Electronic Supplementary Material (ESI) for Nanoscale.

Supporting information

Carbonized polymer dots derived from metformin and L-arginine for tumor cell membrane- and mitochondria- dual targeting therapy

Manling Chen,[a] Yang Li,[b] Yangcheng Liu,[c] Baohua Jia,[d] Xue Liu,*[a] and Tianyi Ma *[d]

[a] *Institute of Clean Energy Chemistry, Key Laboratory for Green Synthesis and Preparative Chemistry of Advanced Materials, College of Chemistry, Liaoning University, Shenyang 110036, Liaoning, P. R. China. E-mail: liuxue@lnu.edu.cn*

[b] *Department of Cell Biology, Key Laboratory of Cell Biology of Ministry of Public Health, Key Laboratory of Medical Cell Biology of Ministry of Education, China Medical University, Shenyang 110122, Liaoning, P. R. China*

[c] *School of Pharmaceutical Science, Liaoning University, Shenyang 110036, Liaoning, P. R. China*

[d] *School of Science, STEM College, RMIT University, Melbourne, VIC 3000, Australia. E-mail: tianyi.ma@rmit.edu.au*

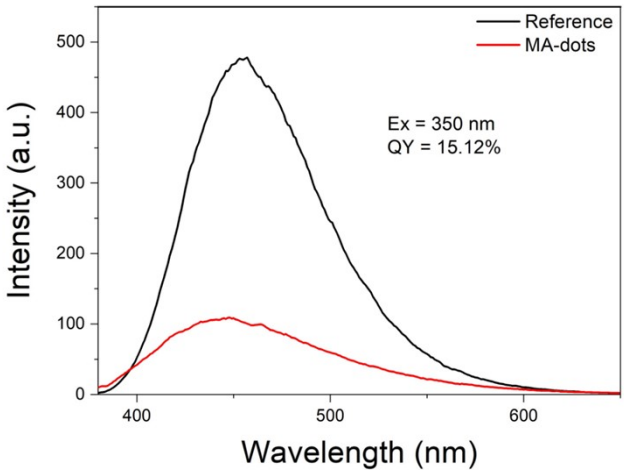

Fig. S1 The quantum yield (QY) of the MA-dots.

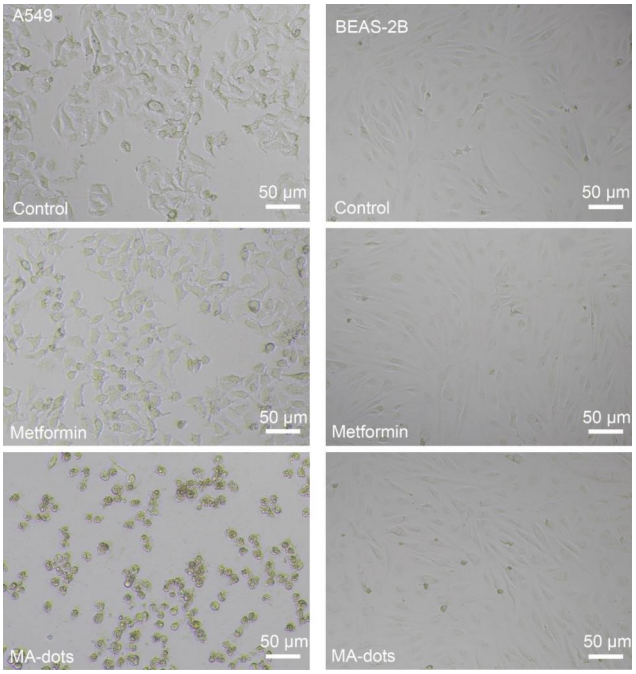

Fig. S2 Images of the A549 and BEAS-2B cells. The cells were treated with the MA-dots or metformin (100 μg mL^{-1}) for 24 h. Scale bars = 50 μm.

