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Supplemental information

Genetically encoded biosensors for evaluating NAD^+/NADH ratio in cytosolic and mitochondrial compartments

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Supplemental Information

Inventory of Supplemental information:

Supplemental Figure Legend

(1) Figure S1. Related to Figure 1.

(2) Figure S2. Related to Figure 4.

(3) Figure S3. Related to Figure 5.

Supplemental Figure Legend

Figure S1. Characterization of mt-SoNar in live cells, Related to Figure 1. A, Representative images of mt-SoNar or mt-cpYFP expression in H9C2 cells after adenovirus mediated gene expression. **B**, Representative images of H9C2 cardiomyoblasts loaded with TMRE (20 nM) and after incubation with PBS or 0.3% digitonin. The quick diffusion of TMRE signal upon the addition of 0.3% digitonin indicates the permeabilization of mitochondrial inner membrane. **C-D**, The effect of mt-SoNar on HEK293 cell viability (**C**) or proliferation (**D**). N=3.

Figure S2. Effects of pathological stress on NAD^+/NADH ratios, mitochondrial membrane potential and cell death, Related to Figure 4. A-B, AcAc/β-OHB ratio (**A**) and pyruvate/lactate ratio (**B**) by biochemical assays before and after phenylephrine (PE, 4 hr) incubation. N=3 rats. *: $P < 0.05$ versus Control. **C**, Membrane potential in response to acute addition of caffeine (10 mM). N=3 rats. **D**, Effects of PE (10 μM for 15 min or 4 hr) on cell death. N=3 rats.

Figure S3. Effects of nicotinamide riboside (NR) on intracellular NAD^+/NADH ratios, Related to Figure 5. Total intracellular NAD^+ level, NADH level and NAD^+/NADH ratio (**A**), or mitochondrial (**B**) or cytosolic (**C**) NAD^+/NADH ratio after NR (1 mM) incubation for 0, 4, 24 or 72 hr. N=5-9 rats. *: $P < 0.05$ versus Control (0 hr, value is set as 1.0).

