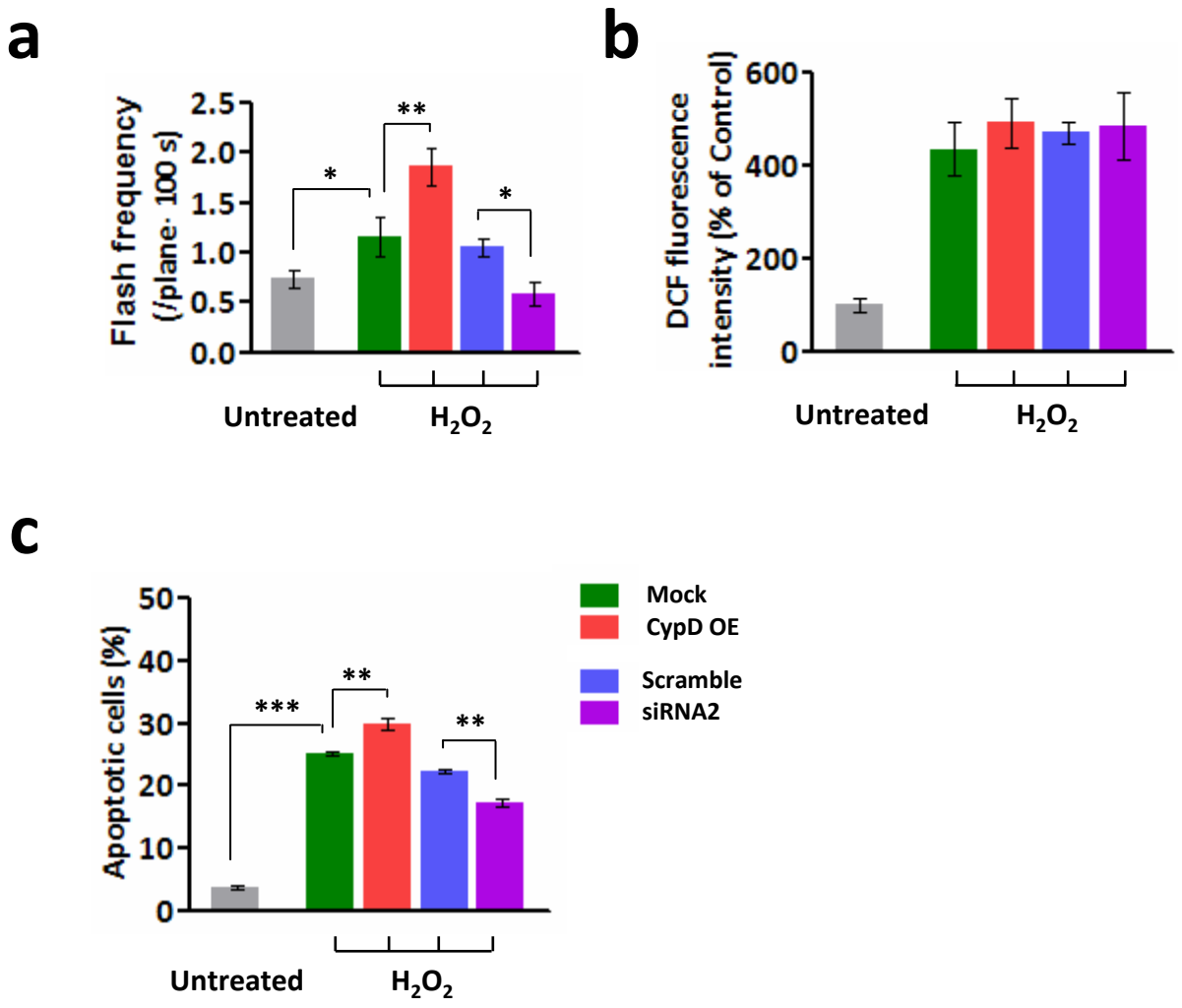
a
Flash frequency (/plane· 100 s)
2.5
2.0
1.5
1.0
0.5
0.0
*
**
*
Untreated
H2O2
b
DCF fluorescence intensity (% of Control)
600
400
200
0
Untreated
H2O2
c
Apoptotic cells (%)
50
40
30
20
10
0
***
**
**
Untreated
H2O2
Mock
CypD OE
Scramble
siRNA2

