# Cell-cycle-specific Cellular Responses to Sonoporation

**Supplementary Information:**

## I. Cell viability evaluation

The main purpose of the current study is to get a comprehensive understanding of the impact of cell cycle phase on the cellular responses simultaneously occurring in cell membrane and cytoskeleton induced by microbubble sonoporation. In order to achieve this goal, the interaction between a single bubble and a single cell was visualized in real-time by using an *in-situ* fluorescence imaging system coupled with ultrasound exposure instruments. In this paper, a total of 50 sonoporated cells and 45 non-sonoporated cells were adopted for fluorescence intensity analysis, and the results are illustrated in Fig. 6. Similar to what was claimed by CX Deng's group (Ref. 9), all of these cells should be viable at the end of 4-min observation period after ultrasound exposure because they met the following conditions: (1) the GFP intensity expressed in cell cytoskeleton dropped quickly to reach a level exhibited by the cells dead before ultrasound exposure; and (2) the PI intensity in cells tended to reach relatively stable plateau which should be much lower than the PI intensity observed for the cells dead before ultrasound exposure.

The normalized GFP intensity of the cell dead before ultrasound exposure should be around 0.19, which could not get down to 0 because of interference of the background fluorescence. The normalized PI intensity for dead cells should be higher than 2.2. The following is a sample for an S-phase cell that was dead after sonoporation. The cell was sonoporated by 2 bubbles. It is obvious that its normalized GFP intensity quickly drops lower than 0.2 within 60 s (Figure b), while its normalized PI intensity keeps enhancing to higher than 2.4 at $t$ = 180 s.

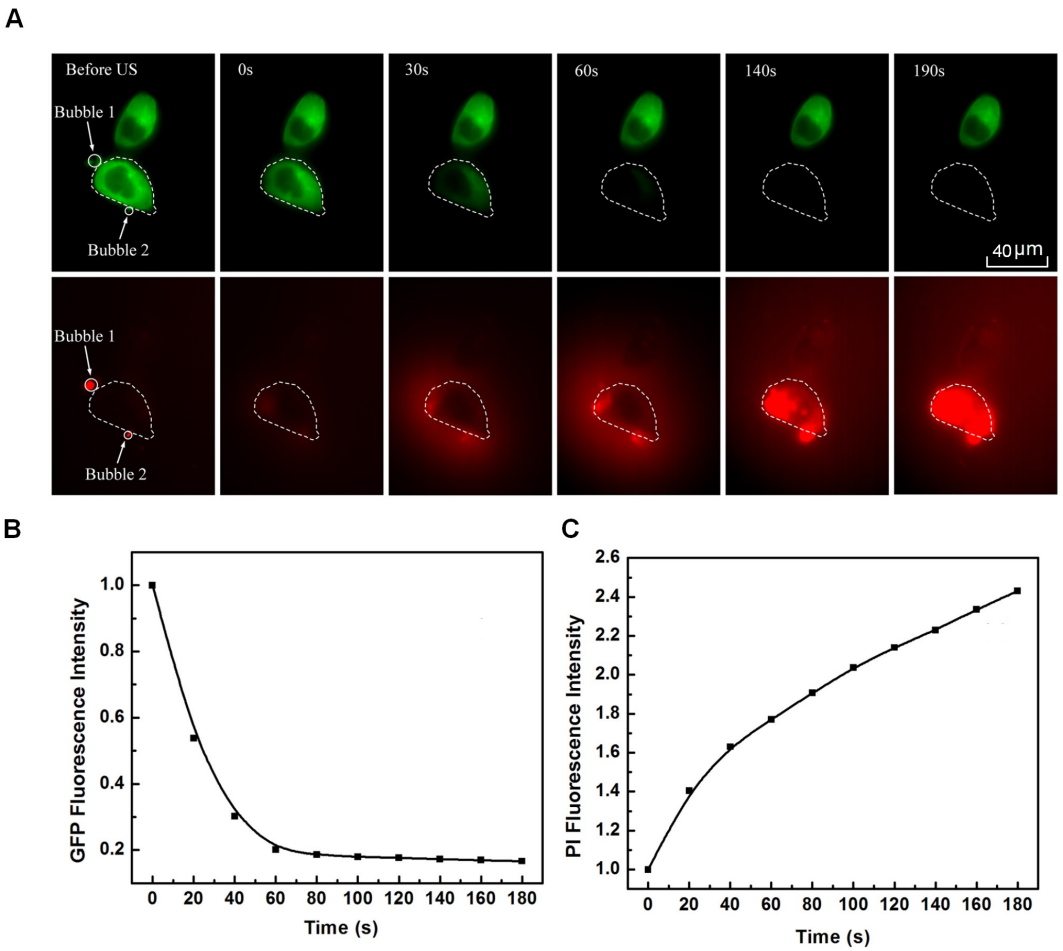


**Figure S1**. Fluorescence imaging analysis for a cell dead after sonoporation：(A) Spatiotemporally localized intracellular PI delivery (red in the bottom row) and α-tubulin cytoskeleton disassembly (green in the top row) in a cell triggered by microbubble-mediated sonoporation.; (B) Sonoporation-induced GFP intensity changes representing the temporal evolution of the integrity of α-tubulin cytoskeleton structure for the dead cell; and (C) Sonoporation-induced PI fluorescence intensity changes indicating the variation in the membrane permeability of the dead cell.

