

## **Bio-Enzyme Responsive L-Arginine-Based Carbon Dots: The Replenishment of Nitric Oxide for Nonpharmaceutical Therapy**

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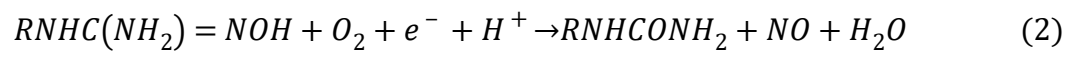
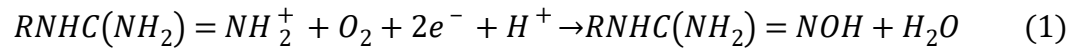
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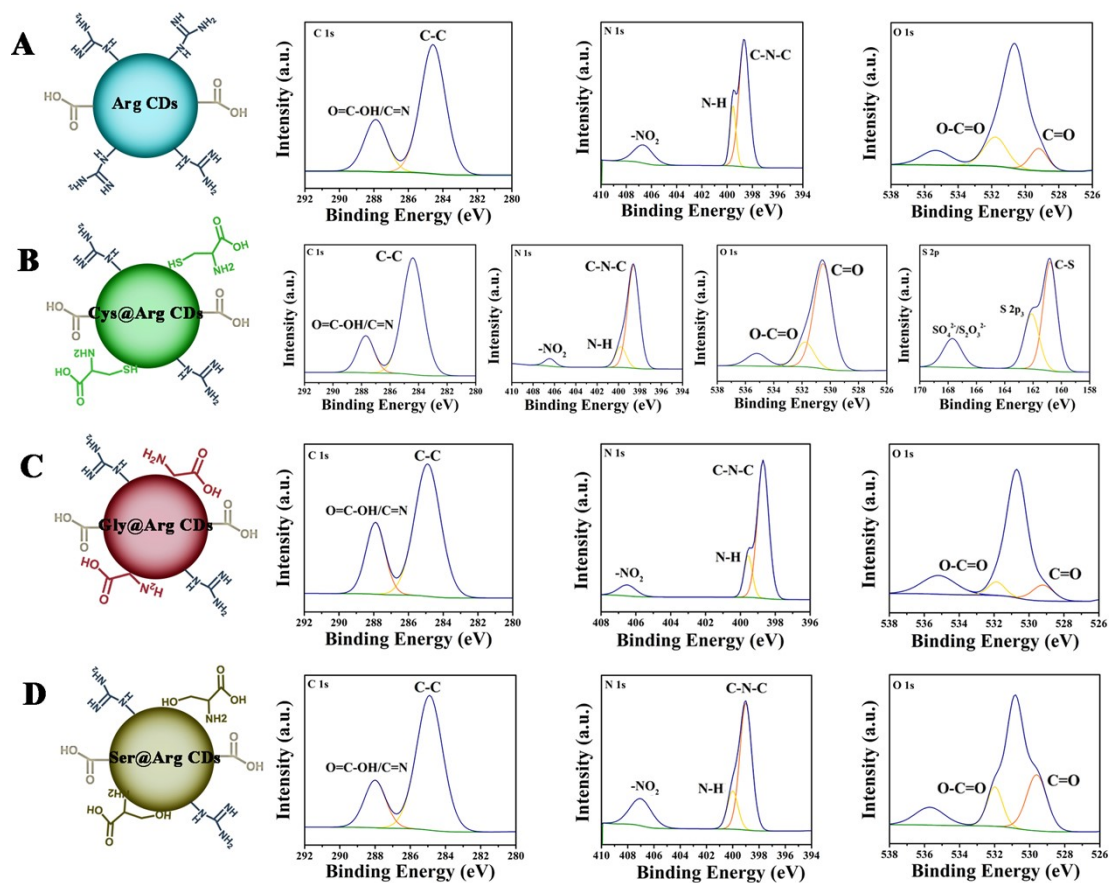
Chao Yu: E-mail: yuchao@cqmu.edu.cn

