Supporting information

Biomineralized nanosilica-based organelles endow living yeast cells with non-inherent biological functions

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Experimental Procedures

1. Materials and synthesis

1.1 Materials

The reagents used in this work include $NaSiO_3 \cdot 9H_2O$ (Shanghai, Xilong chemical factory, Guangdong, China) and Maltose (BR Aoboxing universeen Bio-tech.co.LTD, Beijing, China). Maltose was purchased from Beijing Spiritualizing Star Biotechnology Company Limited, China. Yeast was purchased from ANGEL YEAST CO., Ltd.. Doxorubicin Hydrochloride (DOX-HCl, Beijing Huafeng United Technology Co., Ltd., China) was used as the model drug to examine the pH-responsive anticancer drug carrier. All chemicals were analytical pure and were used without further purification.

1.2 Synthesis of the engineered yeast cells (EYCs)

In a typical synthesis, 0.6 g of yeasts dry powder was cultured in an aqueous solution of Maltose (0.5 wt % 200 mL) at 30 ^oC for 30 min. Then 0.5684 g of $Na_2SiO_3 \bullet 9H_2O$ was added slowly into the mixture under moderate stirring. After that the mixture was transferred to a shaking table and vibrating for 24 h at the shaking speed of 150 r/min at 30 ^oC. Subsequently, the cells were collected by centrifugation (3000 r/min) and washed with distilled water three times. Finally, the cells collected were dried at 40 ^oC for 12 h, in order to dehydrate H_2SiO_3 obtained to produce SiO_2.

1.3 Synthesis of DOX@EYCs

DOX was easily loaded into the EYCs by adding a certain amount of EYCs into the DOX solution. Typically, 40 mg of EYCs were added into 10 mL of DOX solution (100 mg/L). The suspension was incubated in a thermostatic oscillator at the speed of 150 r/min at 30 ^oC for 24 h. Centrifugation was conducted till the

supernatant was colorless to thoroughly wash off the free DOX and then the products were dried under vacuum at 40 ^{o}C for 24 h.

1.4 Synthesis of DOX@EYCs@FA

Folic acid (FA) was used to DOX@EYCs for active tumor targeting based on the fact that folate receptor (FR) is over-expressed on the membrane of many cancer cells yet rarely found in normal cells. FA cannot directly deposit onto DOX@EYCs because both of them are negatively charged in the solution. Herein, a polymer with positive charge is essential to bridge FA and the cell wall. In this study, the polyelectrolyte, poly (diallyldimethylammonium chloride) (PDDAC, Mw = 100,000-200,000 Dalton, Aldrich) are used. The typical adsorption conditions used to obtain the DOX@EYCs encapsulated by PDDAC layers were that 10 mg DOX@EYCs were incubated with 10 mg/mL of PDDAC in 0.10 M NaCl at 30 ^{o}C with shaking at 150 rpm for 30 min. After adsorption, the cells were washed using 0.10 M NaCl to get rid of the residual polyelectrolytes. Then the DOX@EYCs@PDDAC with positive charge could be obtained. Subsequently, the above DOX@EYCs@PDDAC were incubated with 0.1M of FA in 0.10 M NaCl at 30 ^{o}C with shaking at 150 rpm for 30 min. FA could be adsorbed onto the surface of the DOX@EYCs@PDDAC by electrostatic force. After adsorption, the cells were washed using 0.10 M NaCl to get rid of the residual FA and then the DOX@EYCs@PDDAC wrapped folic acid (DOX@EYCs@FA) could be synthesized simply.

2. Characterizations

The identity of the samples were verified by the X-ray powder diffraction (XRD) on a Bruker D8 & Advance X-ray diffractometer with Cu Ka radiation (λ = 1.5406 Å). The morphology of the obtained yeast cells was observed via an AMARY-1000b (Germany Bruker) scanning electron microscope (SEM). Fourier transform infrared spectrometer (FT-IR) analysis was conducted on PekinElmer spectrum 400 Fourier transformer infrared

spectrometer in the wavenumber range 4000-400 cm^{-1}. The TG analysis was performed on an EXSTARTG/DTA 6300 instrument. The transmission electron microscopy (TEM), high-resolution transmission electron microscopy (HRTEM), energy dispersive spectroscopy (EDS) and selected area electron diffraction (SAED) images were received on a microscope (JEOL, JEM-2010).eds The EYCs were crushed for 2 hours by Ultrasonic Homogenizer. Then the broken cells was centrifuged and washed to remove the cells debris and separate the $nSiO_2$ from yeast cells. The HRTEM and STEM-EDX samples were prepared by crushing the yeast cells and then dipping 3 mL distilled water solution onto the carbon-coated copper grids. Fluorescence of the sample were observed on a Leica TCS SP8 Confocal laser scanning electron microscope (CLSM). A TU-1900 UV/Vis spectrophotometer was adopted to obtain the absorbance of DOX, and further determine the concentration of the DOX solution.

X-Ray photoemission spectroscopy (XPS) measurements of films of biogenic SiO_2 nanoparticles cast on to Cu substrates were carried out on a VG MicroTech ESCA 3000 instrument at a pressure better than 1×10^{-9} Torr. The overall resolution was ~1 eV for the XPS measurements. The core level spectra were background corrected using the Shirley algorithm and the chemically distinct species were resolved using a nonlinear least squares fitting procedure. The core level binding energies (BE) were aligned with the adventitious carbon binding energy of 285 eV.

2.1 Bio-TEM observation

The EYCs were harvested by centrifugation and fixed by glutaraldehyde at room temperature, then rinsed with PBS and dehydrated through a graded ethanol series, and finally cleared with propylene oxide. Then, the cell sample was embedded in EPOM812 and polymerized in an oven at 60 ^{o}C for 48 h. Ultrathin sections of approximately 60 nm in thickness were cut with a diamond knife on a Leica UC6 ultramicrotome and transferred to the copper grid.

2.2 Trypan blue exclusion test

When cells are damaged or dead, trypan blue can penetrate the cell membrane, and bind to the disintegration of DNA and color it blue, while the living cells can prevent the dye from entering cells. So it is possible to discriminate dead cells and living cells by trypan blue. Cell suspension at a high concentration (150 cells/mL) was prepared. 1mL trypan blue solution (0.8 mM in PBS) and 1 mL cell suspension were mixed. About 3 min later, one drop of the mixed solution was dipping on a clean glass slide carefully, then the stained products were observed by light microscope. The living cells show achromaticity while the whole body of the dead cells show blue.

