Supporting Information

Exploration of Zinc Oxide Nanoparticles as a Multitarget and Multifunctional Anticancer Nanomedicine

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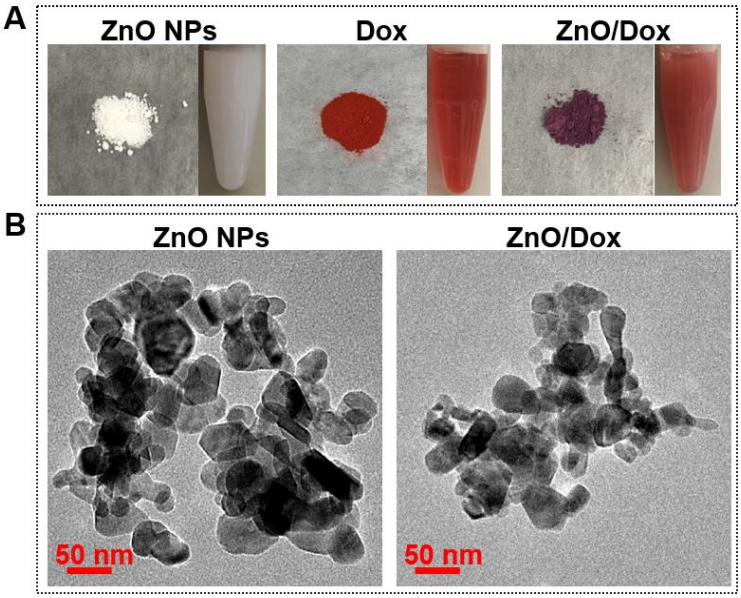

Figure S1. Photographic images (A) and TEM micrographs (B) of ZnO NPs and ZnO/Dox.

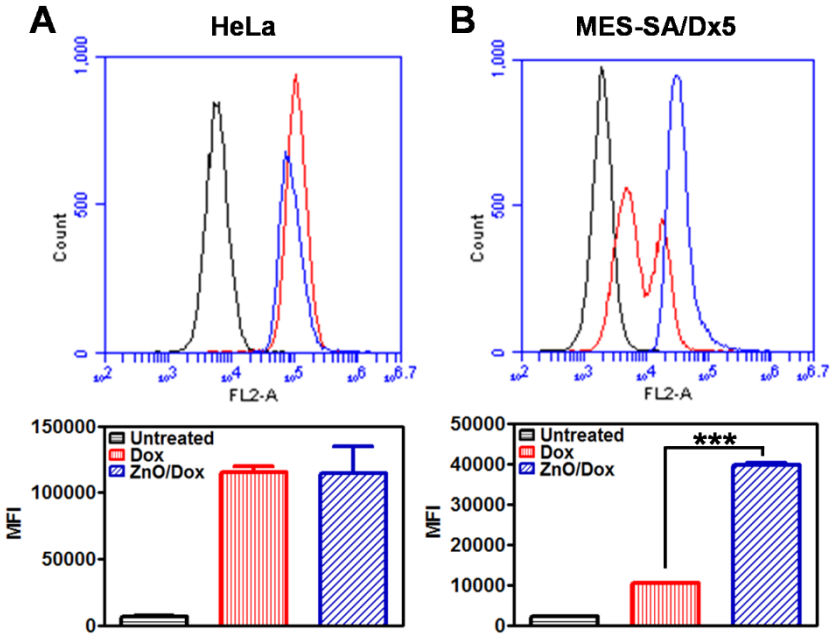

Figure S2. Cellular uptake of Dox and ZnO/Dox determined by flow cytometry. (A) HeLa cells and (B) MES-SA/Dx5 cells. Incubation time: 1h. Data are presented as the mean ± SD. ***P< 0.001.