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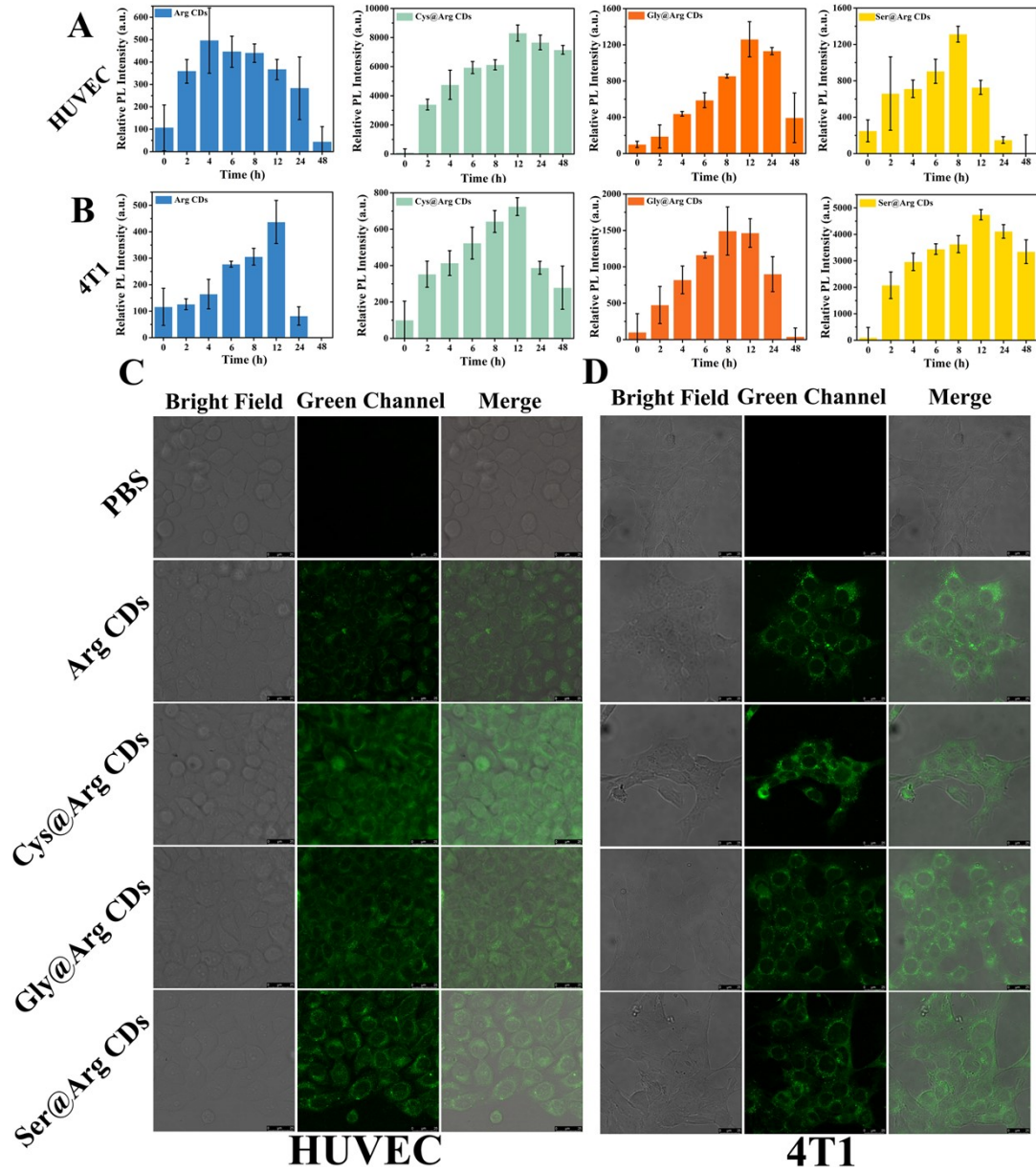