2.3 Cell proliferation test

The EYCs and the controlled S. cerevisiae cells were cultured in YPD medium (2 % peptone and 2 % glucose) at 30 ^{o}C with shaking at 150 rpm. The cell density was measured by turbidity (OD600) with the UV spectrophotometer at the wavelength of 600 nm.

2.4 Confocal Laser Scanning Microscopy (CLSM).

Due to the quantum size effect, the silica showed fluorescence when excited at the wavelength of about 395 nm. The samples were observed with a Leica TCS NT confocal laser scanning microscope (CLSM) (Germany).

2.5 Protein Characterization.

To identify the proteins in the yeast cells responsible for synthesis of nSiO_2, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a 5 % (w/v) polyacrylamide gel in 1M Tris-

HCl buffer (pH = 6.8) stacking gel and a 12 % (w/v) polyacrylamide in 1.5 M Tris-HCl buffer (pH = 8.8) resolving gel. $nSiO_2$ were harvested after the EYCs were crushed by Ultrasonic cell crusher and washed with double distilled water. And the samples (40 μL) were mixed with 10 μL 5x SDS-PAGE loading buffer (1M Tris-HCl, 50 % glycerol, 10 % SDS, 25 % (v/v) β-mercaptoethanol, 1 % bromophenol blue) and heated at 100 ℃ for 5 min, followed by loading 20 μL of the resulting solution on the gel. Experiments were performed at 120 V for 1.5 h. Proteins in SDS-PAGE gels were visualized with Coomassie brilliant blue staining for 15 h. The gels were destained (AcOH / Methanol / double-distilled H_2O = 2 : 1: 17). Matrix assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF-MS) was conducted on a MALDI TOF/TOF 5800 mass spectrometer (AB SCIEX, USA) to determine the type of proteins.

2.6 Flow cytometric analysis

EYCs and the controlled S. cerevisiae cells were washed with distilled water and cold PBS, respectively. Dilute the cell density to $1*10^6$ cells/mL through cell counting. Then the cells were stained with propidium iodide (PI, 10 μg/mL in PBS, Sigma-Aldrich, Co., MO, USA) and fluorescein isothiocyanate (FITC, 10 μg/mL in PBS, Sigma-Aldrich, Co., MO, USA) at 37 ^oC for 30 minutes. After that the stained cells were filtered through 41 μm Nylon net filters and analyzed by a COULTER Epics XL flow cytometry system (Beckman Coulter, Inc., CA, USA) and the percentages of cells viability were recorded.

2.7 MTT colorimetric assay

After treating the EYCs@DOX@FA with Hep G2 cells and BRL-3A cells, MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, 20 μL, 5 mg/mL, (Sigma-Aldrich, Co., MO, USA)) in filtered PBS was added to each well of the control and the DOX@EYCs@FA cells and incubated at 37 ^oC, for 5 hours under 5 % CO_2. After the incubation, the medium was removed and then 150 microliters of DMSO were added to each well to dissolve

the dark blue crystals completely. The absorbance of the solution in each well was measured by a microplate reader at the wavelength of 570 nm. The degree of cell proliferation was reflected by the average value of absorbance and the cytotoxic effects of DOX@EYCs@FA were calculated.

2.8 Subcellular localization of DOX from DOX@EYCs@FA in Hep G2 cells by CLSM imaging

For CLSM observations, Hep G2 ($5*10^4$ cells per dish) were seeded in laser confocal dish and then treated with DOX@EYCs@FA at the same final concentration of 1 μg/mL (with respect to DOX) for 12 h. Then the media were removed and the cells were washed three times with PBS to remove the residual nanoparticles. Then, Hoechst 33342 (10 μM, 1mL) was added and cells were incubated at 37 °C for 30 min. After that, the media were removed and the cells were washed three times with PBS. Finally, 200 nM red Chloromethyl-X-rosamine was added into the cells and then the cells were incubated at 37 °C for another 15 min. cells were visualized under Leica TCS SP8 Confocal laser scanning electron microscope. Blue luminescent emissions from Hoechst 33342 was excited at the wavelength of 405 nm. Red luminescent emissions from DOX was excited at the wavelength of 488 nm. Orange luminescent emissions from Chloromethyl-X-rosamine was excited at the wavelength of 599 nm.

2.9 *In vivo* antitumor efficacy

The BALB/c nude mice (8 weeks old; 20-25 g) were provided by Department of Human Anatomy, Xinxiang Medical University, Henan, P. R. China. The BALB/c nude mice were inoculated with hepatocellular carcinoma cells (5×10^6) in the back through subcutaneous injection for tumor induction. Tumor lengths were measured by a caliper. When the tumors grew up to about 10 mm in length, the model mice were used in the experiments. Then, the tumor-bearing mice were optionally divided into three groups (5 mices/group). Then the seven groups were intratumorally administrated with Saline, native yeasts, EYC, DOX@Native yeasts, DOX@EYCs,

DOX@EYCs@FA and free DOX, respectively. DOX@Native yeasts, DOX@EYCs, DOX@EYCs@FA and free DOX were administrated by a single intratumoral injection at a DOX-equivalent dose of 10 mg/kg at a fixed depth of needling insertion (2 mm under the tumor surface). At 16 days post-injection, the tumors were collected, washed by PBS, and then measured and recorded. As expected, the control groups treated with the native yeasts showed the rapid increase in the size of the tumor with the increase of time. All experiments were performed in compliance with the institute's (Xinxiang Medical University) policy on animal use and ethics.

2.10 Histological examination

At the end of the *in vivo* antitumor, all the mice of the five groups were sacrificed and the organs including heart, liver, spleen, lung and kidney were resected. Tissue samples were digested in concentrated nitric acid until fully digested. Then the other organs were fixed in 10 % formalin solution for H&E staining. After the hematoxylin-eosin (H&E) staining, the sections were examined by Eclipse TE 300 microscope (Nikon, Japan).

3. Figures and Tables

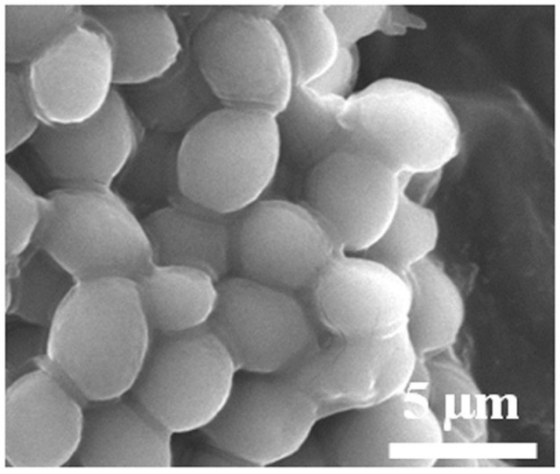

Figure S1. SEM image of the native yeast cells.

