

Supplementary figure

Figure S1

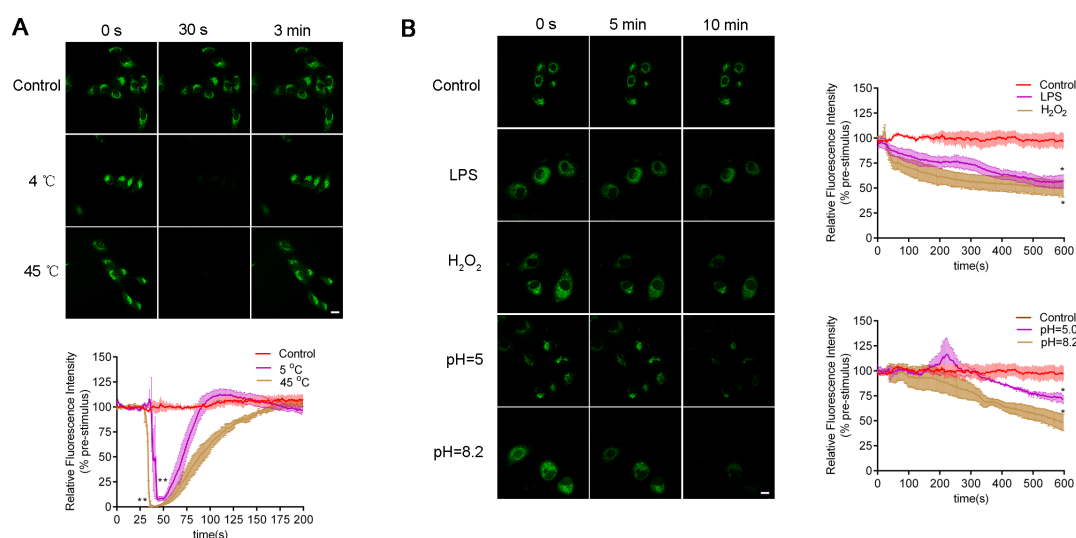


Figure S1. Various stimuli increase intracellular Cl^- levels in 16HBE14o- cells.

16HBE14o- cells were cultured on Glass Bottom Cell Culture Dish (801002, NEST, China).

Cells were subsequently loaded with 5 mM

MQAE(N-(ethoxycarbonylmethyl)-6-methoxyquinolium bromide; ab145418, Abcam) in the

dark for 1 h at 37 °C and washed twice to remove the unbound probe. Then, various

stimuli including cold (5 °C), hot (45 °C), acid (pH=3.6), alkali (pH=8.2), hydrogen

peroxide (H_2O_2 , 15 mM) and LPS (35 $\mu\text{g}/\text{ml}$) HEPES buffers were added to the culture

dish. Confocal microscopy scanning of the process was accomplished within 3-10 min. In

each set of experiment, images were taken using the same parameter settings.

A-B) Left panel shows representative confocal microscopy images of

16HBE14o- cells, which were loaded with MQAE (a Cl^- indicator), before and

after application of coldness (4°C), hot (45°C), acid (pH=5), alkali (pH=8.2),

H_2O_2 (15 mM) and LPS (35 $\mu\text{g}/\text{ml}$) to the basolateral bath. Right panel shows

summary plots of relative fluorescence intensity of MQAE indicating the

change levels of intracellular Cl^- ($n=3$ independent experiments; $*P < 0.05$; $**P < 0.01$).

Figure S2

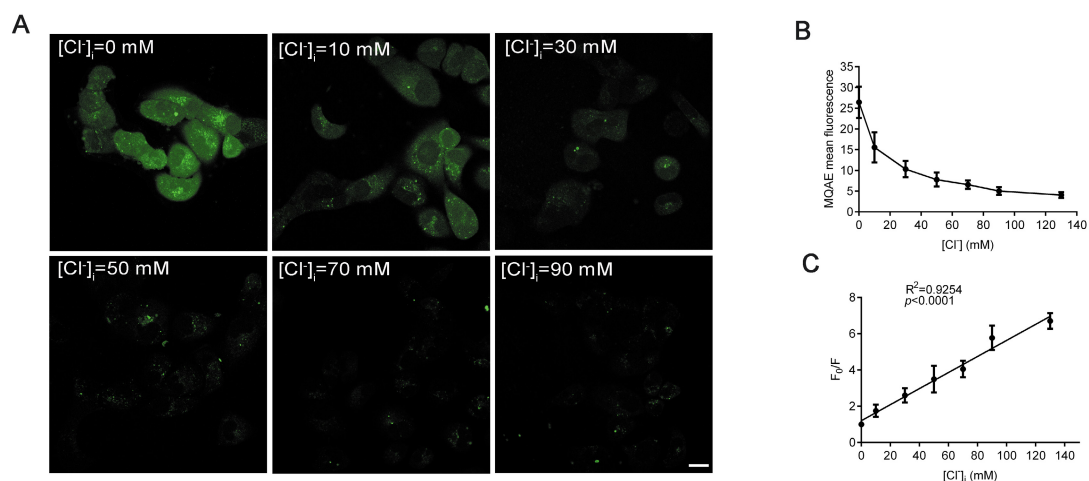


Figure S2. Intracellular quenching of MQAE by chloride ion in 16HBE14o- cells. **A-B)** Correlation between the intracellular chloride concentrations and the mean fluorescence intensities of MQAE were obtained in 16HBE14o- cells ($n=100-180$ cells for each group; Scale bars: $20\ \mu\text{m}$). Cells were incubated with buffers at various chloride concentrations (0, 10, 30, 50, 70, 90 and 130 mM) in the presence of the ionophores tributyltin ($10\ \mu\text{M}$) and nigericin ($5\ \mu\text{M}$), for 1 h. **C)** Stern-Volmer plot analysis ($F_0/F-1=K_{\text{Cl}^-} [\text{Cl}^-]$) for the double ionophore strategy. The regression line gives the Stern-Volmer constant (K_{SV} ; slope of the line) of $22.71 \pm 1.53\ \text{M}^{-1}$ ($n=3$ independent experiments). Data are presented as mean \pm SD.