where  $R = CH_2CH_2CH_2CH(NH_2)COOH$

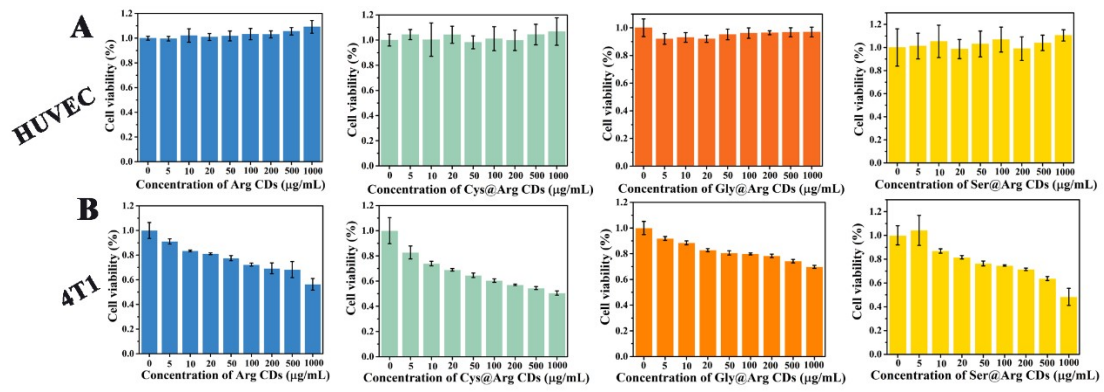
Eqs. (1) and (2).



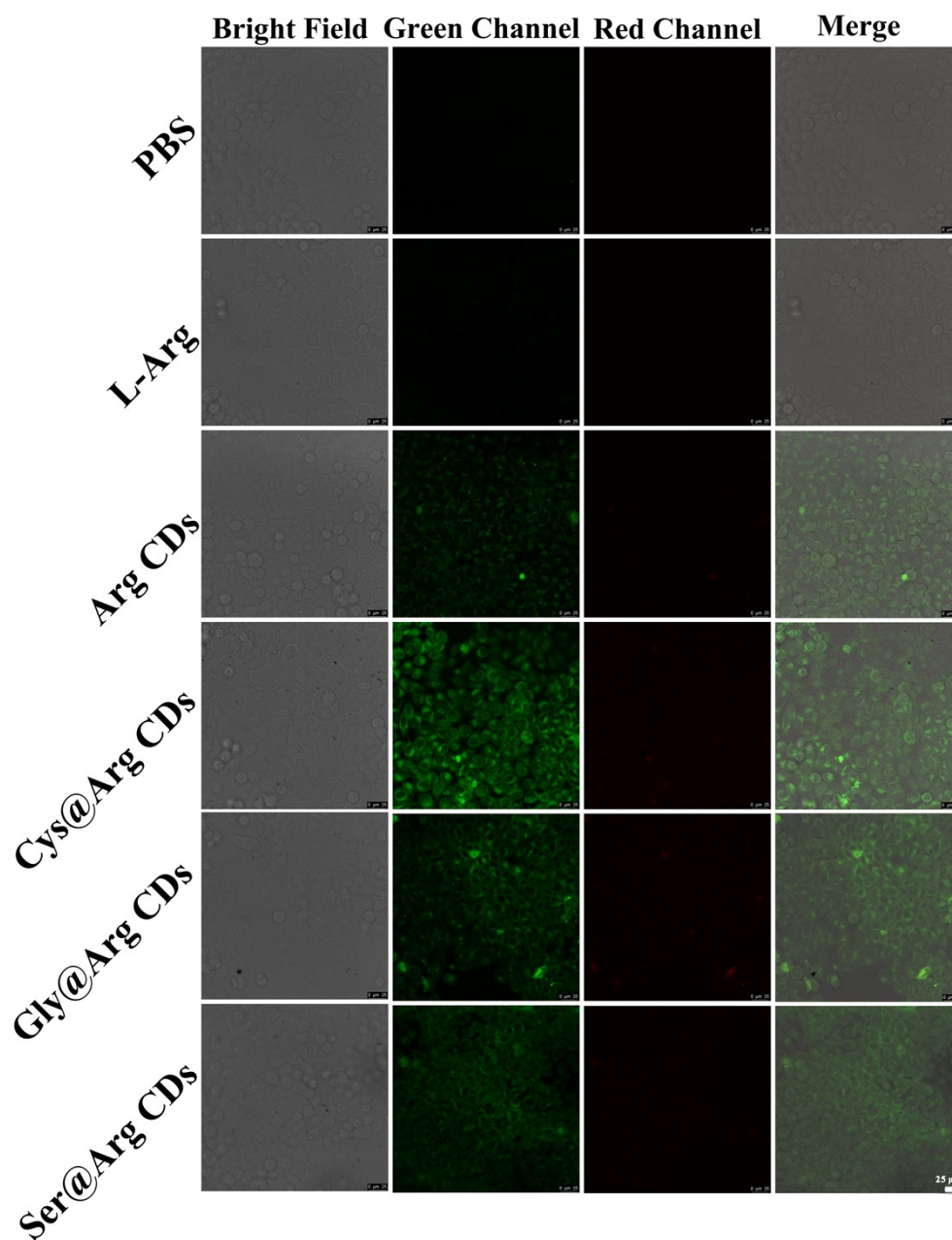
**Fig. S1.** XPS analysis of each component in (A) Arg CDs, (B) Cys@Arg CDs, (C) Gly@Arg CDs and (D) Ser@Arg CDs, respectively.