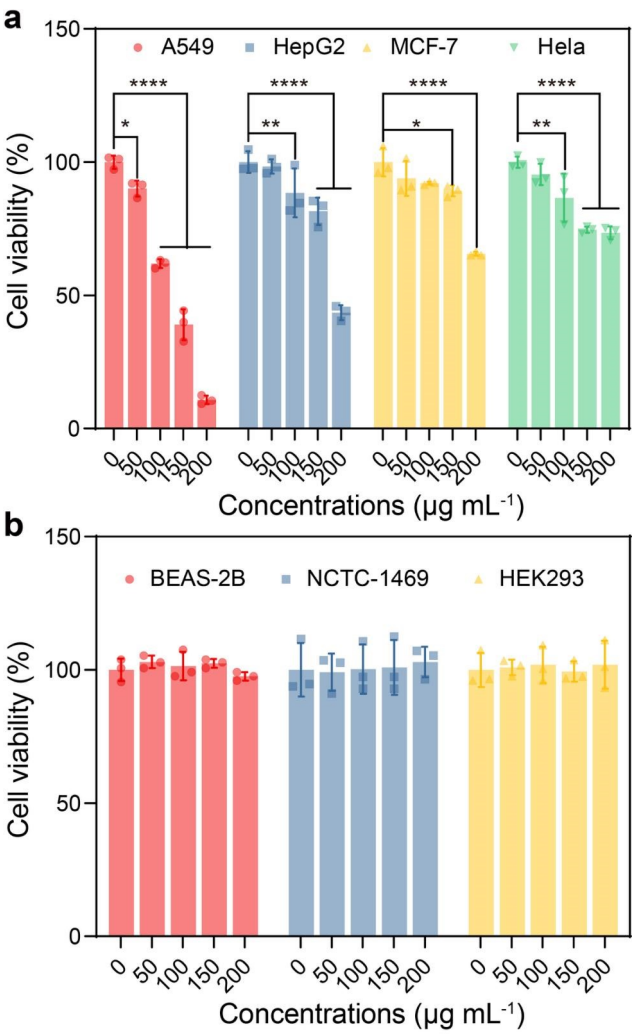

Fig. S3 Cytotoxicity analysis of the MA-dots by MTT method. The viability of the A549 cells, HepG2 cells, MCF-7 cells, Hela cells (tumor cell lines) (a), BEAS-2B cells, NCTC1469 cells, and HEK293 cells (normal cell lines) (b) treated with various concentrations of the MA-dots (0, 50, 100, 150, and 200 μg mL^{-1}) for 24 h. n = 3. Data display mean ± s.d. (one-way ANOVA, *p < 0.05, **p < 0.01, and ****p < 0.0001).

