Supporting Information

for

Selective Decoating-Induced Activation of Supramolecularly Coated Toxic Nanoparticles for Multiple Applications

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Materials and Methods

Materials. All chemicals were supplied by Sigma Aldrich and Aladdin (China). CB[8] was synthesized by our lab according to a literature procedure. UCNPs were purchased from Xi'an Ruixi Biological Technology Co., Ltd (catalog number: R-UY365). TNF-α, IL-1β, and IL6 by Elisa kits from Hefei Laier Biotechnology Co., Ltd. (China), including TNF-α (catalog number: LE-M1065), IL-1β Elisa kit (catalog number: LE-M0444), IL6 Elisa kit (catalog number: LE-M0458) The normal human liver cell line L-02, murine breast cancer cell line 4T1, and murine melanoma cell line B16 were purchased from ATCC (Shanghai, China), where they were verified by mycoplasma detection, isozyme detection, DNA fingerprinting and cell vitality test. Cell culture media were purchased from Gibco (USA). Wild type zebrafish and FAP zebrafish were supplied by Institute of Chinese Medical Sciences, University of Macau. C57BL/6 mice and BALB/c mice were purchased from Faculty of Health Science, University of Macau. All of the animal experiments were approved by the Animal Ethics Committee, University of Macau.

Synthesis of MV-PLA. 4,4-bipyridine (9.57 mmol, 1.50g) and iodomethane (8.83 mmol, 1.25g) were dissolved in 150 mL of acetonitrile, and stirred for 24 h at room temperature. After filtering, the filtrate was evaporated under vacuum to ~15 mL. The precipitate was washed with 10 mL of ethanol for three times, and dried under vacuum. Finally, pure yellow power (monomethyl violet) was obtained (yield 44%). ^{1}H NMR (600 MHz, DMSO-d6): δ 9.18 (d, 2H), 8.91 (d, 2H), 8.69 (d, 2H), 8.08 (d, 2H), 4.47 (s, 3H). Subsequently, monomethyl violet (2 mmol, 596.24 mg) was dissolved in 10 mL of acetonitrile, and 2-bromoethanol (10 mmol, 780 μL) was added into the solution dropwise. After stirring for 48 h under 85 ºC, the reaction mixture was evaporated under vacuum and filtrated. The resultant yellow precipitate was washed by diethyl ether for three times, and dried in vacuum to get pure product (MV-OH, yield 62%). ^{1}H NMR (600 MHz, DMSO-d6): δ 9.37 (d, 4H), 8.96 (d, 4H), 4.81 (t, 2H), 4.51 (s, 3H), 3.97 (t, 2H). Finally, MV-OH (0.03 mmol, 12.69 mg), DL-lactide (2.1 mmol, 302.4 mg), and stannous octoate (0.015 mmol, 0.5 μL) were mixed together and heated to 135 ºC under protection of nitrogen. After reaction for 72 h, the mixture was dissolved in trichloromethane, and then precipitated in cool mixed solvent of diethyl ether and methanol. After purifying for three times, the precipitate was dry under vacuum to get

MV-PLA. The structure was analyzed by ^{1}H NMR spectrum, and the molecular weight was 2250 g/mol with PDI of 1.22.

Synthesis of Azo-HA. HA (1 mmol, 776.6 mg) were added into 50 mL of formamide, and the solution was heated to 70 ºC to sufficiently dissolve HA. Then, p-aminoazobenzene (1 mmol, 197.2 mg), N,N-Diisopropylethylamine (2 mmol, 258.48), and HATU (1.5 mmol, 570.3 mg) were added into HA solution, and stirring under 70 ºC for 24 h. The obtained reaction solution was dialyzed in dialysis bag (molecular weight cut-off = 12000) to purify the Azo-HA. Subsequently, the solution was lyophilized for future use. The structure was analyzed by ^{1}H NMR spectrum, and the molecular weight was 5.475×10^5 (±0.574%) measured by gel permeation chromatography.

Preparation of HA-MV-NPs. Firstly, MV-NPs were constructed *via* micro-emulsion method. Briefly, MV-PLA (0.01 mmol, 16.67 mg) was dissolved in 2 mL of DMSO, and added dropwise into 20 mL of polyvinyl alcohol solution (1%, w/v). After stirring for 30 min, MV-NPs was obtained. Then, Azo-HA (0.002 mmol, 26.9 mg) and CB[8] (0.002 mmol, 2.7 mg) were added into resultant solution, and stirring for 30 min again to get crude HA-MV-NPs. Subsequently, the mixture was dialyzed in dialysis bag to remove the organic solvent. Finally, the solution in dialysis bag was lyophilized for future use. The morphology, size distribution, and zeta potential of HA-MV-NPs were measure by TEM and DLS, respectively. The changes of particle size incubated in PBS or cell culture medium were also evaluated by DLS.

***In vitro* safety evaluation in cell.** Safety evaluation experiments was carried out on L-O2 cells. The cytotoxicity experiments of MV-NPs and HA-MV-NPs were measured by MTT assay after treatment for 24 h.[1] The cell viability was evaluated under microplate reader and the result was analyzed by GraphPad 6.0. Furthermore, after treatment with MV-NPs and HA-MV-NPs at concentration of 0.2 mM for 12 h, the cell was stained 2′,7′-dichlorofluorescin diacetate (DCFH-DA) for 30 min. Then, the cells were washed with PBS for three time and analyzed by flow cytometry at exciting wavelength of 488 nm. The result was analyzed by using FlowJo 10. Finally, in order to investigate cell apoptosis, the cells were collected after incubation with MV-NPs and

HA-MV-NPs at concentration of 0.4 mM for 12 h. After washing for three times with PBS, the cells were suspended in 100 μL of binding buffer, and then stained by 10μL of propidium iodide and 10 μL of annexin V-fluorescein isothiocyanate (V-FITC) for 15min. Subsequently, the mixture was diluted with 400 μL of binding buffer and analyzed by flow cytometry. The result was analyzed by using FlowJo 10.