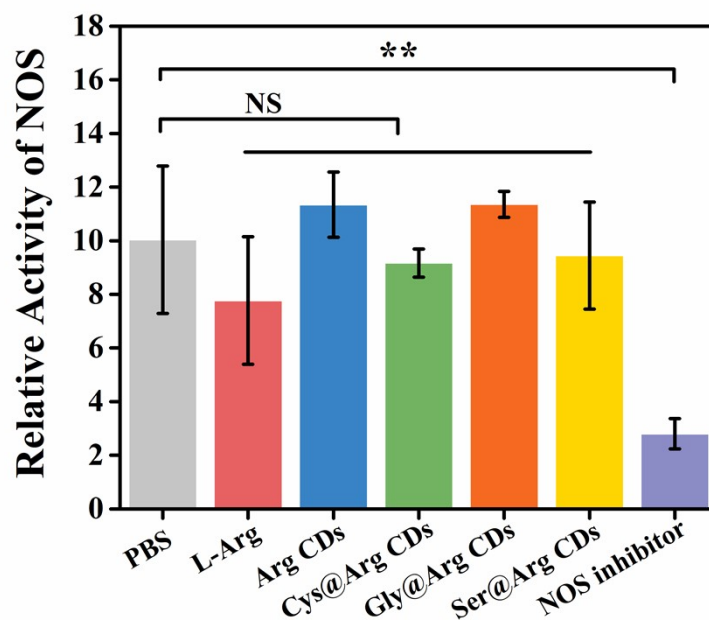
**Fig. S2.** (A) Using CLSM to investigate the cellular uptake efficiency of L-Arg-based CDs in HUVEC and (B) 4T1 cells with a variety of incubation durations (0, 2, 4, 6, 8, 12, 24, and 48 h),  $n = 3/\text{group}$ . The intracellular location of each L-Arg-based CDs in (C) HUVEC and (D) 4T1 cells after incubating corresponding optimal time (scale bar = 25  $\mu\text{m}$ ).



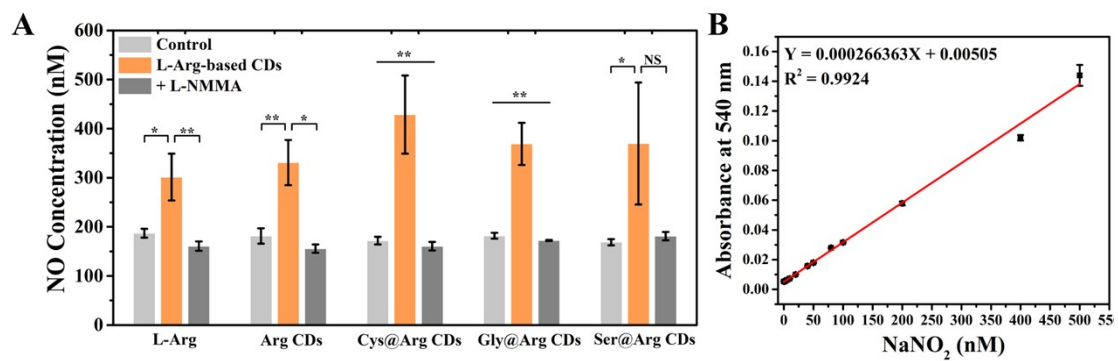
**Fig. S3.** Cytotoxicity test of Arg CDs, Cys@Arg CDs, Gly@Arg CDs and Ser@Arg CDs to (A) HUVEC (upper) and (B) 4T1 cells (lower). (n = 5/group)