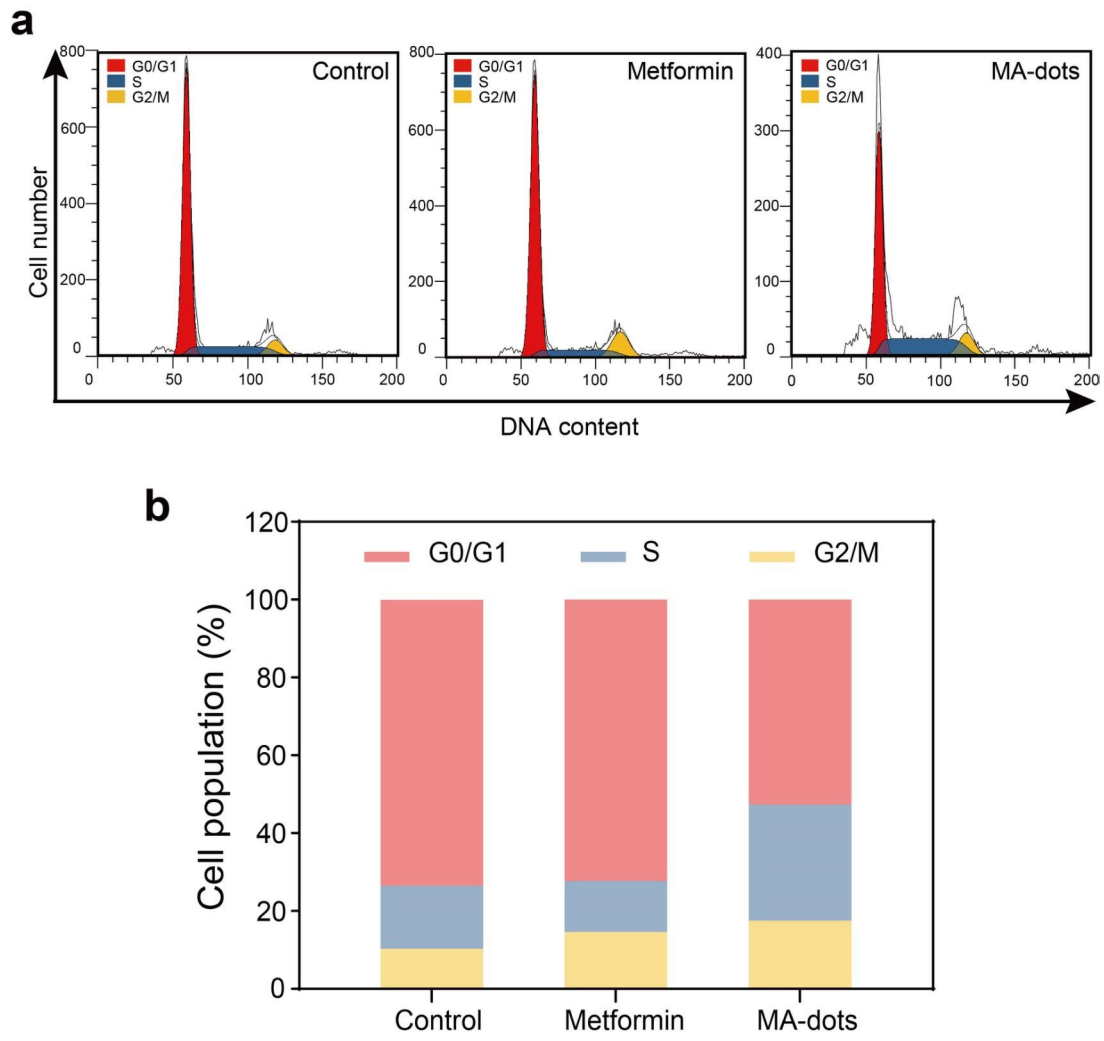

Fig. S4 Cell-cycle analysis of the A549 cells stained with propidium iodide. (a) A549 cells were treated with the MA-dots or metformin (100 μg mL^{-1}) for 24 h. (b) Summary of the cell percentage in each cell cycle phase. *n* = 3.

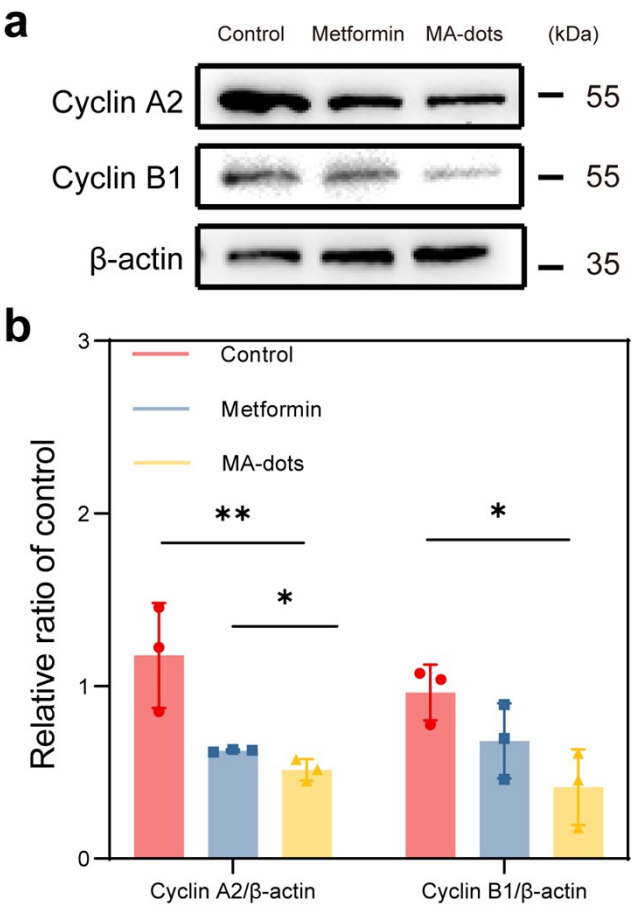

Fig. S5 Western blot analysis of cell cycle arrest-associated proteins in A549 cells. (a) The A549 cells were treated with the MA-dots or metformin (100 μg mL^{-1}) for 24 h. (b) Images were analyzed by Image J software. $n = 3$. Data display mean ± s.d. (one-way ANOVA, *$p < 0.05$ and **$p < 0.01$).