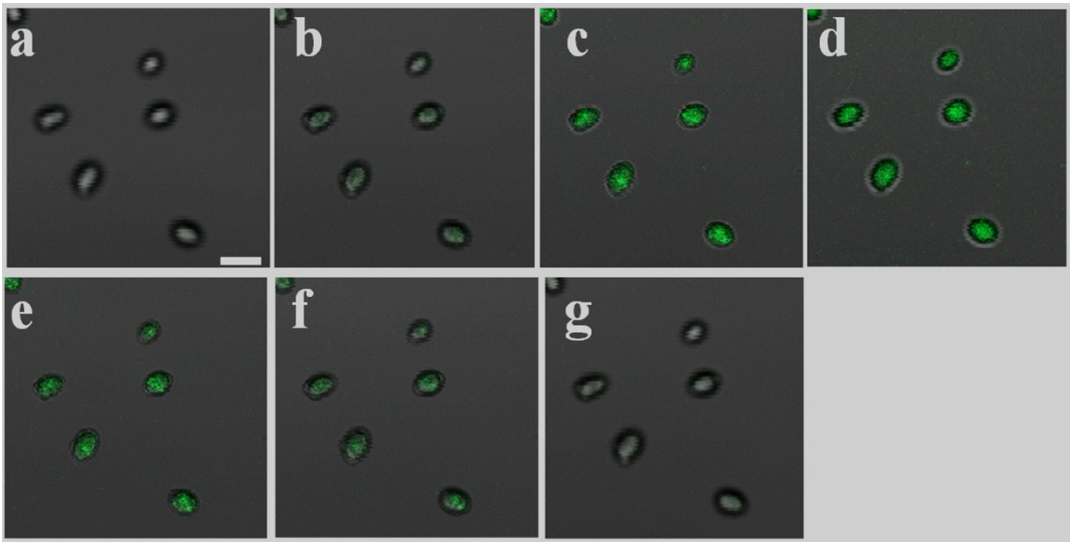

Figure S2. Confocal laser scanning images of the EYCs. (a-g) Different Z-axis focal planes excited by 395 nm laser beam. (The scale bars are 5 μm) The different Z-axis (CLSM) images show that the biogenic SiO_2 nanoparticles could be observed inside the yeast cells base on their autofluorescence.

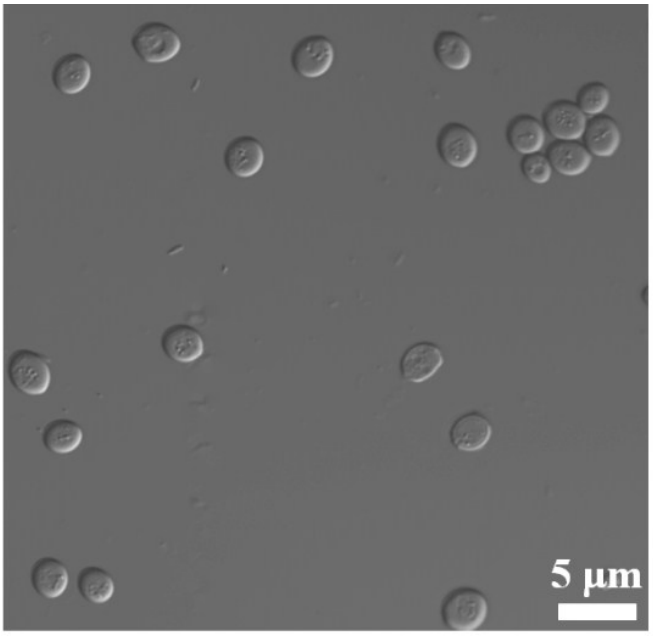

Figure S3. Confocal laser scanning image of native yeast cells.

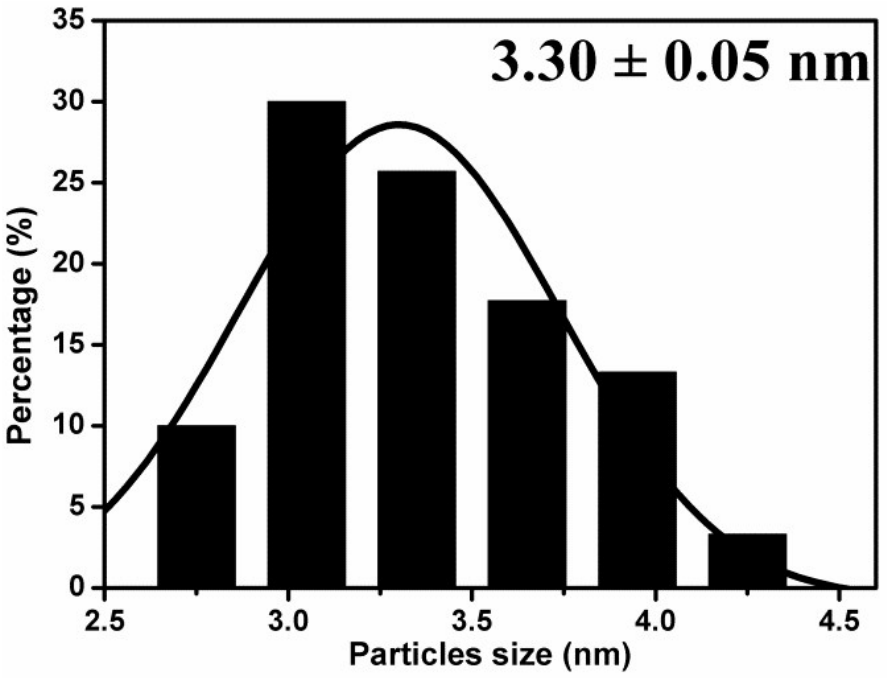

Figure S4. The particle size histograms recorded from representative silica nanoparticles.