**Fig. S4.** Using CLSM to observe the fluorescence intensity of L-Arg-based CDs *per se* in green channel (Ex = 405 nm, Em = 420-480 nm) and red channel (Ex = 488 nm, Em = 520-600 nm) in HUVEC (scale bar = 25  $\mu$ m). (n = 3/group)

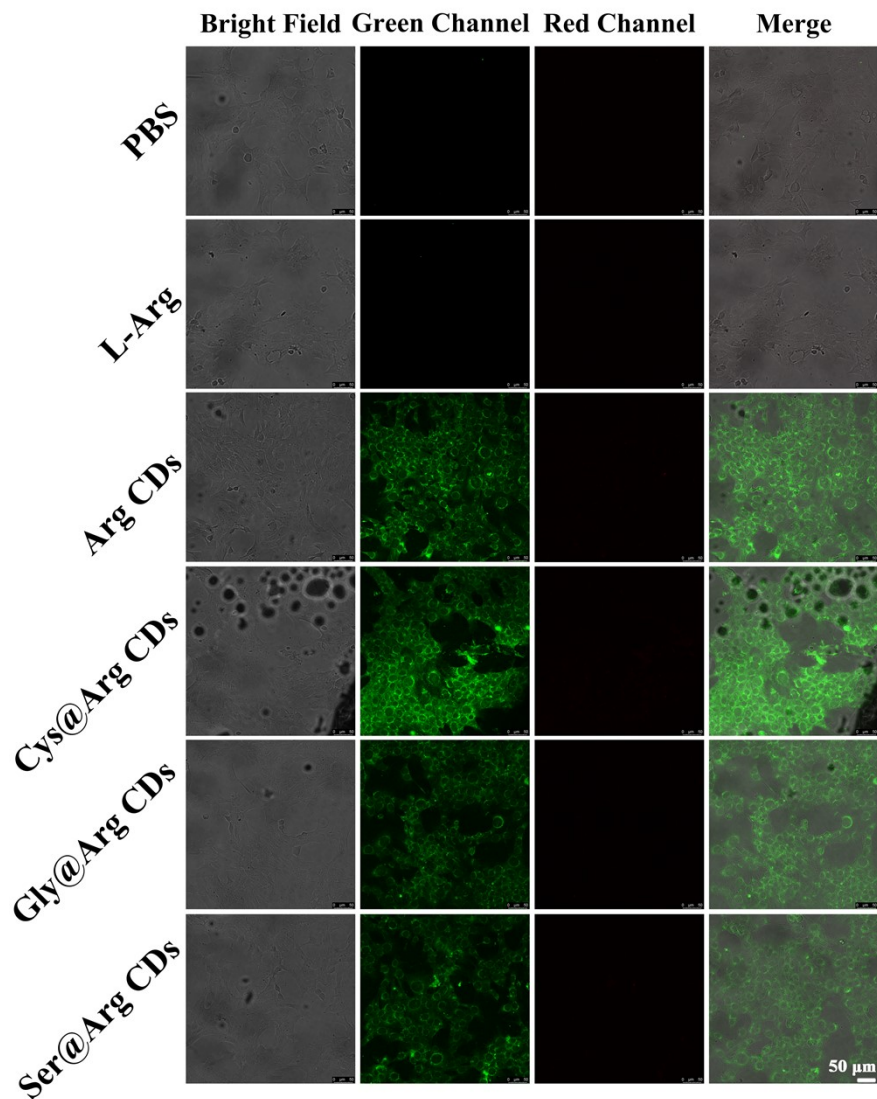


**Fig. S5.** Using Nitric Oxide Synthase Assay Kit to verify the effect on NOS activity of L-arginine-based CDs and NOS inhibitor (L-NMMA) in HUVEC. (n = 3/group, \*\*P < 0.01)