***In vivo* safety evaluation in zebrafish.** Wild-type zebrafish and Tg (fabp10:dsRed) zebrafish were used for safety evaluation of HA-MV-NPs. For bio-uptake of MV-NPs and HA-MV-NPs, cyanine 5 NHS ester (Cy5) was encapsulated into MV-NPs (Cy5-loaded MV-NPs) and HA-MV-NPs (Cy5-loaded HA-MV-NPs). Two days post fertilization (dpf), zebrafish were randomly and investigator-blindly separated into 24-well plates (10 fish per well). Subsequently, zebrafish were incubated with Cy5-loaded MV-NPs and Cy5-loaded HA-MV-NPs at different concentrations of Cy5 (0.25 μM, 0.5 μM). After incubation for 2 days, the red fluorescence in zebrafish was observed by using an Olympus DSU (Disk Scanning Unit) Confocal Imaging System. For toxicity evaluation of HA-MV-NPs, 2 dpf Tg (fabp10:dsRed) zebrafish was also randomly separated into 24-well plates, and incubated with MV-NPs and HA-MV-NPs at different concentrations (100 μM, 200 μM). After incubation for 2 days, the hepatoxicity was observed by fluorescent microscope, and liver size and red fluorescence intensity were measured by Image-Pro Plus 6.0. Finally, the dose of MV was increased to 2 mM, the survival rates of wild-type zebrafish incubated with HA-MV-NPs were determined.

***In vivo* safety evaluation in mouse.** Female C57BL/6 mice were randomly separated into 5 groups (n = 6), and the mice were treated with saline, MV-NPs (10 mg/kg, 20 mg/kg, 40 mg/kg) and HA-MV-NPs (40 mg/kg) at first day. The survival rate was recorded up to 30 days. Then, the changes of body weight were measured in mice treated with saline, MV-NPs (10 mg/kg), and HA-MV-NPs (10 mg/kg) for 30 days. At the end of experiment, all mice were sacrificed, and the blood were collected for testing hepatic damage biomarkers (ALT and AST), renal function biomarkers (UA and BUN), and inflammatory cytokines (TNF-α, IL-1β, IL-6). The liver, spleen, lung, and kidney were collected for performing H&E staining, and Masson's trichrome staining were conducted on

lung tissues, according to the standard method.

Herbicidal activity. The herbicidal activity of HA-MV-NPs was carried out in grass lawn of E. arundinacea. The seeds were cultivated in flowerpots according to reported procedure.[2] Briefly, the cultivation condition was set at 24 ± 0.5°C with a humidity of 70 ± 6%, and illuminated with simulated sunlight (10:14 h light/dark cycle). After cultivation for 2 weeks, the lawns were clipped to the same height and then the flowerpots were randomly separated into four groups, which were treated as follows: control group, grass received only water; MV-NPs groups, grass treated with MV-NPs; HA-MV-NPs group, grass treated with HA-MV-NPs under irradiation by simulated sunlight without UV (410-800 nm spectral output, 30 W radiant output); and HA-MV-NPs (UV) group, grass treated with HA-MV-NPs under irradiation by simulated sunlight (360-800 nm spectral output, 30 W radiant output). The pH of water was ~7.0. Furthermore, the herbicidal activity of MV-NPs and HA-MV-NPs in natural condition was also carried out with a similar procedure. The dose of MV was set as 2 mg/mL according to the manufacturer's instructions (Syngenta Crops, Lda). The herbicidal activity was monitored by visual assessment for 5 days.

Antibacterial activity. Single colony of E. coli and S. aureus was transferred in 10 mL of liquid lysogeny broth (LB) culture, and grown at 37 °C for 12 h with shaking. Then, the optical density at 600 nm (OD600nm) of bacterial suspension was diluted into 0.3 with liquid LB culture containing different concentrations of MV-NPs and HA-MV-NPs at predetermined concentration, and seeded in 96-well plates. After incubation for 24 h, the OD600nm was recorded to determine the growth condition of both bacteria. As for spread plate method, bacteria were mixed with liquid LB culture containing MV-NPs and HA-MV-NPs, and grown at 37 °C for 12 h with shaking. 100 μL of bacterial suspension was subsequently spread on solid LB culture, and incubated for 24 h to get images by photo camera and count the bacterial colonies. Furthermore, the *in vivo* antibacterial activity of HA-MV-NPs was evaluated in BALB/c mice. The mice were shaved on the abdomen, and inoculated intradermally with 100 μL of S. aureus (10^9 CFUs/mL) at midlogarithmic growth phase. At the second day after inoculation, the mice were randomly separated into 3 groups (n=6), and subcutaneously in suit injected with PBS, MV-NPs, and HA-MV-NPs at dosage of 10 mg/kg. All

mice were sacrificed at day 10 after inoculation, the lesion regions were measured by digital photographs, and the lesion skins were collected for further H&E staining and Gram staining, according to the standard guidelines.