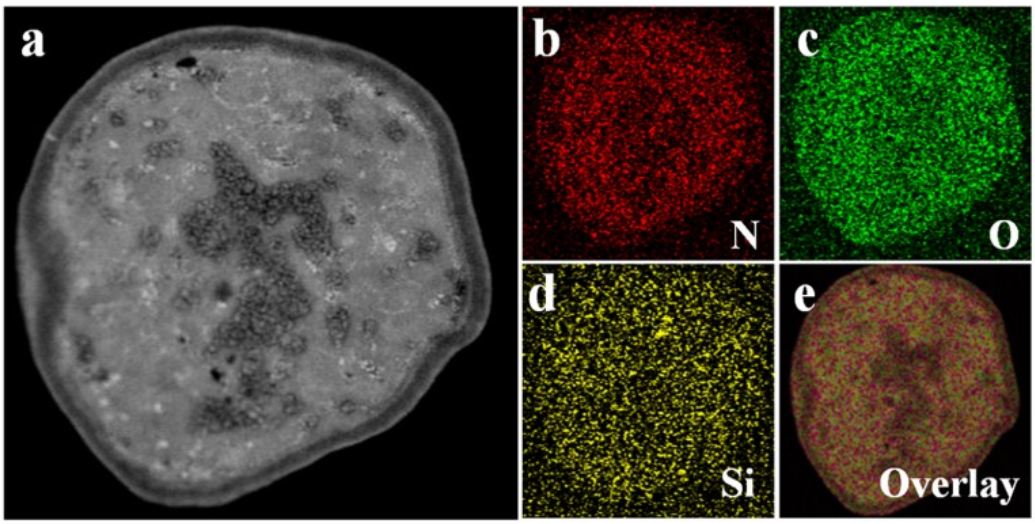

Figure S5. TEM image (a) and EDX elemental maps of EYC's fine slices (b-e).

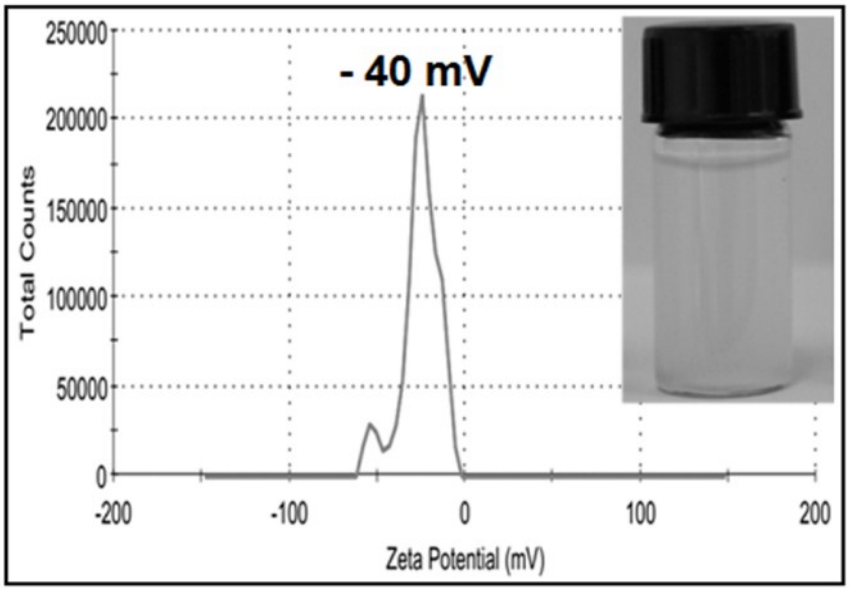

Figure S6. Zeta potential of SiO_2 nanoparticles dispersion in PBS. (Inset is the transparent silica nanoparticles colloidal solution in PBS.)

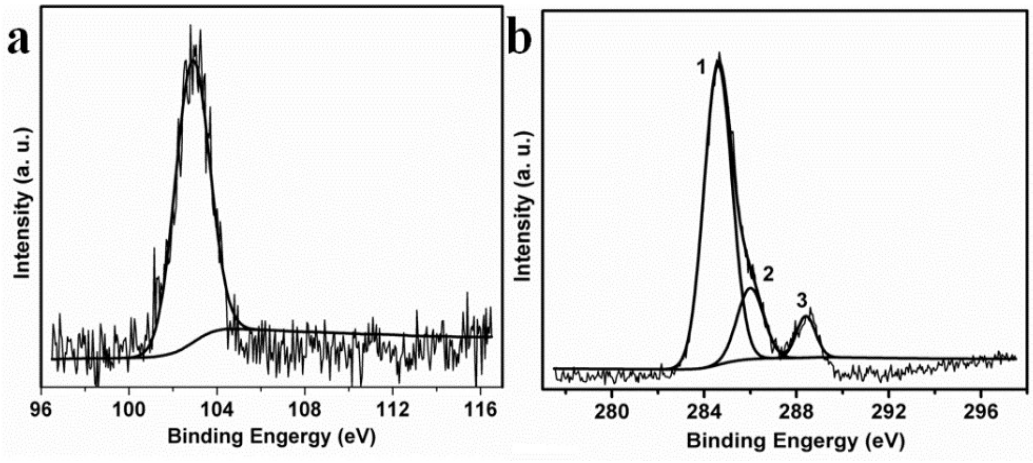

Figure S7. XPS data showing the Si 2p (a) and C 1s (b) core level spectra recorded from the biosynthesized silica nanoparticle into yeast cells; (a) the Si 2p spectrum could be attributed to 2p3/2 binding energies (BES) of 102.9 eV, which agrees excellently with values reported for SiO_2 [1]; (b) it shows that curves 1, 2 and 3 correspond to the chemically distinct C 1s core levels originating from the hydrocarbon chains, a-carbon and –COOH groups present in the intracellular proteins with binding energies (BEs) of 284.3, 285.7 and 288.5 eV respectively, indicating that the as-prepared intracellular nanoparticles should be protein/SiO_2 composite.

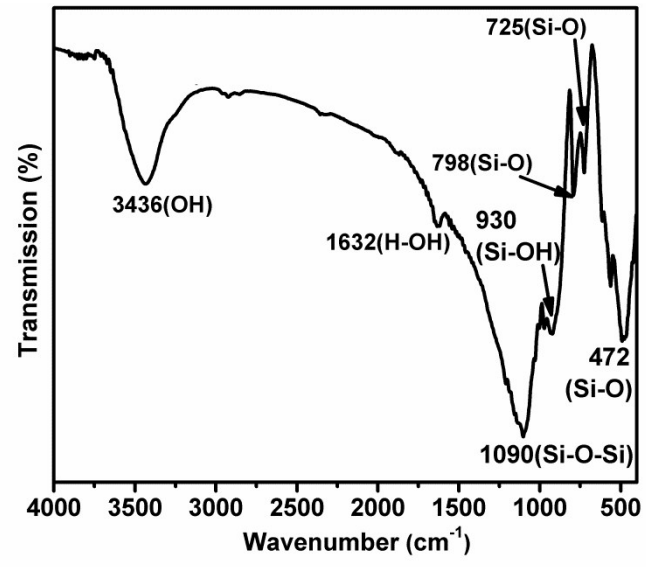

Figure S8. FT-IR of intracellular silica nanoparticles.