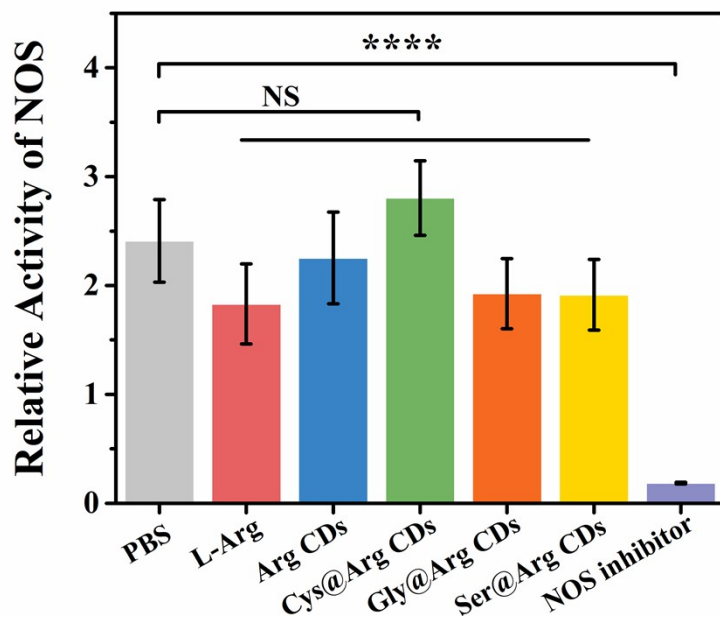


**Fig. S6.** (A) Using classic Griess Reagent to evaluate NO production in cell lysate supernatant after indicated treatment. (B) The standard curve which was made based on standard sample (NaNO<sub>2</sub>) for calculating NO concentration. (n = 3/group, \*P < 0.05, \*\*P < 0.01)

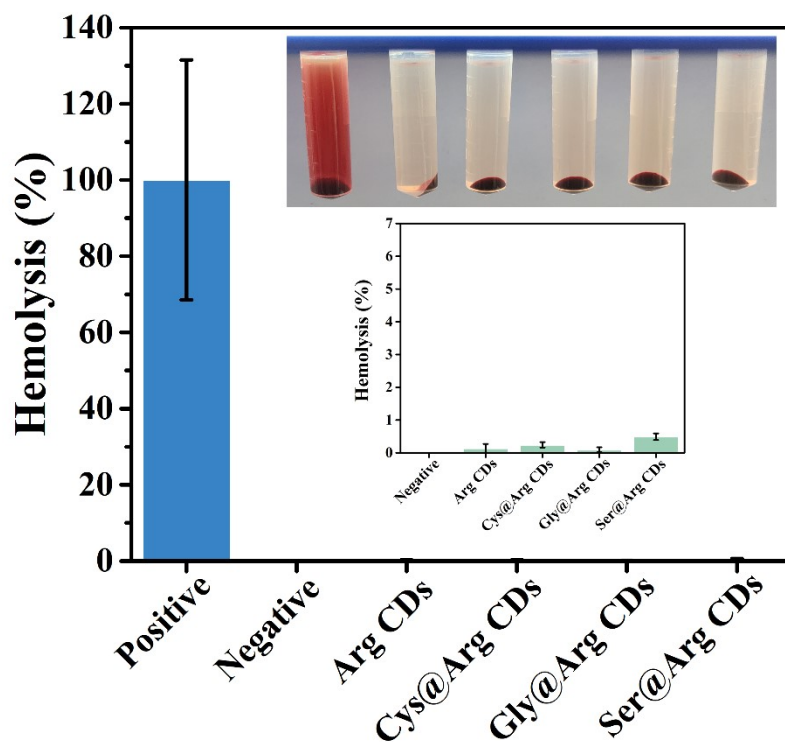




**Fig. S7.** Using CLSM to observe the fluorescence intensity of L-Arg-based CDs *per se* in the green channel (Ex = 405 nm, Em = 420-480 nm) and red channel (Ex = 488 nm, Em = 550-620 nm) in 4T1 cells (scale bar = 50  $\mu$ m). (n = 3/group)



**Fig. S8.** Using Nitric Oxide Synthase Assay Kit to verify the effect on NOS activity of each L-arginine-based CDs and NOS inhibitor (L-NMMA) in 4T1 cells. (n = 3/group, \*\*\*\*P < 0.0001)



**Fig. S9.** Hemolytic activities of Arg CDs, Cys@Arg CDs, Gly@Arg CDs and Ser@Arg CDs to red blood cells (RBCs). Hemolysis assay with ultra-pure-water and physiological saline as positive control and negative control, respectively. Insets: photographs of corresponding RBC solutions and partial enlarged figure. (n = 3/group)