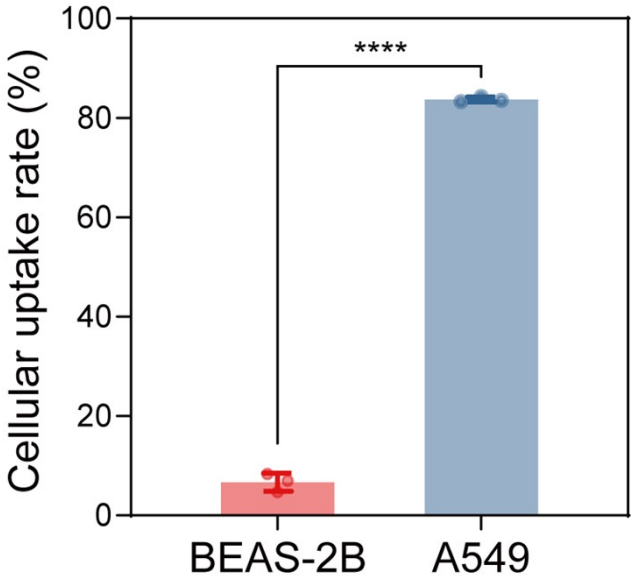

Fig. S6 Uptake rates of the MA-dots in the BEAS-2B and A549 cells analyzed by flow cytometry. *n* = 3. Data display mean ± s.d. (Unpaired Student's t-test, ****$p <$ 0.0001).

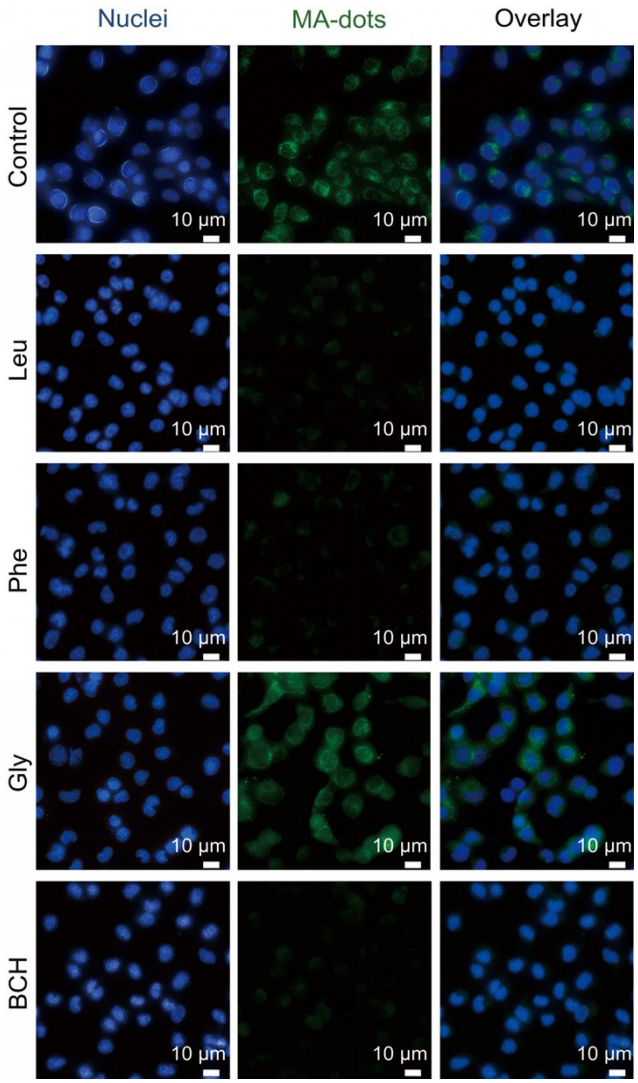

Fig. S7 Fluorescence images of the A549 cells pretreated with Leu, Phe, Gly, or BCH before incubating with the MA-dots. Blue fluorescence (λ_{ex} = 365 nm): nuclei, green fluorescence (λ_{ex} = 488 nm): the MA-dots. Scale bars = 10 μm.