***In vivo* controlled release.** Similar to the preparation procedure of HA-MV-NPs, UCNPs@MV-NPs were firstly constructed. Briefly, MV-PLA (0.01 mmol, 16.67 mg) was dissolved in DMSO (1 mL), followed by the addition of chloroform (0.1 mL) containing UCNPs (5 mg/mL). Subsequently, the mixture was added dropwise into 5 mL of polyvinyl alcohol solution (1%, w/v). After stirring for 30 min, the solution was dialyzed to remove the organic solvent and the UCNPs@MV-NPs solution was obtained. Then, Azo-HA (0.002 mmol, 26.9 mg) and CB[8] (0.002 mmol, 2.7 mg) were added into resultant solution, and stirred for 30 min to get UCNPs@HA-MV-NPs.

UCNPs@HA-MV-NPs were irradiated by IR light at 980 nm for 5 h (0.5 W/cm2), and the samples irradiated for different times were detected by UV/vis spectrophotometer (DR 6000, HACH Company). To verify the *in vivo* controlled release behavior of UCNPs@HA-MV-NPs, xenografted murine melanoma model was constructed. 100 μL of B16 cells (10^6/mL) were injected to the armpits of C57BL/6 mice, and the mice were randomly separated into 4 groups (n=6) at day 10 after inoculation. Then, the four groups were i.v. treated with PBS, IR, HA-MV-NPs, and HA-MV-NPs +IR at day 0 and day 2, respectively. IR Irradiation (980 nm) lasts for 15 min at power of 0.5 W and repeated for 2 times each day with interval of 10 min. The survival rate, body weight and tumor size were recorded. At the end of experiment, all mice were sacrificed, and the tumor tissues, liver, spleen, lung and kidney were collected for histological and immunohistochemical studies according to standard procedures.

References

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L.-R. CHD4 has oncogenic functions in initiating and maintaining epigenetic suppression of multiple tumor suppressor genes. *Cancer Cell* **2017,** *31* (5), 653-668. e7.
(2) Gao, C.; Huang, Q.; Lan, Q.; Feng, Y.; Tang, F.; Hoi, M. P.; Zhang, J.; Lee, S. M.; Wang, R. A user-friendly herbicide derived from photo-responsive supramolecular vesicles. *Nat. Commun.* **2018,** *9* (1), 1-13.

