Electronic Supplementary Material (ESI) for Analytical Methods.

Electrochemical detection of 8-hydroxy-2'-deoxyguanosine as a biomarker for oxidative DNA damage in HEK293 cells exposed to 3-chloro-1,2-propanediol

Xiulan Sun[1]*, Lijuan Zhang[1], Hongxia Zhang[2]，He Qian[1], Jian Ji[1] , Lili Tang[1], Zaijun Li[1], Genyi Zhang[1]*

1 State Key Laboratory of Food Science and Technology, School of Food Science of Jiangnan University, Synergetic Innovation Center of Food Safety and Nutrition, Wuxi, Jiangsu 214122, China

2 School of Foreign Studies, Shanxi University of Technology, Hanzhong, Shanxi 723000, China

***Corresponding author.**

Xiulan Sun*: Tel: +86 510-85329015 ; Fax: +86 510-85328726.

E-mail: sxlzzz@jiangnan.edu.cn

Genyi Zhang*: Tel: +86 510-85329015 ; Fax: +86 510-85328726.

E-mail: genyiz@gmail.com

1. Assessment of oxidative damage in 3-MCPD-treated HEK293 cells

1.1 Measurement of cell viability by MTT assay

By applying quantitative calorimetric assay with MTT at a density of 3.6×10^3 /well on 96-well plates, we measured cell viability. After incubation for 12 h, the cells were treated with varying concentrations of 3-MCPD (0.5-100 mM.) for 24 h, then treated with 100 μL MTT (1 mg/ml in DMEM) and incubated for 4 h at 37℃. The dark blue formazan crystals which formed in intact cells were solubilized with DMSO for 10 min, and the absorbance at 490 nm was measured with a microplate reader (MULTISKAN MK3, Thermo Scientific, Shanghai, China). Each experiment was replicated at least five times. Cell viability was calculated based on the absorbance of the 3-MCPD-treated cells relative to that of vehicle-treated control cells.

1.2 Morphological changes

We seeded HEK293 cells at a density of 3.6×10^4 cells/dish in lasered dishes and incubated for 12 h. After treatment with 3-MCPD at varying concentration (1, 10, 50, and 100 mM) for 24 h, we observed the morphological changes by laser scanning confocal microscope (LSCM) (Zeiss LSM710, Germany). The group stimulated by 100 μM H_2O_2 serves here as the positive control.

1.3 Measurement of reactive oxygen species

ROS was detected with Reactive Oxygen Species Assay Kit according to the manufacturer's instructions. We used 2',7'-dichloro-fluorescein diacetate (DCFH-DA) to detect the intracellular reactive oxygen species (ROS). Cells were seeded at a density of 1×10^6 cells/well in a 6-well plate and incubated for 12 h. After treatment

with various concentrations of 3-MCPD (1, 10, 50 and 100 mM) for 24 h, we washed the cells with PBS twice and loaded them with 10 μM DCFH-DA in serum-free medium for 60 min at 37℃ without light, followed by three more rinses in PBS. We monitored the formation of the fluorescent-oxidized DCF derivative using FACS Calibur flow cytometer, with excitation at 485 nm and emission at 525 nm (Becton Dickinson, America). Using the median fluorescence intensity of 20,000 cells, we quantified ROS generation. We chose the group stimulated by Rosup as the positive control.

1.4 Comet assay

Using the Comet Assay Kit according to the manufacturer's instructions, we trypsinized cells treated with 3-MCPD for 24 h, then diluted with ice-cold PBS. The positive controls here are100 μM H_2O_2-treated cells. We mixed 3-MCPD- and H_2O_2-treated cells with low-melting-point agarose to coat the slides. Next, we treated the slides with lysis buffer and alkaline solution, electrophoresed them under neutral conditions, and stained them with DNA dye. Under an Eclipse 80i Fluorescence Microscope (Nikon, Japan), we visualized stained slides. For analysis, we selected two slides per concentration and a total of 50 comets per group (25 cells per slide). CASP image analysis software was applied to the captured images. We measured the olive tail moment (OTM), calculated as comet tail moment length multiplied by the relative DNA content of the tail, as an indicator of DNA damage (strand breaks).

Figures：

Fig.S1. CVs of the GCE in 0.1 M pH 6.0 PBS. Scan rate: 50 mV s^{-1}, scan cycles: 10.