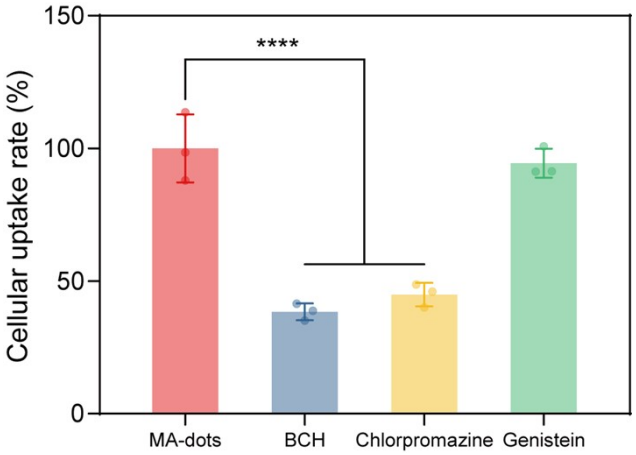

Fig. S8 Uptake rates of the MA-dots in the A549 cells treated with BCH, chlorpromazine, or genistein analyzed by flow cytometry. *n* = 3. Data display mean ± s.d. (one-way ANOVA, ****$p < 0.0001$).

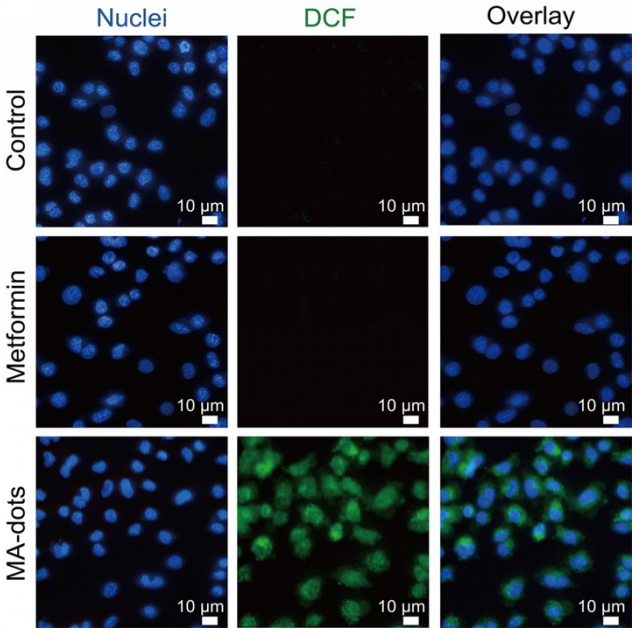

Fig. S9 Fluorescence images of the ROS in the A549 cells stained by DCFH-DA as a ROS-responsive marker. The cells were treated with the MA-dots or metformin (100 μg mL^{-1}) for 24 h. Blue fluorescence (λ_{ex} = 365 nm): nuclei, green fluorescence (λ_{ex} = 488 nm): DCF. Scale bars = 10 μm.