Supplementary Materials and Tables

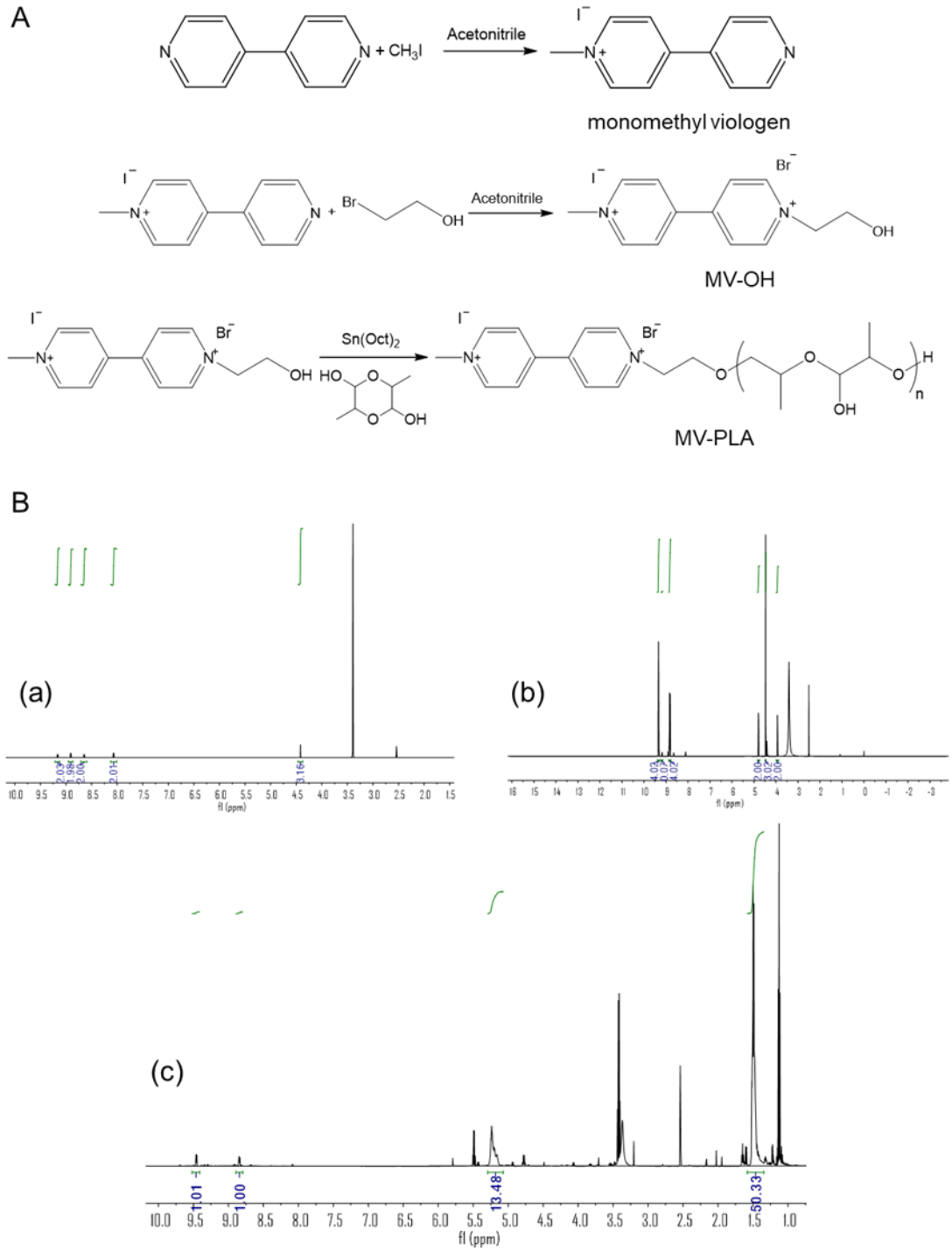

Figure S1. Preparation of MV-PLA. (A) Synthetic process of MV-PLA. (B) ^{1}H NMR spectra of monomethyl viologen (a), MV-OH (b), and MV-PLA (c).

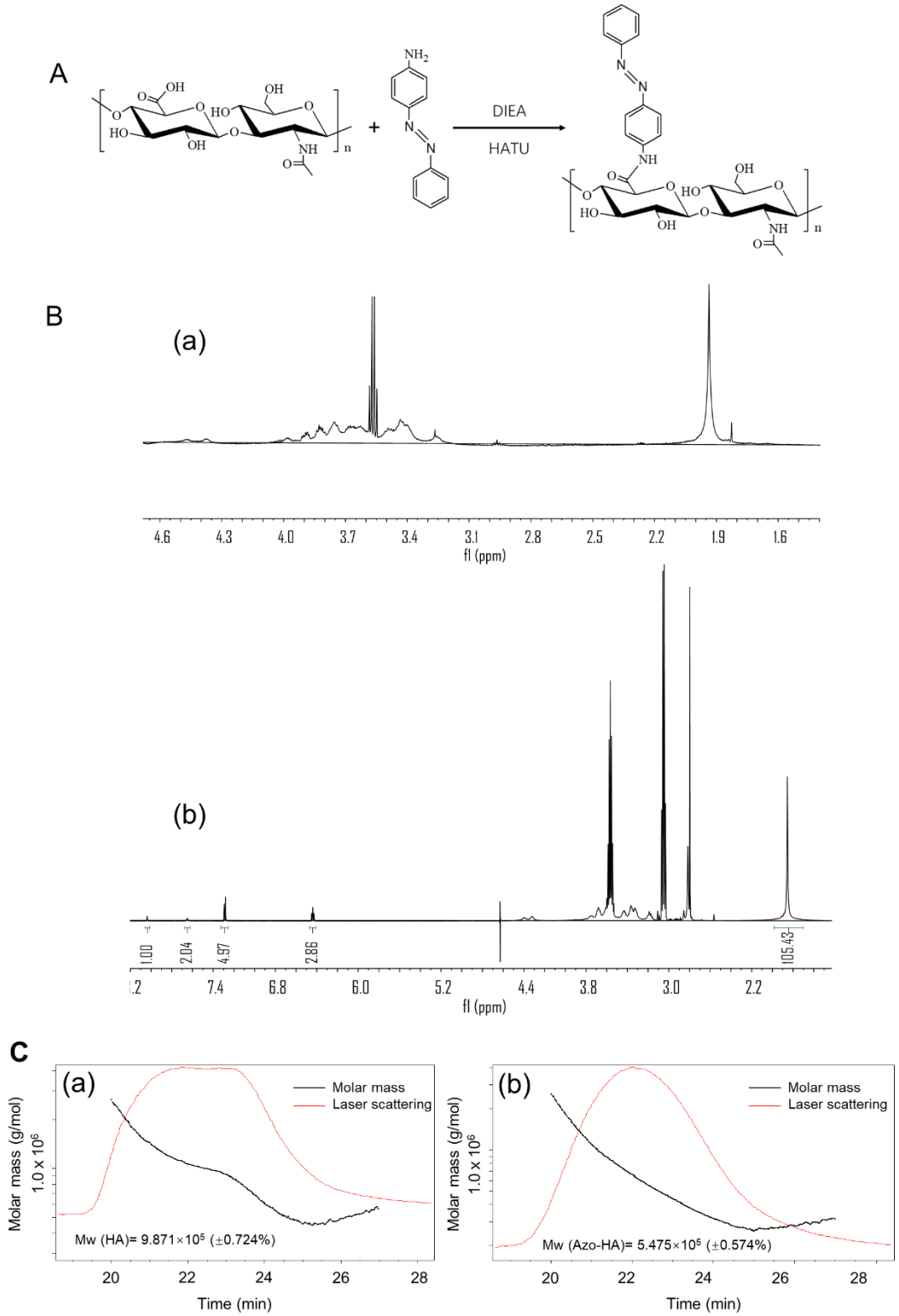

Figure S2. Preparation of Azo-HA. (A) Synthetic process of Azo-HA. (B) ^{1}H NMR spectra of HA (a) and Azo-HA (b). (C) The molecular weight of HA (a) and Azo-HA (b) determined by gel permeation chromatography.