Fig. S4

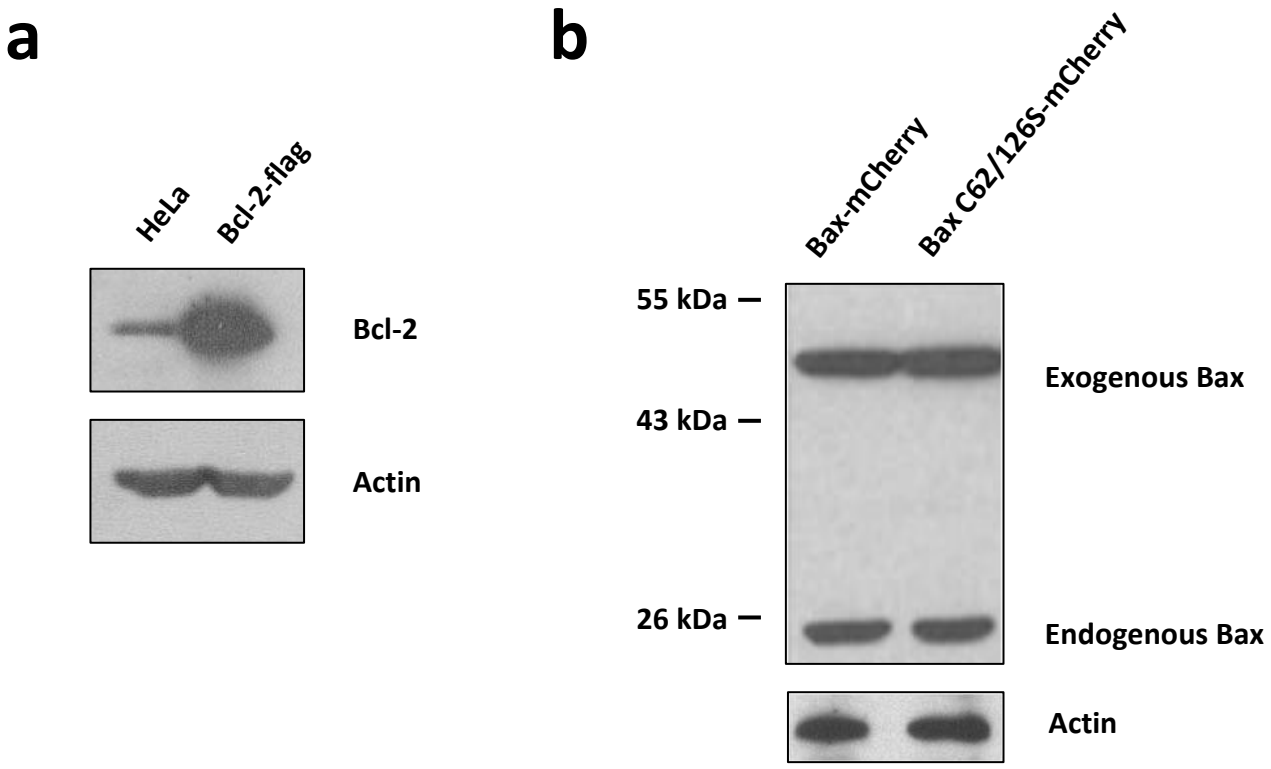
a
HeLa
Bcl-2-flag
Bcl-2
Actin
b
Bax-mCherry
Bax C62/126S-mCherry
55 kDa
43 kDa
26 kDa
Exogenous Bax
Endogenous Bax
Actin

Fig. S5