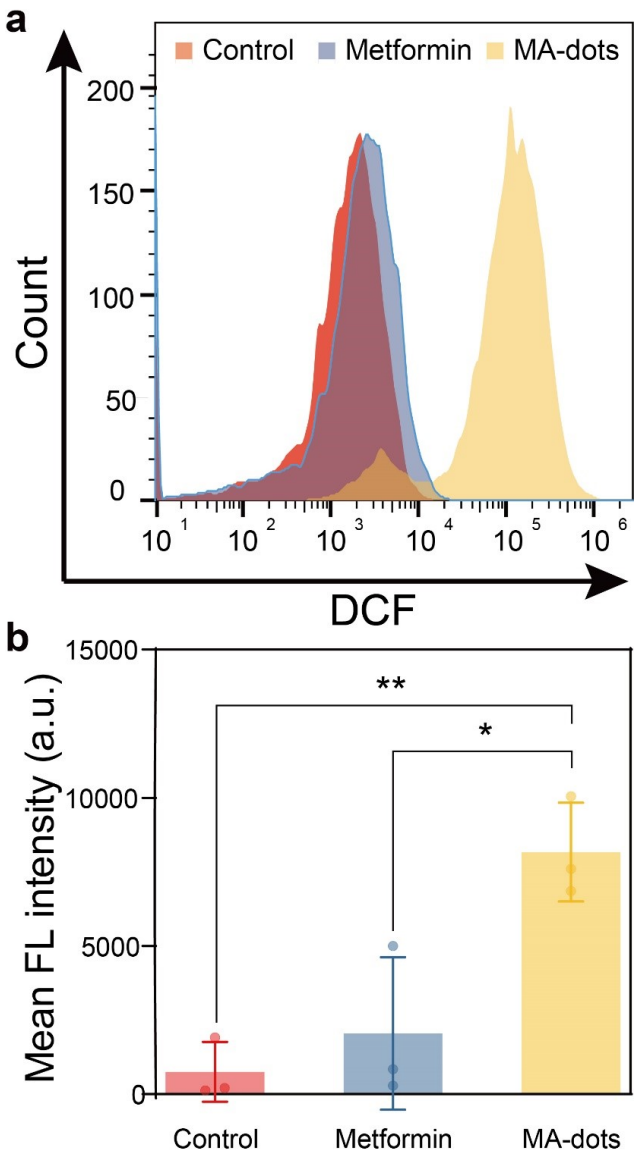

Fig. S10 (a) Flow cytometry analysis of the ROS levels in the A549 cells stained by DCFH-DA as a ROS-responsive marker. (b) Mean fluorescence (FL) intensity of the ROS levels in the A549 cells stained by DCFH-DA as a ROS-responsive marker. The cells were treated with the MA-dots or metformin (100 μg mL^{-1}) for 24 h. n = 3. Data display mean ± s.d. (one-way ANOVA, *p < 0.05 and **p < 0.01).

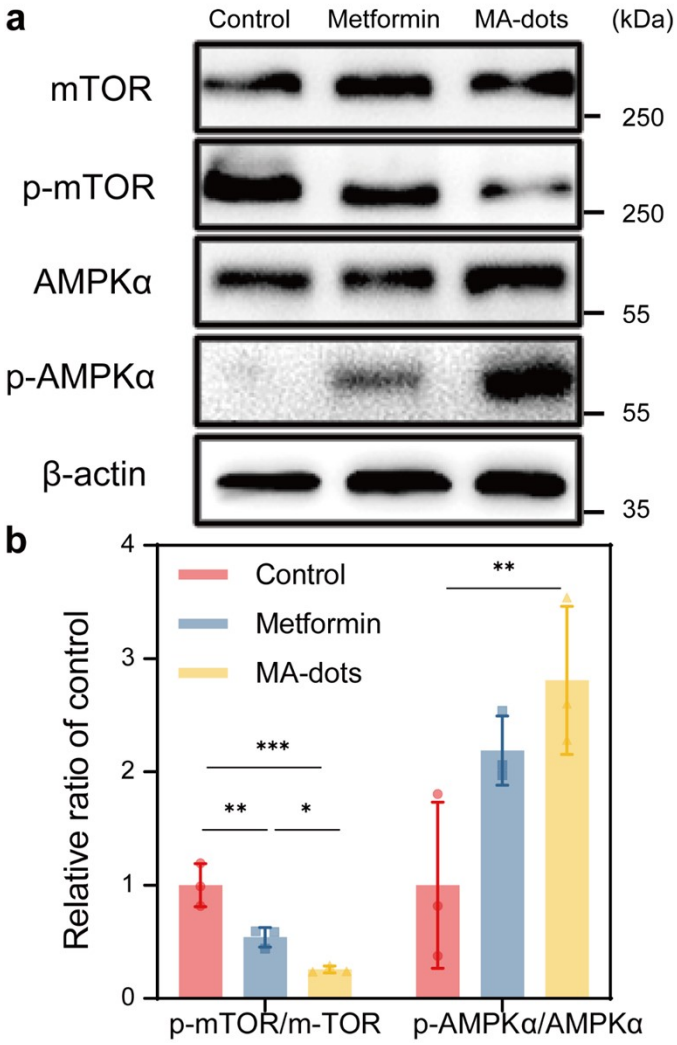

Fig. S11 Western blot analysis of the AMPK, p-AMPK, mTOR, and p-mTOR expression in the A549 cells. (a) The cells were treated with the MA-dots or metformin (100 μg mL^{-1}) for 24 h. (b) Images were analyzed by Image J software. $n = 3$. Data display mean ± s.d. (one-way ANOVA, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$).