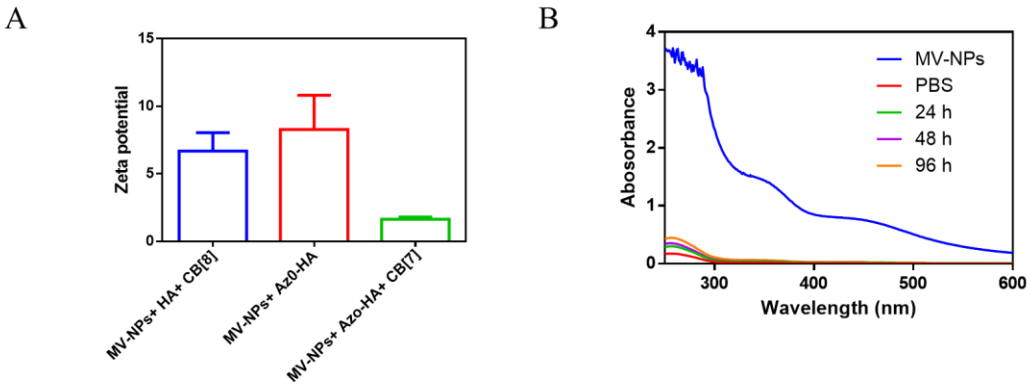

Figure S3. Characterizations of MV-NPs. (A) The zeta potentials of MV-NPs + HA + CB[8], MV-NPs + Azo-HA, and MV-NPs + Azo-HA+ CB[7]. The molar ratio was 5: 1: 1 for MV-NPs, HA and CB[8], 5: 1 for MV-NPs and Azo-HA, and 5: 1: 1 for MV-NPs, Azo-HA and CB[7]. (B) MV-NPs was placed in a dialysis bag (MWCO= 5000 Da), and 1 mL of PBS was taken out at different time points (0, 24, 48, and 96 h) for UV-vis absorbance measurements.

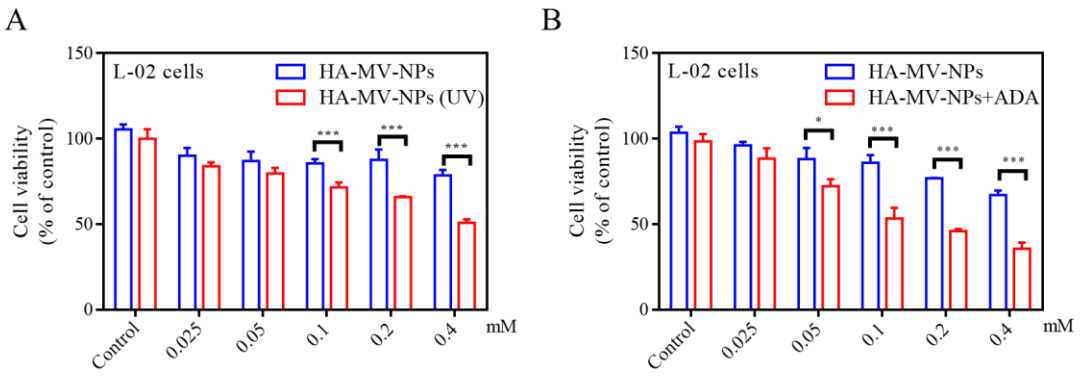

Figure S4. The reversed cytotoxicity of HA-MV-NPs after treatment with competing guest or UV irradiation. (A) L-O2 cells incubated with HA-MV-NPs at different concentrations (0.025, 0.05, 0.1, 0.2, and 0.4 mM) for 24 h, with or without UV irradiation for 5 min. All data were presented as mean ± s.d (n = 3). Statistical difference was analyzed by using Two-Way ANOVA. *P≤0.05, **P≤0.01, and ***P≤0.001. (B) L-O2 cells incubated with HA-MV-NPs at different concentrations (0.025, 0.05, 0.1, 0.2, and 0.4 mM) for 24 h, with or without addition of 0.4 mM of ADA. All data were presented as mean ± s.d (n = 3). Statistical difference was analyzed by using Two-Way ANOVA. *P≤0.05, **P≤0.01, and ***P≤0.001.