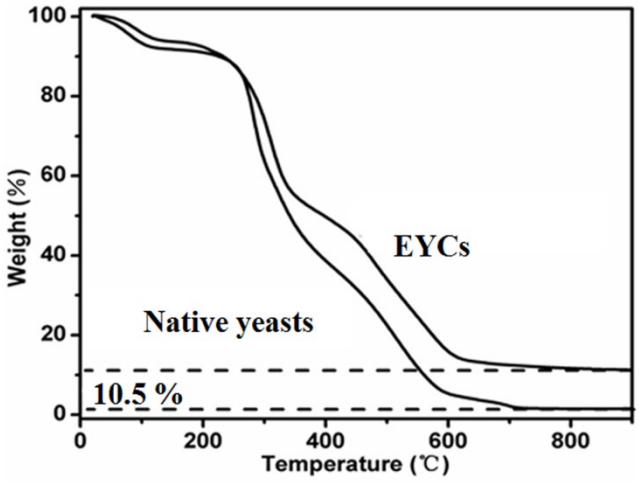

Figure S9. Thermo-gravimetric (TG) analyses of the EYCs and native yeast cells. TG curves of the EYCs and native yeast cells were determined under air atmosphere from 30 to 900 °C, respectively. The mass loss of the EYCs and native yeast cells at 200-700 °C is assigned to the combustion of the yeast cell. After 700 °C, the native yeast cells are burned out and the amount of $nSiO_2$ is about 10.5 % by weight in the EYCs.

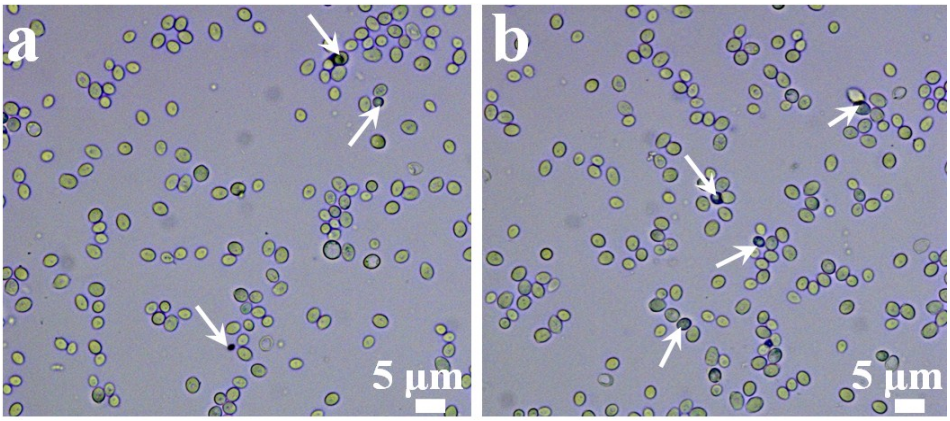

Figure S10. Light micrograph of the control S. cerevisiae cells (a) and EYCs (b) stained by trypan blue. The white arrows mark the dead cells, which were stained blue by trypan blue.

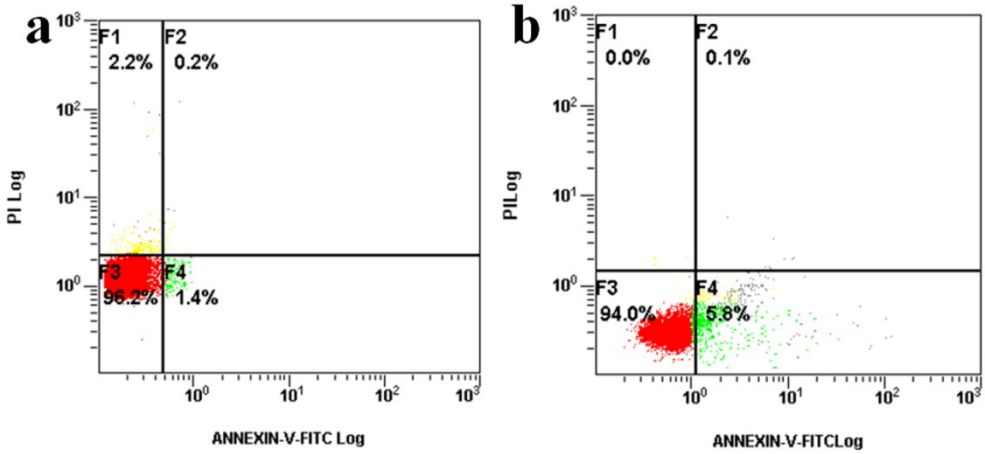

Figure S11. Flow cytometric quadrant analysis of pure yeast cells (a) and EYCs (b). F1, F2, F3, and F4 zones represent necrosis, apoptosis, normality and early apoptosis, respectively.

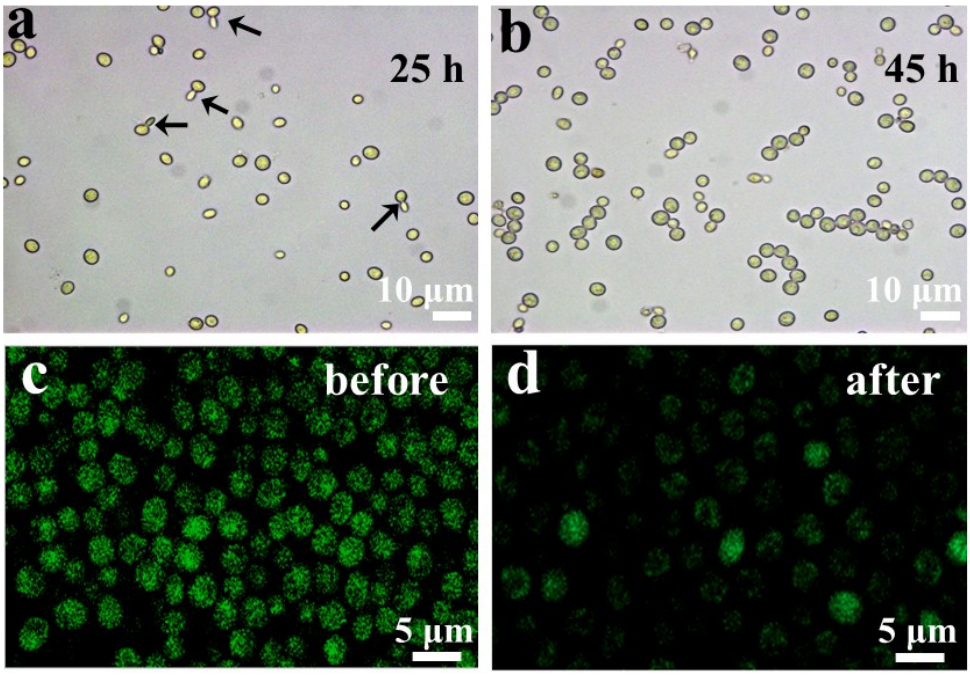

Figure S12. The light micrographs of the EYCs after incubation for 25 h (a) and 45 h (b) in YPD broth; The CLSM of EYCs before (c) and after (d) proliferation.