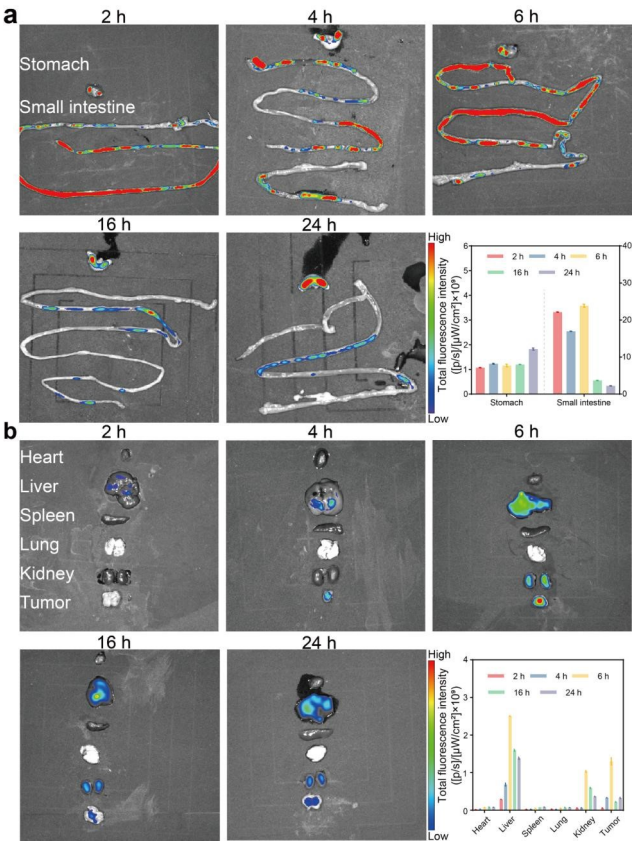

Fig. S12 *In vivo* distribution of the MA-dots in the major organs (stomach, small intestine, heart, liver, spleen, lungs, and kidneys) and tumors. *Ex vivo* fluorescence imaging and quantitative evaluation of fluorescent intensities of the major organs and tumors in the tumor-bearing mice after oral administration of the MA-dots. Fluorescence images were captured at excitation/emission wavelengths of 480/520 nm by an animal optical imaging system. The fluorescence was from the MA-dots.

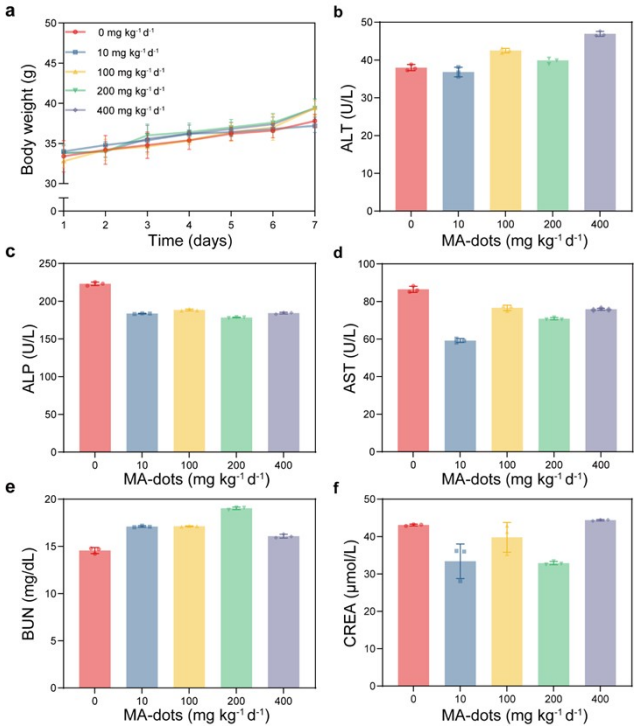

Fig. S13 *In vivo* toxicity studies of the MA-dots in mice models. (a) Body weight change curves of the mice treated with various concentrations of the MA-dots (0, 10, 100, 200, and 400 mg kg^{-1} d^{-1}). *n* = 5. Hepatic function analysis through the serum levels of alanine aminotransferase (ALT) (b), alkaline phosphatase (ALP) (c), aspartate aminotransferase (AST) (d), blood urea nitrogen (BUN) (e), and creatinine (CREA) (f) in the mice on Day 8 treated with various concentrations of the MA-dots. *n* = 3.