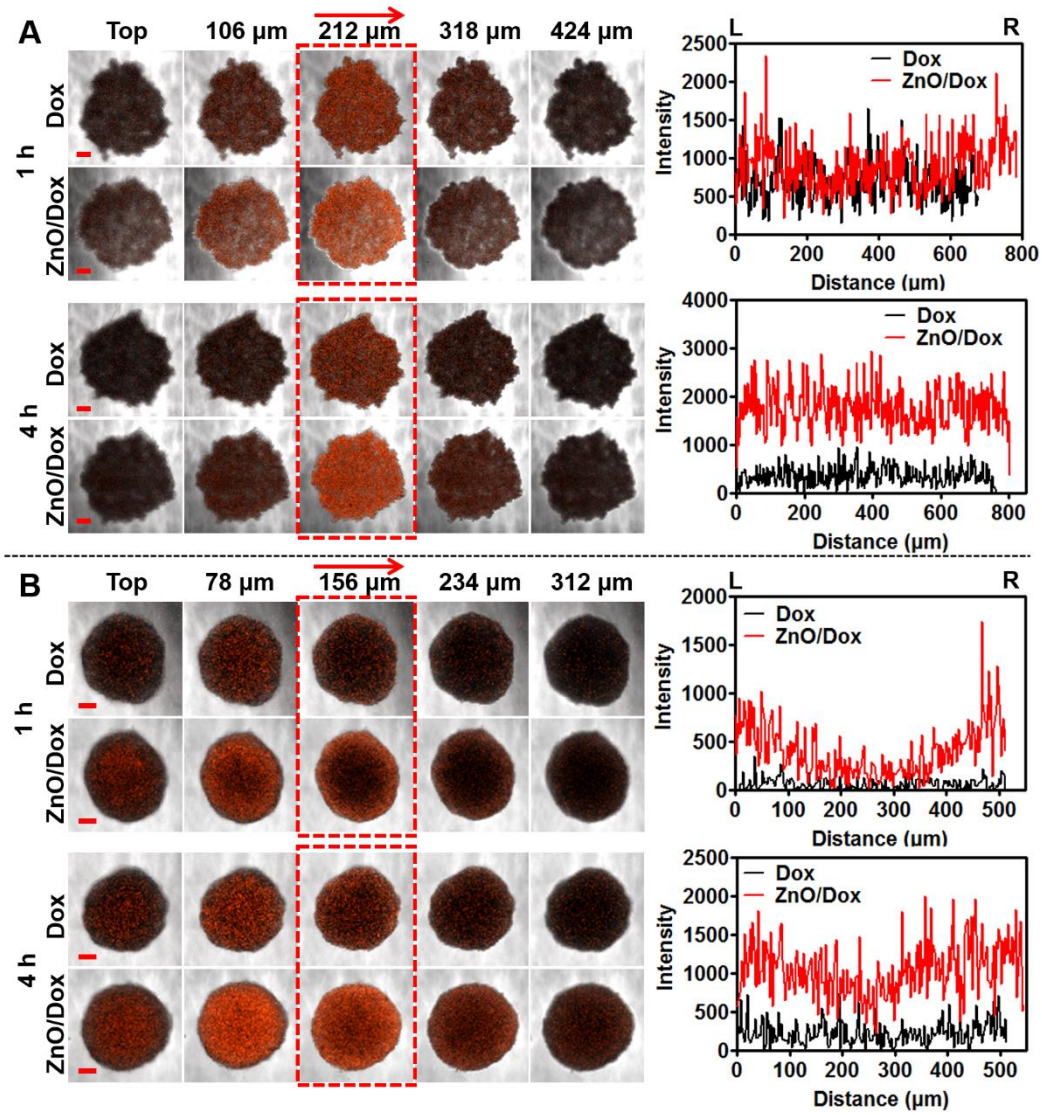

Figure S3. Drug penetration through (A) MDA-MB-231 and (B) NCI/ADR-RES cell spheroids. The right panels are the fluorescence intensity of the selected Z-stack images analyzed by a NIS-Elements AR software. L: left, R: right. The scale bar represents 100 μm.

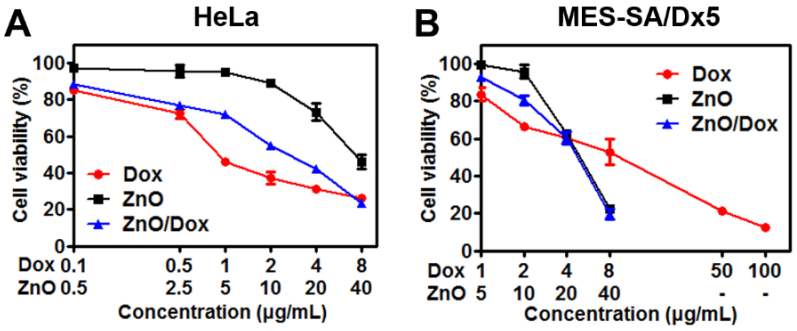

Figure S4. Cytotoxicity of Dox, ZnO NPs, and ZnO/Dox in (A) HeLa and (B) MES-SA/Dx5 cell monolayers. Cells were incubated with the formulations for 24 h, followed by MTT Assay. Data are presented as the mean ± SD.