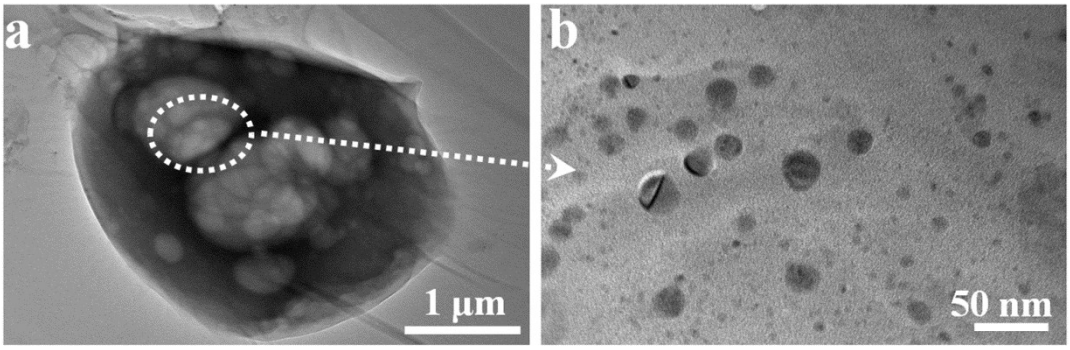

Figure S13. (a) TEM and (b) HRTEM image of the EYC's fine slices after the EYCs cultured for 45 h.

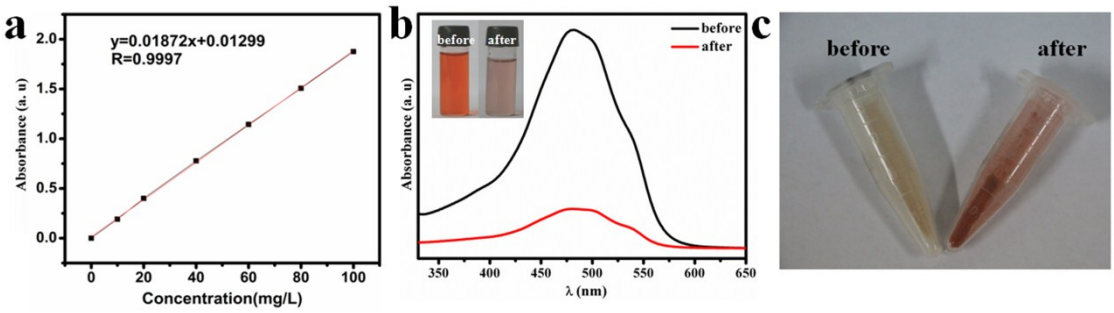

Figure S14. (a) Absorbance at 480 nm vs. free DOX concentration (optical path = 1 cm). Solid line is the liner fit using the analysis tool in Origin software and the R2 = 0.9997; (b) UV-Visible spectra of DOX solution before and after loading into the EYCs (5 mg sample added into 5 mL DOX solution with a concentration of 250 μg/mL). Inset is the digital image of the corresponding solution; (c) Digital images of EYCs before and after loading DOX.

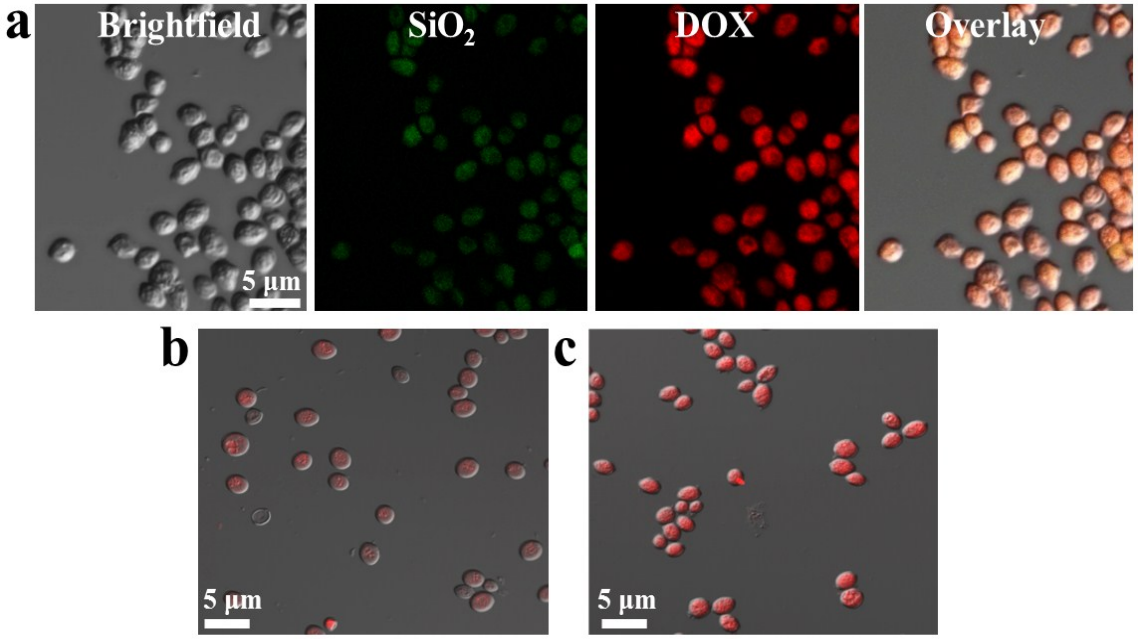

Figure S15. (a) CLSM images of the EYCs after loading DOX;
CLSM images of (b) the native yeast cells and (c) EYCs after loading DOX, respectively.

Table S1. Amount of DOX adsorbed into EYCs and YCs (yeast cells) under the same condition.

Samples	Adsorption efficiency of DOX adsorbed (%)	Amount of DOX adsorbed (μg/mg)
EYCs	70.80	17.70
YCs	23.10	5.78

Table S2. The electro-conductibility of DOX@EYCs in the solution of PDDCA and the electro-conductibility of DOX@EYCs@PDDCA in the solution of FA. The results of the electro-conductibility show that the DOX@EYCs wrapped by folic acid (DOX@EYCs@ FA) could be synthesized by the covering of PDDCA.