Fig. S2. Agarose gel electrophoresis of genomic DNA of HEK293 cells stimulated by (a-e) 0, 1, 10, 50, and 100 mmol/L 3-MCPD for 24 h

Fig.S3. Effects of 3-MCPD on survival, morphology, ROS levels and DNA in HEK293 cells. Exponentially-growing HEK293 cells were treated with the indicated concentration of 3-MCPD for 24 h. (A) Cell viability was determined by MTT assay. The data share a mean ± SD (n=5) (*P < 0.05 vs. control). (B) Morphological changes in HEK293 cells stimulated by 3-MCPD. (C) Intracellular ROS levels, using DCF fluorescence assay for flow cytometric measurement. (D) Analysis of intracellular ROS results; the data share a mean ± SD (n=3) (*P < 0.05 vs. control). (E) Detection of DNA damage using comet assays. (F) Olive tail moment (Tail DNA% × Tail Moment Length) as quantified for each cell. The data share a mean ± SD (n=50) (*P < 0.05 vs. control).

Fig.S4. MRM chromatograms of (A-C) 8-OH-dG standard solution and (D-F) cell sample analyzed by UPLC-MS/MS. A and D: pathways of m/z 284.21 → 117.13; B and E: pathways of m/z 284.21 → 140.15; C and F: pathways of m/z 284.21 → 168.13.

Fig.S5. (A) CVs of 2.5 mM $[Fe(CN)_6]^{3-/4-}$ at the bare GCE (a) and P3AT/GCE (b). (B) CVs of 2.5 mM $[Fe(CN)_6]^{3-/4-}$ at the P3AT/GCE at varying scan rates: 5, 10, 20, 40, 60, 80, 100, 150, 200 and 300 mV s^{-1}. Inset: calibration plots of the $[Fe(CN)_6]^{3-/4-}$ anodic and cathodic peak current versus the square root of scan rate on P3AT/GCE.