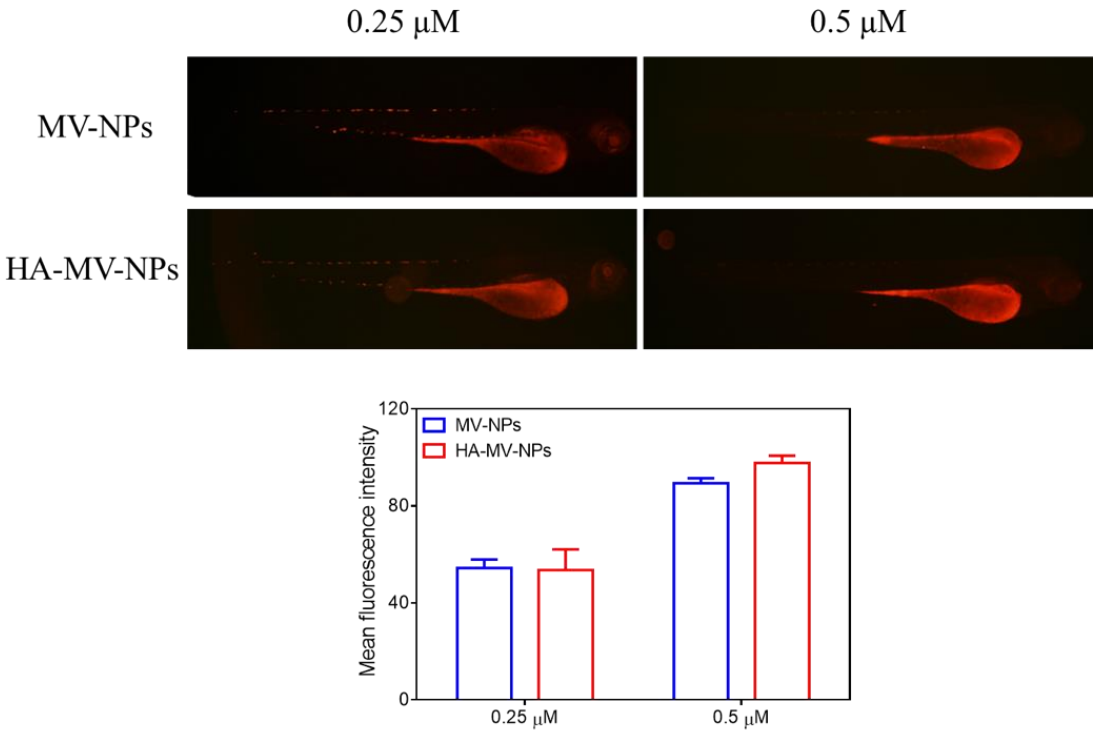

Figure S5. Uptake behavior of wild type zebrafish towards to MV-NPs and HA-MV-NPs, and quantitative analysis determined by mean fluorescence intensity. All data were presented as mean ± s.d. (n = 10).

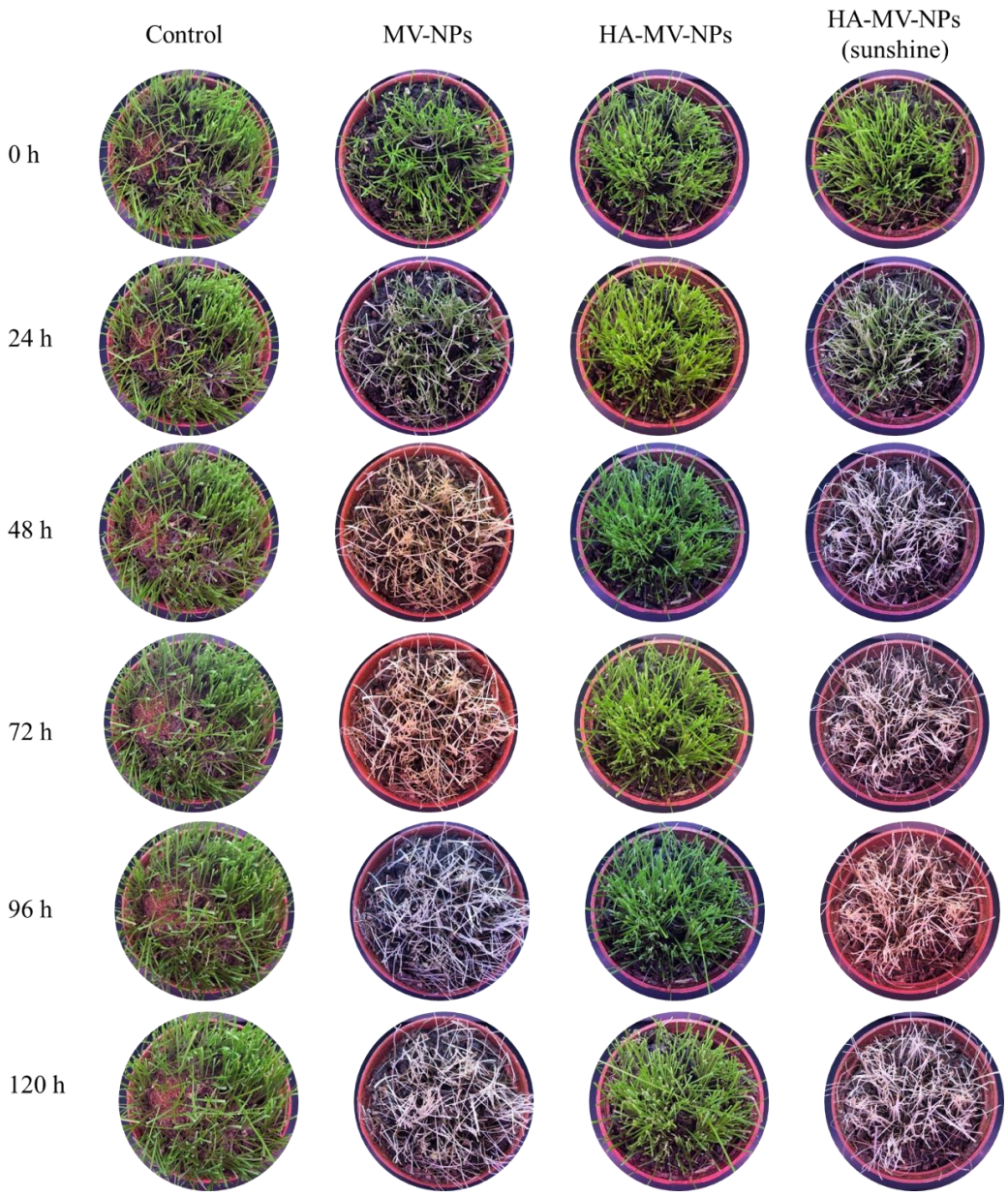

Figure S6. Herbicidal activity of MV-NPs and HA-MV-NPs under natural sunlight. The grass was sprayed with control (water), MV-NPs and HA-MV-NPs at a single dose of 2 mg/mL (normalized to MV), monitored under natural conditions (with exposure to sunlight) for 5 days.

Note: The herbicidal experiment was performed in Macau from November 21, 2018 to November 26, 2018, and the weather conditions were listed as follows.