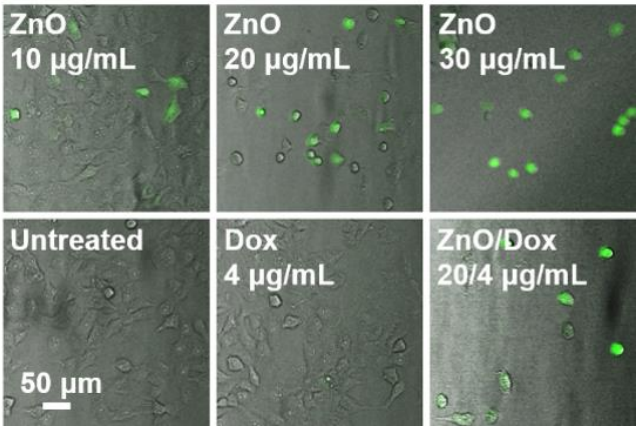

Figure S5. Intracellular levels of ROS. The NCI/ADR-RES cells were incubated with the formulations for 24 h. The intracellular ROS production was stained by H2DCFDA and analyzed by fluorescence microscopy.

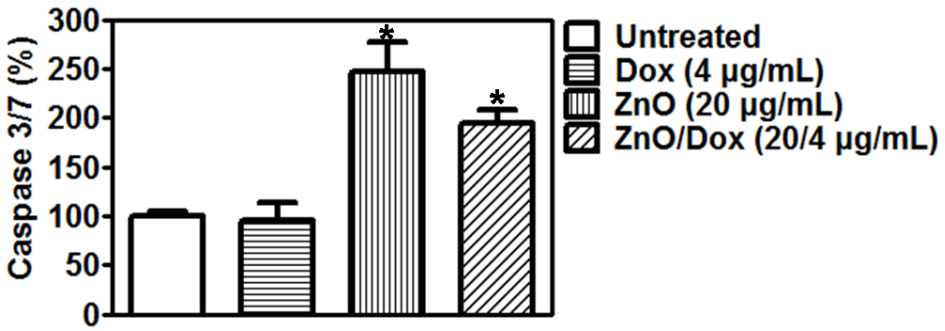

Figure S6. Intracellular levels of caspase 3/7. The NCI/ADR-RES cells were incubated with the formulations for 24 h. The caspase levels were measured by the Apo-ONE® Homogeneous Caspase-3/7 Assay (Promega). Data are presented as the mean ± SD. *P< 0.05. All P-values shown are *vs* the untreated group.