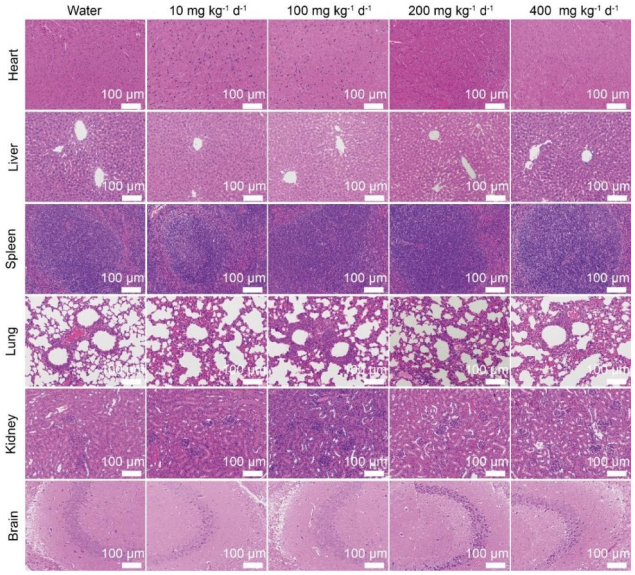

Fig. S14 *In vivo* biocompatibility studies of the MA-dots in mice models by histological analysis. H&E staining images of the heart, liver, spleen, lung, and kidney from the mice on Day 8 treated with various concentrations of the MA-dots (0, 10, 100, 200, and 400 mg kg^{-1} d^{-1}). Scale bars = 100 μm.

Table S1 Elemental compositions of metformin, L-arginine, and the MA-dots.

	C 1s (%)	N 1s (%)	O 1s (%)
Metformin	37.06	54.99	0
L-arginine	41.33	32.15	18.37
MA-dots (theoretical value)	39.20	43.57	9.19
MA-dots (actual value)	61.01	29.24	9.75

Table S2 Nitrogen compositions of metformin, L-arginine, and the MA-dots.

	Percentage (%)		
	Primary amine	Secondary amine	Tertiary amine
Metformin	20	60	20
L-arginine	50	50	0
MA-dots (theoretical value)	35	55	10
MA-dots (actual value)	24.57	29.52	45.91

Table S3 IC_{50} (half maximal inhibitory concentration) values on various tumor cell lines of free metformin or metformin delivery nanosystems.

Drug	IC_{50} (μg mL^{-1})	Tumor cells	Treatment time (h)	References
O-CMC-metformin	>612.79	MiaPaCa-2 cells (pancreatic cancer cell lines)	72	[1]
Free Metformin	371.00	C26 cells (colon26 cell lines)	48	[2]
Gel + Metformin	3951.70	C26 cells	48	[2]
Gel+ME/FU	191.91	C26 cells	48	[2]
Free Metformin	766.00	CT26 cells	48	[3]
Met-lip	245.00	CT26 cells	48	[3]
Free Metformin	908.59	A375 cells (melanoma cell lines)	24	[4]
MET FCA NPs	784.87	A375 cells	24	[4]
MET&DOX FCA NPs	434.59	A375 cells	24	[4]
Free Metformin	1808.57	SK-MEL-28 cells (melanoma cell lines)	24	[4]
MET FCA NPs	837.04	SK-MEL-28 cells	24	[4]
MET&DOX FCA NPs	625.05	SK-MEL-28 cells	24	[4]
Free Metformin	>>828.10	MB49 cells (mouse bladder cancer cell lines)	48	[5]
IR775@Met@Lip + Laser	>207.03	MB49 cells	48	[5]
Free Metformin	3218.00	Hela cells (cervical cancer cell lines)	48	[6]
MET liposomes	1729.07	Hela cells	48	[6]
MET Mito-liposomes	220.27	Hela cells	48	[6]
Free Metformin	3328.96	T-47D cells (breast cancer cell lines)	48	[7]
Nano metformin (Met-loaded PLGA-PEG NPs)	2701.26	T-47D cells	48	[7]
Free Metformin	1656.20	Breast cancer stem cells	48	[8]
Her-LP-MET	<357.74	Breast cancer stem cells	48	[8]
Free Metformin	828.10	MDA-MB-231 cells (breast cancer cell lines)	48	[9]
Met -MSNs	165.62	MDA-MB-231 cells	48	[9]
Free Metformin	1159.00	A549 cells (lung	24	This paper

		cancer cell lines)		
MA-dots	93.60	A549 cells	24	This paper

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