Date	November 21, 2018	November 22, 2018	November 23, 2018
Weather condition	Passing clouds	Passing clouds	Sunny
Date	November 24, 2018	November 25, 2018	November 26, 2018
Weather condition	Scattered clouds	Passing clouds	Scattered clouds

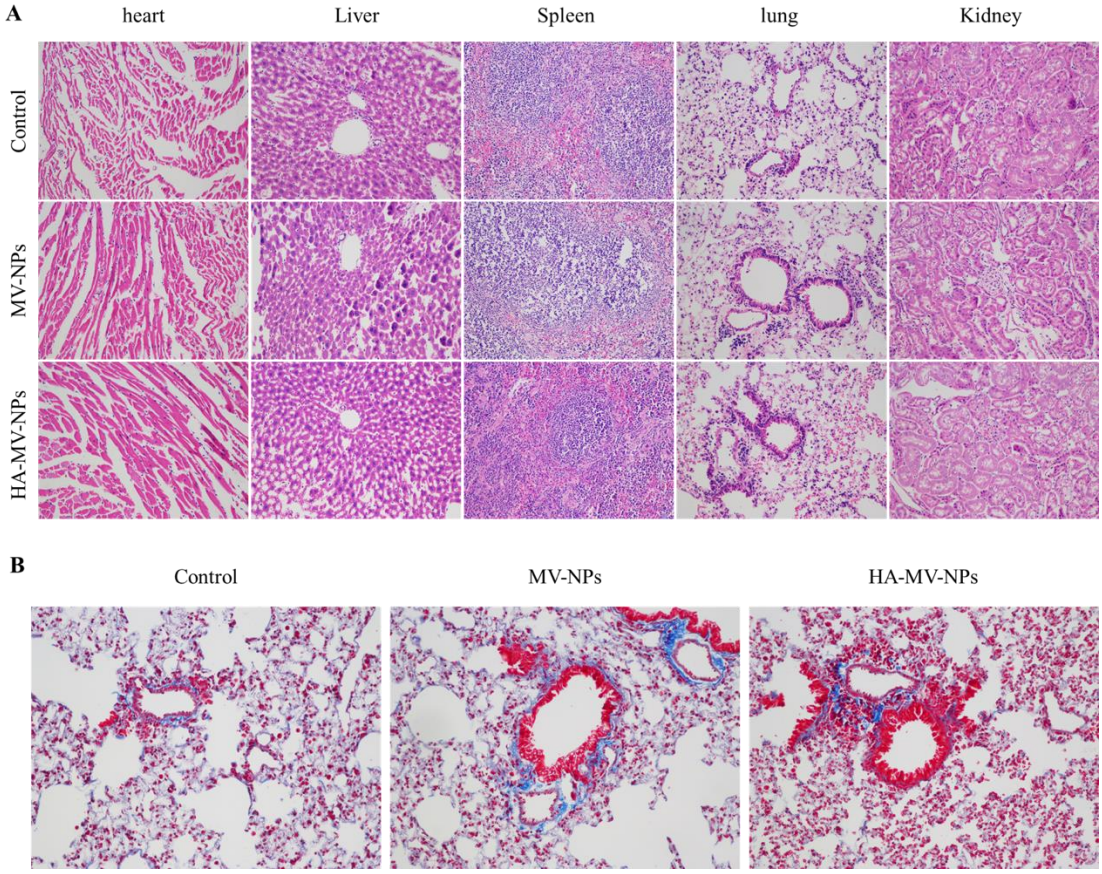

Figure S7. Systemic toxicity evaluation on bacteria-infected mice intradermally in situ administered with MV-NPs and HA-MV-NPs. A) H&E staining on the heart, liver, spleen, lungs, and kidneys collected, and B) Masson's trichrome staining on the lungs.