## II. A finite element model of the coupled bubble-fluid-cell system

In the simulation, the fluid environment around the bubble is assumed to be a homogeneous, incompressible and single-phase Newtonian fluid. When the microbubble is activated by US, the fluid around the bubble should obey the mass and momentum conservation laws:[61]

$$\frac{\partial \rho_l}{\partial t} + \nabla \cdot \left( \rho_l \mathbf{v} \right) = \mathbf{0},$$

$$\rho_l \left( \frac{\partial \mathbf{v}}{\partial t} + \left( \mathbf{v} \cdot \nabla \right) \mathbf{v} \right) = \nabla \cdot \left( -p\mathbf{I} + \mu_l \left( \nabla \mathbf{v} + \nabla \mathbf{v}^T \right) \right) .$$

Given that the bubble in the simulation is encapsulated, a modified Rayleigh-Plesset equation can be used to calculate the vibration of the bubble:[58,59]

$$\rho_l \left( R_b \ddot{R}_b + \frac{3}{2} \dot{R}_b^{\,2} \right) = \left( p_0 + \frac{2\sigma_g}{R} \right) \left( \frac{R_{b0}}{R_b} \right)^{3\gamma} - \frac{2\sigma_g}{R_b} - \frac{4\eta_l \dot{R}_b}{R_b} - p_0 - p_{ac}(t) - S ,$$

where $\rho_l$ and $p_0$ are the density and hydrostatic pressure of the surrounding liquid, $R_b(t)$ is the bubble radius as a function of time $t$, with $R_{b0}$ being its equilibrium value. $\sigma_g$ is the interfacial tension coefficient of the free gas bubble, $\gamma$ is the polytropic exponent of the gas core, $\eta_l$ is the dilatational viscosity coefficient of the fluid.

The effect of encapsulation in the equation is described by the term $S$, which can be written as:[58-60]

$$S = 4\chi \left( \frac{1}{R_0} - \frac{1}{R} \right) + 4\kappa_s \frac{\dot{R}}{R^2} ,$$

where $\chi$ and $\kappa_s$ denote the shell surface elasticity and the shell surface viscosity, respectively.

Since the size of the cell membrane is much greater than the microbubble's radius, the cell membrane can be treated as a homogeneous elastic wall close to the bubble, which should satisfy the stress–strain condition:

$$\sigma_{ij} = \frac{E}{1+v}(\varepsilon_{ij} + \frac{v}{1-2v}\varepsilon_{kk}\delta_{ij}),$$

when $i = j$, $d_{ij} = 1$, and when $i \neq j$, $d_{ij} = 0$. $\sigma_{ij}$ and $\varepsilon_{ij}$ are the stress tensor and strain tensor, respectively. $E$ is the Young's modulus of the cell membrane and $v$ is the corresponding Poisson's ratio. The strain tensor and the displacement of the cell membrane satisfy the following relationship:

$$\varepsilon_{ij} = \frac{1}{2}(u_{i,j} + u_{j,i}).$$

The boundary conditions of velocity continuity and pressure continuity must also be satisfied on the fluid–solid interface:[61]

$$\mathbf{v}_{\text{fluid}} = \frac{\partial \mathbf{u}_{\text{solid}}}{\partial t},$$

$$\sigma \cdot \mathbf{n} = [-p\mathbf{I} + \mu(\nabla \mathbf{v}_{\text{fluid}} + (\nabla \mathbf{v}_{\text{fluid}})^{T})] \cdot \mathbf{n},$$

where $\boldsymbol{v}_{\text{fluid}}$ is the fluid velocity vector, $\boldsymbol{u}_{\text{solid}}$ is the solid displacement vector and $\mathbf{n}$ is the unit normal vector on the fluid–solid interface.

During the interaction between the bubble and the cell, the shear stress is explicitly discussed based on the Nyborg's theory:

$$\tau_{xz_0} = \frac{1}{4}\left(\frac{\rho_l \eta_l}{\pi f}\right)^{\frac{1}{2}}\left(u_{xz_0}\frac{\partial u_{xz_0}}{\partial x}\right)_{z=z_0},$$

where $f$ is the driving frequency, and $u_{xz_0}$ and $\frac{\partial u_{xz_0}}{\partial x}$ denote the $x$ component of solid displacement and its gradient.

The typical parameters used in the simulation are listed as follows: the radius of the bubble is $R_{b0}$=1.5 $\mu$m, the US driving pressure amplitude is $p$=300 kPa, the US frequency is $f$=1MHz, the initial distance between the bubble center and the cell membrane is set to be twice the bubble

radius, and the Young's modulus of the elastic wall ($E$) corresponds to the Young's modulus of cells. Other parameters used in the current work are listed in the following:[66] the fluid density $\rho_l = 10^3 \mathrm{kg/m^3}$, the fluid viscosity $\mu_l = 10^{-3} \mathrm{Pa \cdot s}$, the static pressure in fluid $p_0 = 1.013 \times 10^5 \mathrm{kg/m^3}$, the polytropic exponent $\gamma$=1.07, the interfacial tension coefficient of the free gas bubble $\sigma = 0.072 \mathrm{N/m}$, the microbubble shell surface elasticity and viscosity are $\chi$=0.32 N/m, and $\kappa_s$=4×10$^{-9}$ kg/s, respectively.

## III. Video recording samples of real-time fluorescence observation on cell-cycle-dependent cellular responses induced by microbubble-mediated sonoporation

Video S1: A representative sample of temporal variations in the membrane permeabilization and cytoskeleton disassembly of HeLa cells synchronized in the G1 phase.

Video S2: A representative sample of temporal variations in the membrane permeabilization and cytoskeleton disassembly of HeLa cells synchronized in the S phase.

Video S3: A representative sample of temporal variations in the membrane permeabilization and cytoskeleton disassembly of HeLa cells synchronized in the G2/M phase.