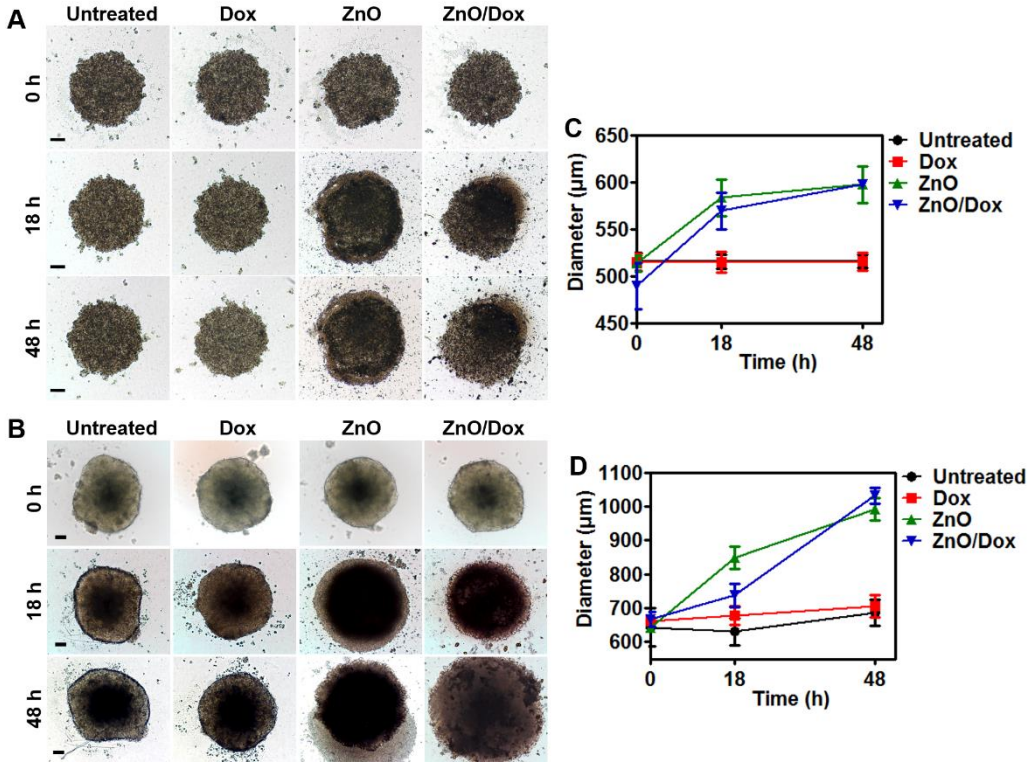

Figure S7. Images of (A) MDA-MB-231 and (B) NCI/ADR-RES cell spheroids after treatment with Dox, ZnO NPs, and ZnO/Dox. The images were captured by a Nikon Eclipse Ti microscope system at 40×. The scale bar represents 100 μm. The

mean size of (C) MDA-MB-231 and (D) NCI/ADR-RES spheroids was measured under microscopy.

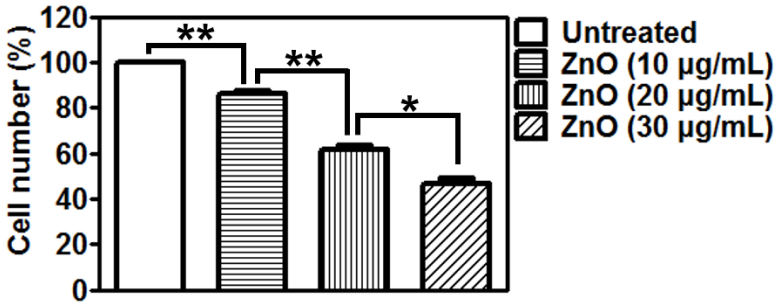

Figure S8. Inhibition of cancer cell adhesion by ZnO NPs. The cell number of the untreated group was served as 100%. Data are presented as the mean ± SD. *P< 0.05, **P< 0.01.

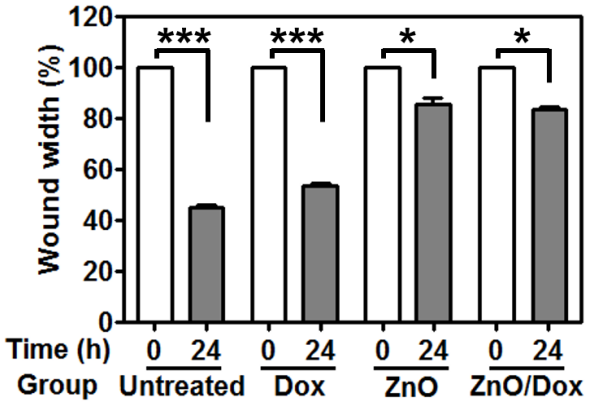

Figure S9. Inhibition of cancer cell migration in the presence of Dox, ZnO, or ZnO/Dox. The wound width was measured after 24 h incubation in the serum-free medium. The wound width at 0 h was used as 100%. Data are presented as the mean ± SD. *P< 0.05, ***P< 0.001.