Sample	pH	Zeta potential (mv)
Native yeast cells	7.27	-16.8
EYCs	7.03	-25.1
DOX	5.39	+1.1
DOX@EYCs	6.26	-15.6
FA	7.33	-17.2
PDADMA	3.83	+19.1
DOX-EYCs@PDDCA	6.60	+23.1
DOX@EYCs@ PDDCA@FA	6.36	-2.6

Figure S16. Digital images (from left to right) of EYCs, free DOX and DOX@EYCs obtained after storage for 7 days under centrifugation at the speed of 10000 r/min for 10 min. No obvious DOX release was observed after storage for 7 days, indicating the stability of the DOX@EYCs.

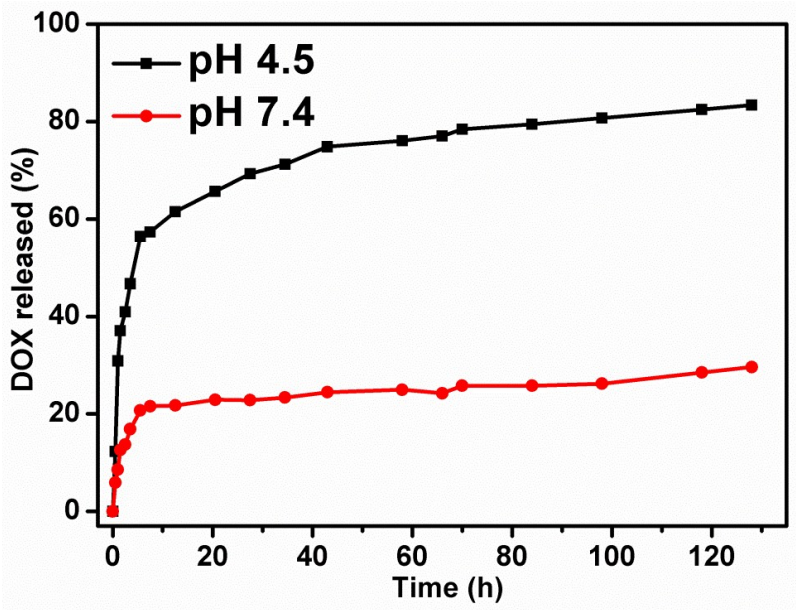

Figure S17. *In vitro* DOX release profiles of DOX@EYCs under different pH.

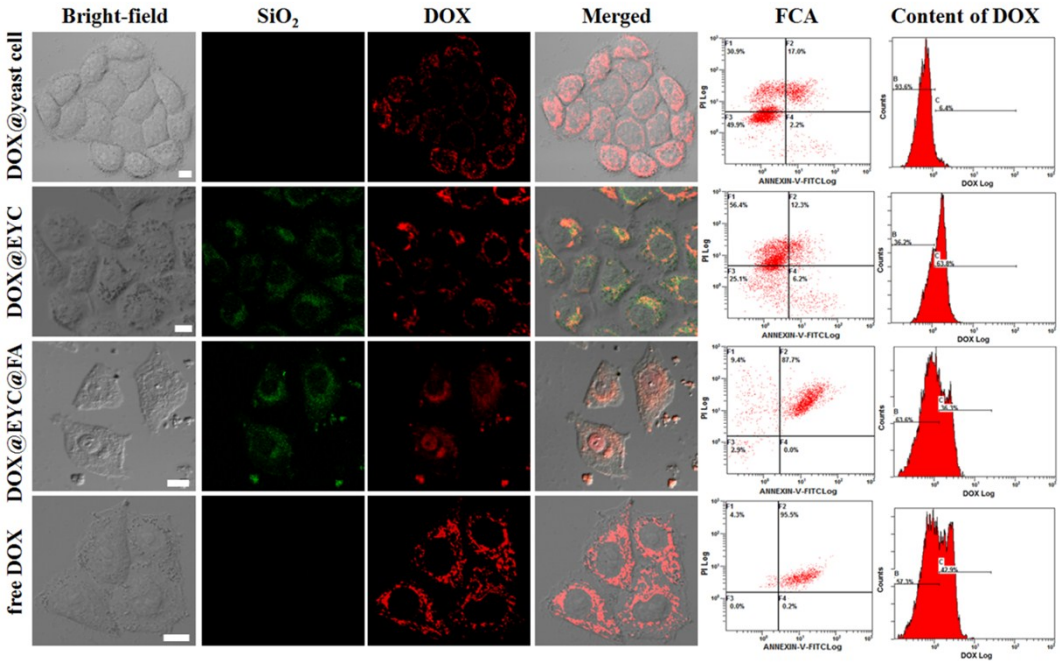

Figure S18. CLSM images of Hep G2 cells incubated with DOX@EYCs@FA for 12 h (from left to right: optical microscope images of Hep G2 cells; green fluorescence arised from $nSiO_2$; red fluorescence arised from DOX molecule; merged images. The scale bars are 5 μm) and Flow cytometry analysis of quantification of DOX levels uptake into Hep G2 cell and evaluation of the death pathways of Hep G2 cells treated with DOX@Native yeasts, DOX@EYC, DOX@EYC@FA and free DOX at the equivalent DOX concentration (60 μg/mL) for the same incubation time period (8 h). F1, F2, F3, and F4 zones represent necrosis, apoptosis, normality and early apoptosis, respectively.