Fig.S1

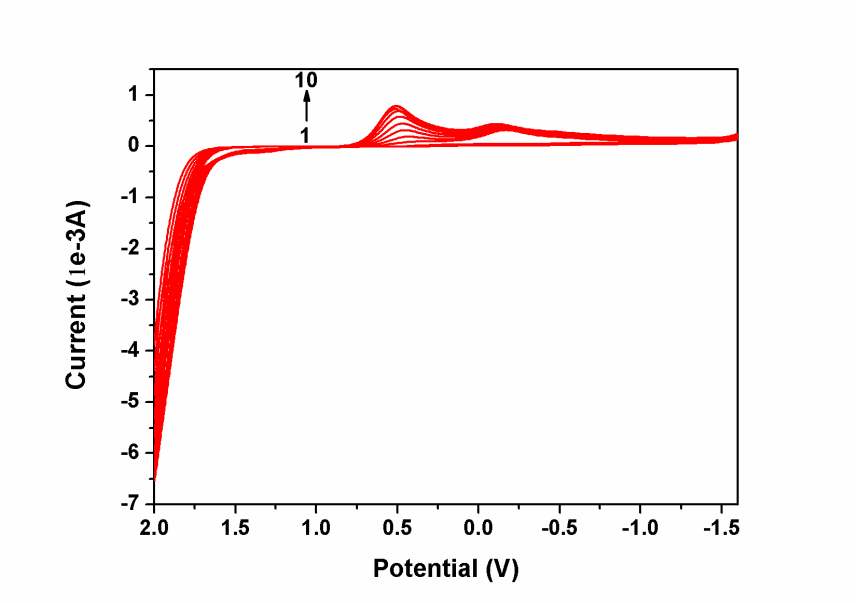


Fig.S2

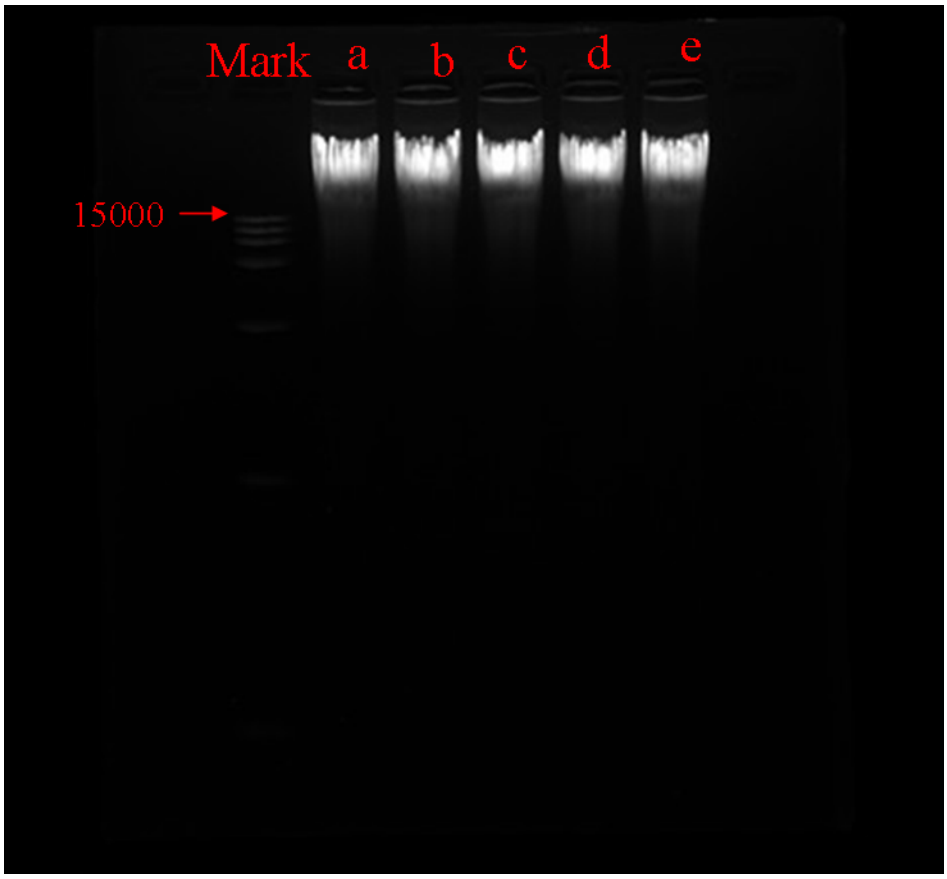


Fig.S3

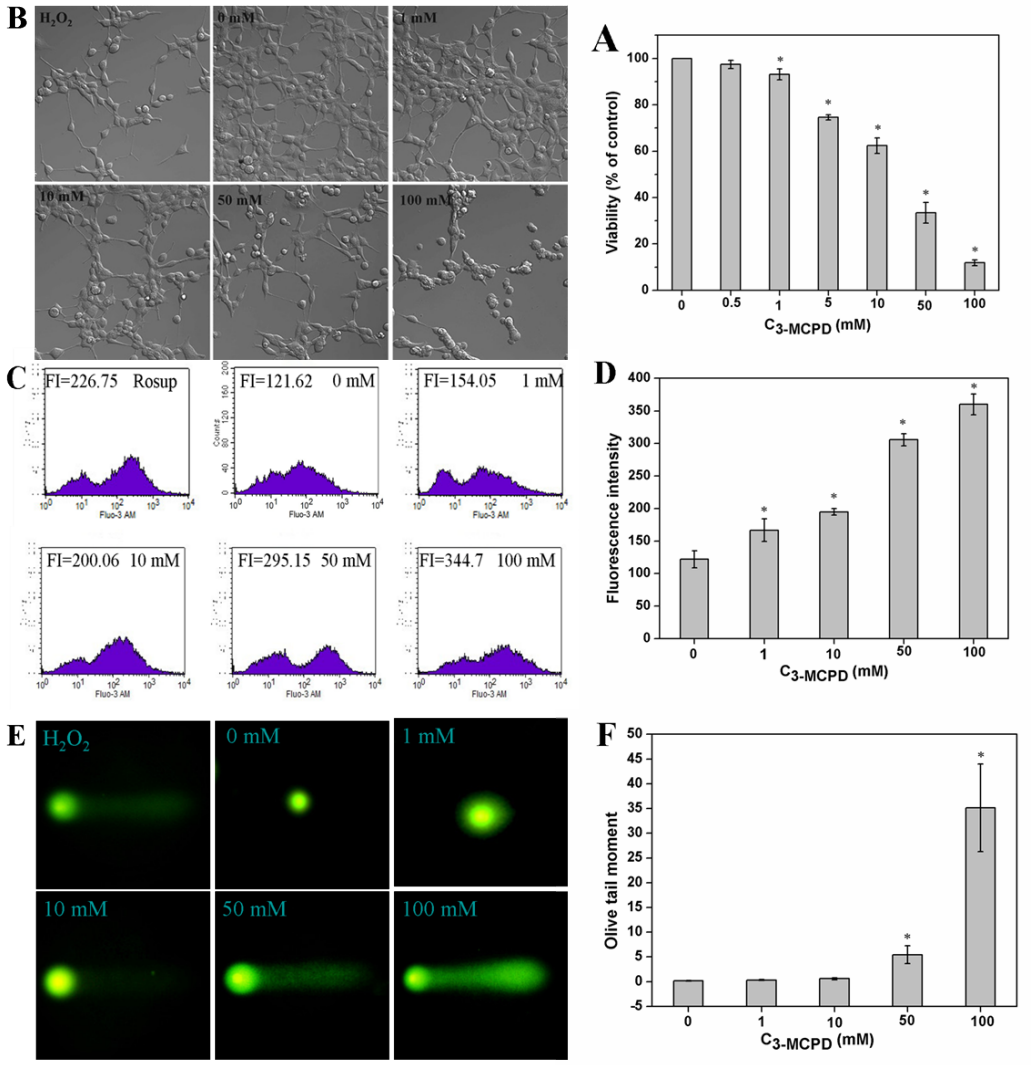

B
H_2O_2
0 mM
1 mM
10 mM
50 mM
100 mM
A
Viability (% of control)
$C_{3\text{-MCPD}}$ (mM)
C
FI=226.75 Rosup
FI=121.62 0 mM
FI=154.05 1 mM
FI=200.06 10 mM
FI=295.15 50 mM