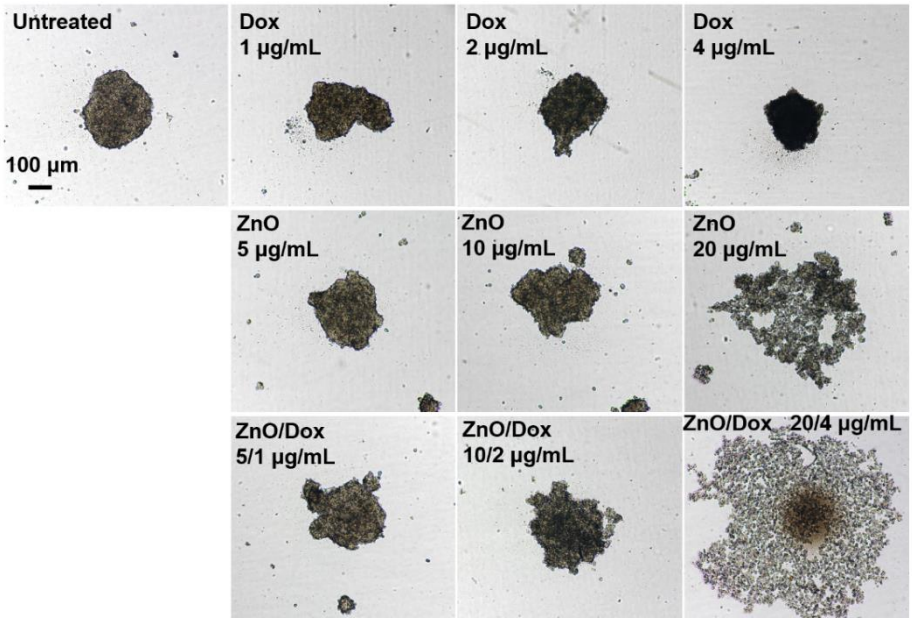

Figure S10. Inhibition of tumor cell spheroid formation. The NCI/ADR-RES cells were seeded into the agarose-coated 96-well plates and incubated in the presence of Dox, ZnO NPs, or ZnO/Dox for 48 h. The scale bar represents 100 μm.

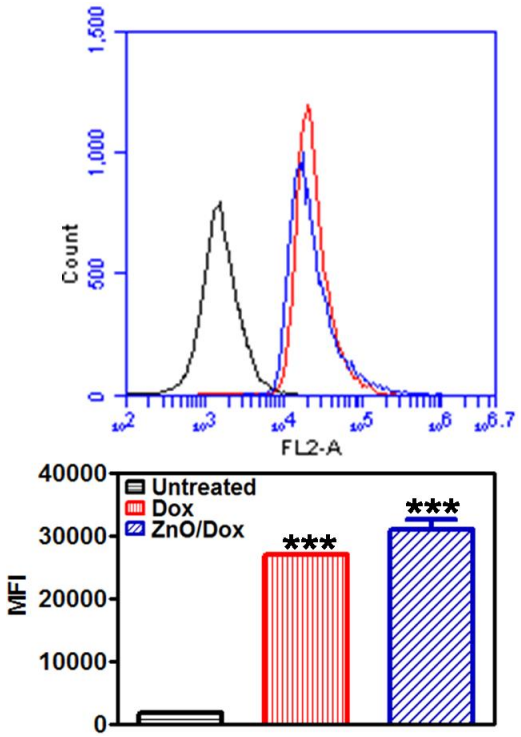

Figure S11. Cellular uptake of Dox and ZnO/Dox in the RAW264.7 cells, determined by flow cytometry. Incubation time: 1 h. Data are presented as the mean ± SD. ***P< 0.001. All P-values shown are *vs* the untreated group.

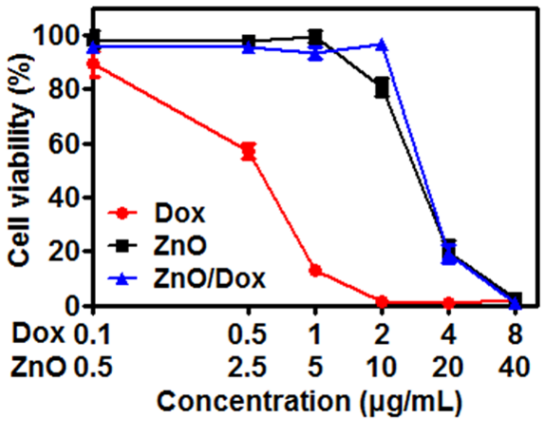

Figure S12. Cytotoxicity of Dox, ZnO NPs, or ZnO/Dox in the RAW264.7 cells. The cell viability was determined by MTT Assay after 24 h cell incubation. Data are presented as the mean ± SD.

Table S1. The IC_{50} of Dox, ZnO, and ZnO/Dox in various cancer cells.

		IC_{50} (μg/mL)			
		MDA-MB-231	**HeLa**	**NCI/ADR-RES**	**MES-SA/Dx5**
Dox		0.77±0.01	0.82±0.02	48.57±1.13	8.21±1.07
ZnO NPs		33.58±1.01	37.10±0.89	19.04±0.87	20.64±0.25
ZnO/Dox	Dox	2.61±0.73	2.76±0.68	3.72±0.57	4.13±0.74
	ZnO	13.05±0.58	13.80±0.49	18.60±0.76	20.63±0.46