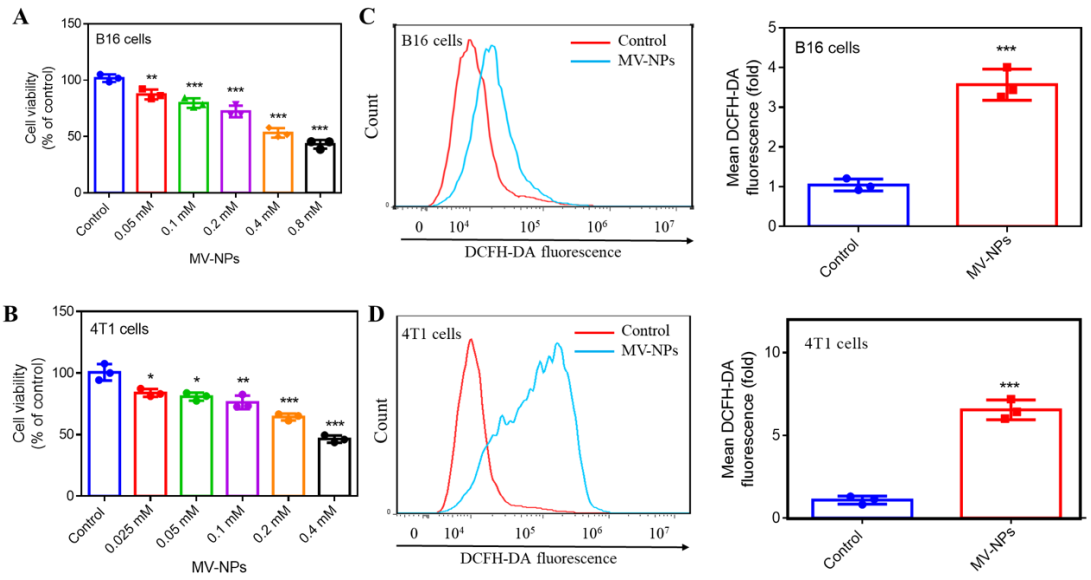

Figure S8. Toxicity of MV-NPs to cancer cells. A) Cell viability of B16 cells treated with different concentrations of MV-NPs for 24 h. B) Cell viability of 4T1 cells treated with different concentrations of MV-NPs for 24 h. C) ROS generation of B16 cells treated with 0.4 mM of MV-NPs determined by flow cytometry. D) ROS generation of 4T1 cells treated with 0.2 mM of MV-NPs determined by flow cytometry. All data were presented as mean ± s.d. (n = 3). Statistical difference of cell viability was analyzed by using One-Way ANOVA. Statistical difference of ROS generation was analyzed by using t test. *P≤0.05, **P≤0.01, and ***P≤0.001.

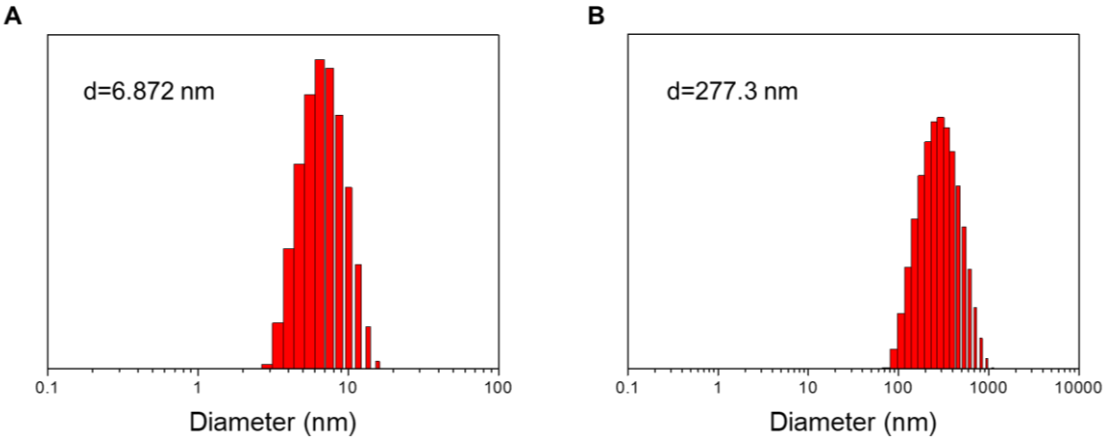

Figure S9. Mean diameter of UCNPs (A) and UCNPs@HA-MC-NPs (B) detected by DLS.

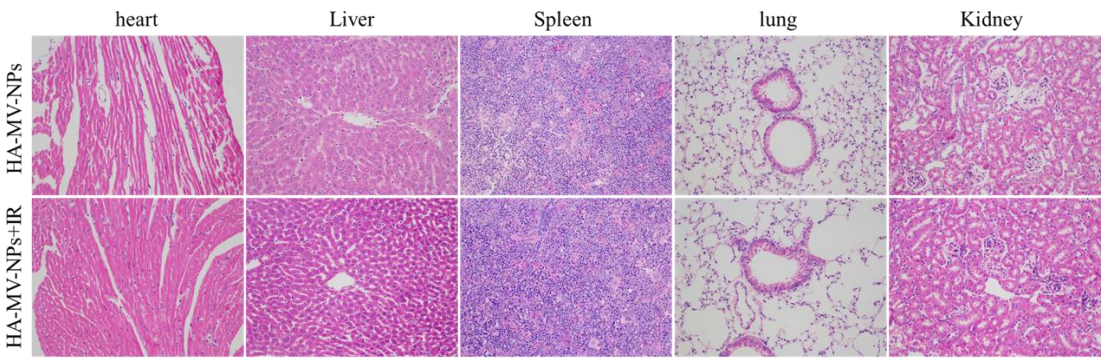

Figure S10. Histological analysis of the heart, liver, spleen, lungs, and kidneys isolated from tumor-bearing mice intravenously injected with HA-MV-NPs with or without IR irradiation.

Table S1. The diameter of MV-NPs and HA-MV-NPs incubated in DMEM containing 10% FBS for 3 days.

Time (h)	MV-NPs		HA-MV-NPs	
	Size (nm)	PDI	Size (nm)	PDI
0	219.7	0.169	243.7	0.130
24	220.4	0.172	239.4	0.133
48	223.0	0.181	243.9	0.074

Table S2. The diameter of HA-MV-NPs incubated in PBS for half a year.

Time (day)	HA-MV-NPs	
	Size (nm)	PDI
0	243.7	0.130
30	261.7	0.185
180	268.3	0.195

Table S3. The IC_{50} of MV, MV-NPs and HA-MV-NPs against *E. coli* and *S. aureus* after incubation for 24 h.

Bacterial	IC_{50} (mM)		
	MV	MV-NPs	HA-MV-NPs
E. coli	0.6085	0.4088	N/A
S. aureus	1.117	0.8136	1.282

Table S4. Physical properties UCNPs (by manufacturer).

Items	Specification	Results
Appearance	solution	solution
1XRD	NaYF4,Yb Tm	NaYF4,Yb Tm
Surface group		OA
Concentration	5mg/ml	≈5mg/ml
Size		6-8nm
Ex；Em		980nm；360/450/470/800nm
Dispersant	Normal hexane	Normal hexane