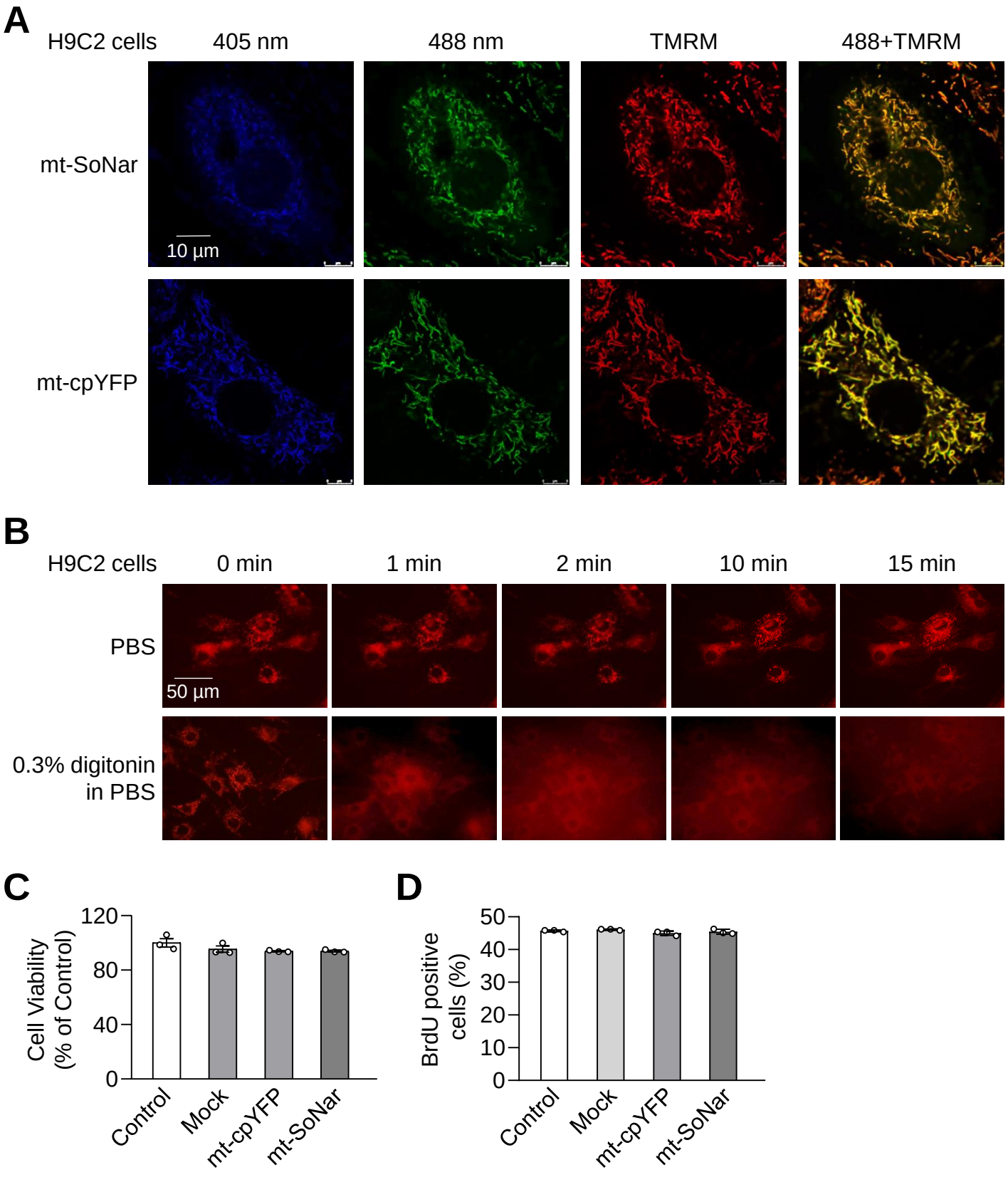
A
H9C2 cells
405 nm
488 nm
TMRM
488+TMRM
mt-SoNar
mt-cpYFP
10 μm
B
H9C2 cells
0 min
1 min
2 min
10 min
15 min
PBS
0.3% digitonin in PBS
50 μm
C
Cell Viability (% of Control)
120
80
40
0
Control
Mock
mt-cpYFP
mt-SoNar
D
BrdU positive cells (%)
50
40
30
20
10
0
Control
Mock
mt-cpYFP
mt-SoNar

Figure S1

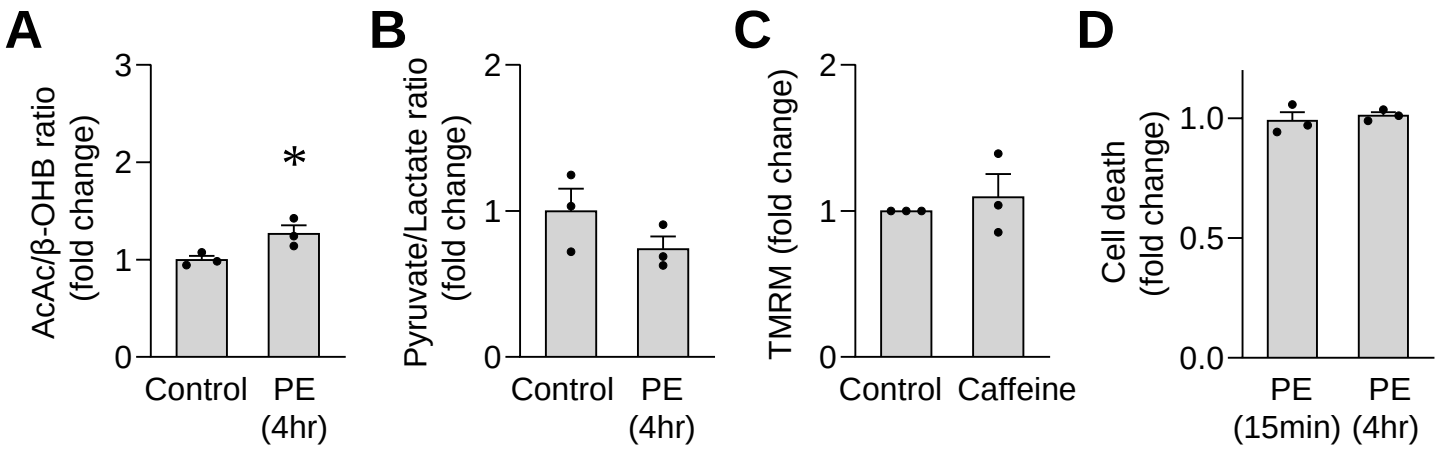
A
AcAc/β-OHB ratio
(fold change)
3
2
1
0
*
Control
PE
(4hr)
B
Pyruvate/Lactate ratio
(fold change)
2
1
0
Control
PE
(4hr)
C
TMRM (fold change)
2
1
0
Control
Caffeine
D
Cell death
(fold change)
1.0
0.5
0.0
PE
(15min)
PE
(4hr)