FI=344.7 100 mM
Fluo-3 AM
D
Fluorescence intensity
$C_{3\text{-MCPD}}$ (mM)
E
H_2O_2
0 mM
1 mM
10 mM
50 mM
100 mM
F
Olive tail moment
$C_{3\text{-MCPD}}$ (mM)

Fig.S4

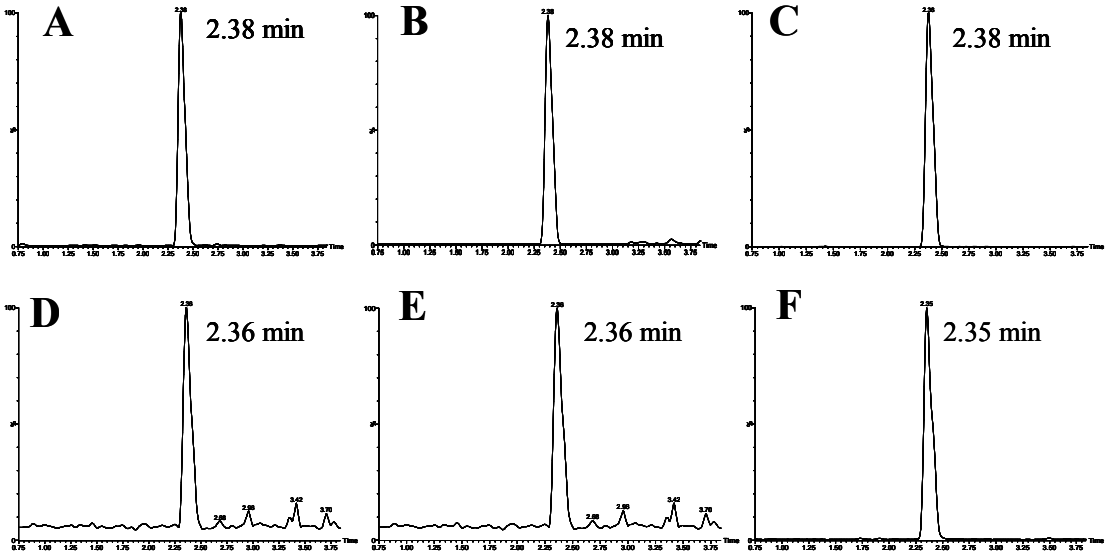
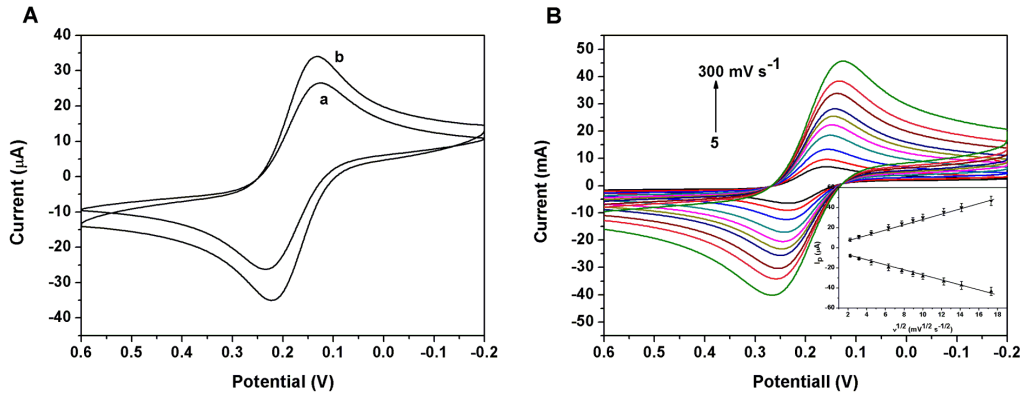


Fig.S5


A
Current (μA)
Potential (V)
a
b
B
Current (mA)
Potentiall (V)
300 mV s-1
5