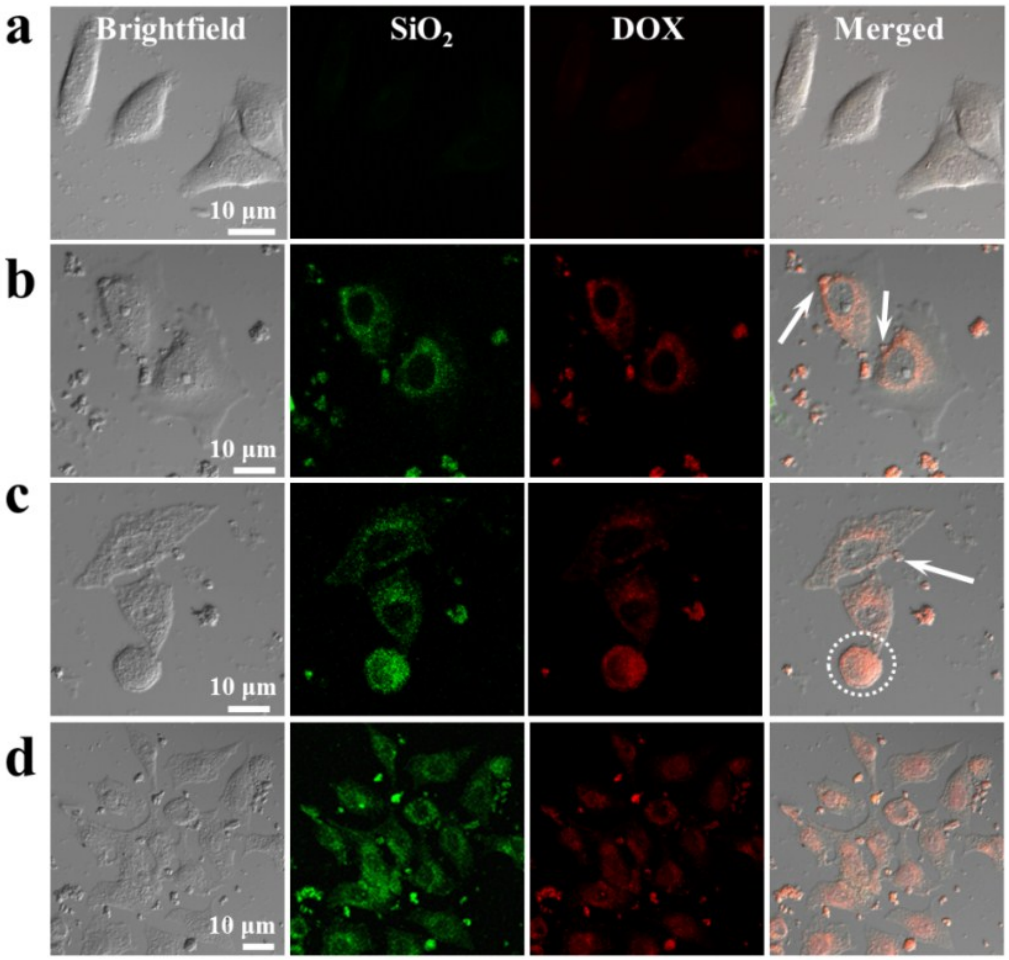

Figure S19. The endocytosis process of the DOX@EYCs@FA uptake in HepG2 cells. The time-dependent CLSM images of Hep G2 cells incubated with DOX@EYCs@FA for 5 h (a), 8 h (b), 12 h (c) and 24 h (d), respectively. (The arrows indicated the EYCs, which was about to be endocytosed by Hep G2 cell. The ring indicated the dead Hep G2 cells, which showed stronger florescence inside the cell.)

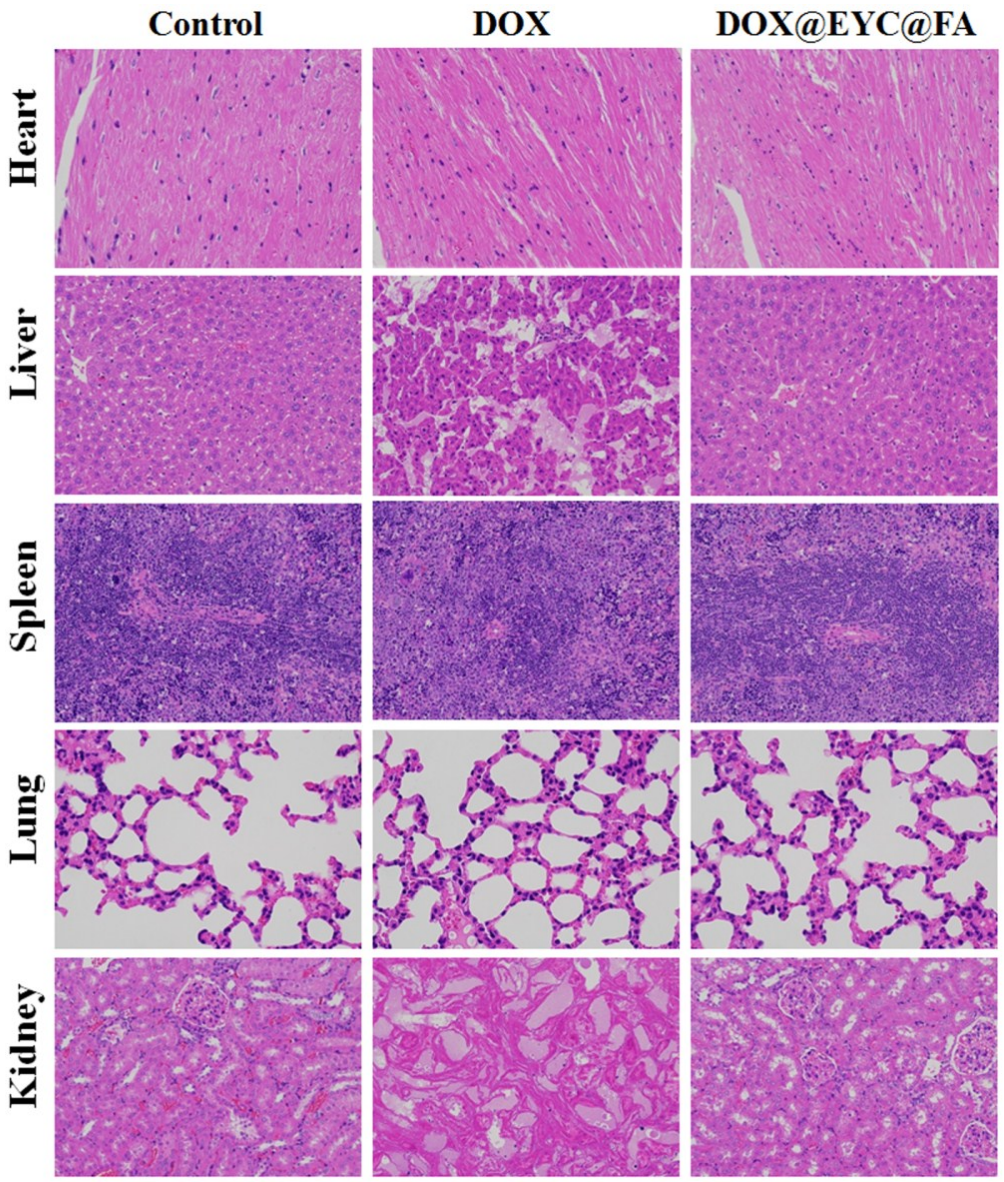

Figure S20. Histological examination of primary organs (heart, liver, spleen, lung and kidney) after various treatments indicated. Sections for light microscopy were stained with hematoxylin-eosin (H&E). Samples were from three different mice of the experiment. Magnification was 200×.

References

1. V. Bansal, D. Rautaray, A. Bharde, K. Ahire, A. Sanyal, A. Ahmad and M. Sastry, *J. Mater. Chem.* **2005**, *15*, 2583-2589.