Figure S2

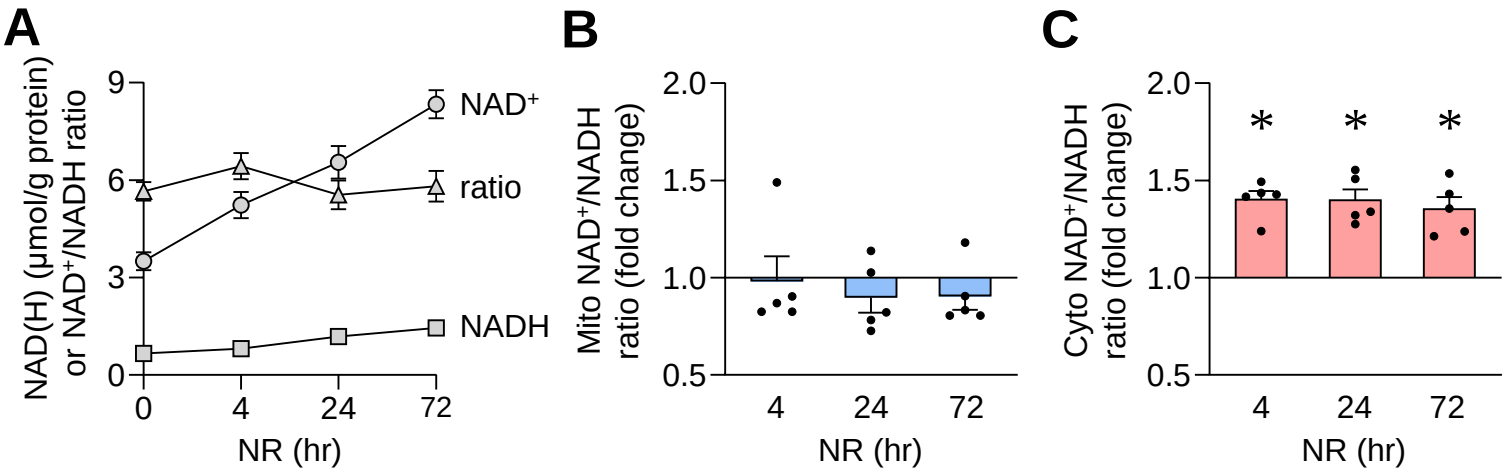
A
B
C
NAD(H) (μmol/g protein)
or NAD+/NADH ratio
9
6
3
0
0
4
24
72
NR (hr)
NAD+
ratio
NADH
Mito NAD+/NADH
ratio (fold change)
2.0
1.5
1.0
0.5
4
24
72
NR (hr)
Cyto NAD+/NADH
ratio (fold change)
2.0
1.5
1.0
0.5
*
*
*
4
24
72
NR (hr)

Figure S3