**Supplementary Information** 

Genetically Engineered Magnetic Nanocages for Cancer Magneto-Catalytic Theranostics Zhang et *al*.

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## Genetically Engineered Magnetic Nanocages for Cancer Magneto-Catalytic Theranostics

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**Supplementary Figure 1.** Plasmid construction and expression of encABC. **a** encA cloned into pRSFDuet-1; encB and encC cloned into co-expression vector. **b** Representative cryo-EM image of encABC (scale bar: 50 nm). **c** SDS-PAGE to identify the compose of encABC. One of three repetitions with similar results is shown for (**b**) and (**c**).



**Supplementary Figure 2.** Morphology of fMIONs and eMIONs. **a** Representative TEM images of enc ABC (I) (Scale bar: 100 nm), eMIONs (II) (Scale bar: 100 nm) and fn (III) (Scale bar: 50 nm), fMIONs (IV) (One of three repetitions with similar results is shown, Scale bar: 50 nm); **b** Size of the fMIONs and eMIONs. **c** Size of the cores of fMIONs and eMIONs. **d,e,f** Stability detection of eMIONs in different conditions: various temperatures (25 °C, 37 °C, 50 °C, 60 °C and 70 °C) (**d**); in cell culture with 10% FBS at 37 °C (**e**); and in cathepsin B (0.2 units) solution at 37 °C (**f**). Data are presented as mean  $\pm$  SD (n = 5 independent experiments).



**Supplementary Figure 3.** Characterization of fMIONs and eMIONs. **a** XRD of eMIONs. **b** HRTEM and corresponding FFT pattern (inset) of fMIONs nanocrystals. One of three repetitions with similar results is shown. **c,d** XRD and XPS analysis to estimate fMIONs, showing its component is  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>.

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6.036 (0.036)	14.6297 (0.1732)	5.657	302 (5)	16343 (1137)	6.1	1.000	0.867	1.312 (0.073)	6.238 (0.021)	14.1576 (0.0952)	6.107	355 (17)	10821 (815)	4.5	1.000v	0.796	0.526 (0
24.372 (0.013)	3.6491 (0.0039)	24.360	462(13)	893 (2)	26	1.000	0.900	0.040 (0.030)	13 230 (0.054)	3.8782 (0.0838)	31.625	1 (2)	39062 (11810)	0.0	0.000	-0.900	27.800 (0
26.671 (0.244)	3.3395 (0.0600)	35.577	148 (3)	2042 (112)	100.0	0.973v	-0.873	30.366 (0.764)	24.108 (0.011)	3.6886 (0.0032)	24.078	150 (23)	726 (218)	0.3	0.000v	0.900	0.094 (0
30.078 (0.006)	2.9686 (0.0011)	30.056	381 (24)	2735 (265)	1.0	0.279v	0.639	0.126 (0.012)	27.503 (0.018)	3.2404 (0.0042)	27.441	121 (15)	1384 (287)	0.6	0.384v	0.897	0.198 (
34.577 (0.009)	2.5919 (0.0013)	34.538	166 (17)	1077 (209)	0.4	0.074v	0.900	0.124 (0.022)	6.922 (0.915)	12.7587 (?)	5.387	32 (8)	3585 (?)	1.5	0.000v	0.900	4.899 (1
35.439 (0.003)	2.5308 (0.0003)	35.390	833 (20)	6782 (268)	2.5	0.000v	0.900	0.156 (0.005)	39.030 (0.011)	2.3058 (0.0012)	38.992	136 (19)	865 (210)	0.4	0.000∨	0.900	0.123 (
40.514 (0.013)	2.2248 (0.0013)	40.495	123 (18)	712 [189]	0.3	0.000v	0.591	0.113 (0.026)	✓ 43.414 (0.009)	2.0826 (0.0008)	43.382	164 (20)	854 (203)	0.4	0.0000	0.900	0.101 (
54.420 (0.006) EC 950 (0.010)	1.6846 (0.0003)	54.351 EC 91C	281 (14)	021 (102)	1.0	1.000	0.900	0.188 (0.013)	49,538 (0.005)	1.3005 (0.0004)	49,549	106 (17)	1651 (192)	9.2	0.000	0.536	0.120.0
62 537 (0.010)	1.4840 (0.0003)	62 495	180 (13)	1603 (199)	0.5	0.155	0.300	0.165 (0.023)	54 105 (0.008)	1.6936 (0.0005)	54 059	203 (16)	1559 (218)	0.5	0.000	0.900	0.148 (
75.280 (0.010)	1.2613 (0.0003)	75.282	161 (12)	210 (?)	0.6	0.483v	-0.055	0.168 (0.016)	56.575 (0.010)	1.6254 (0.0005)	56.562	248 (19)	3223 (357)	1.3	0.564v	0.256	0.207 (
				1000					62.542 (0.011)	1.4839 (0.0005)	62.516	114 (20)	761 (199)	0.3	0.963v	0.829	0.096 (
									66.327 (0.018)	1.4081 (0.0007)	66.309	111 (14)	1301 (253)	0.5	0.371v	0.349	0.205 (
									75.381 (0.010)	1.2599 (0.0003)	75.380	212 (14)	2662 (237)	1.1	0.592v	0.007	0.199 (
									84.024 [0.014]	1.1509 [0.0003]	84.055	129112	1601 [214]	0.7	U.674V	-0.585	0.1951

**Supplementary Figure 4.** Crystallinity evaluation of eMIONs and fMIONs. Results of XRD data analysis by JADE software, showed that the crystallinities of eMIONs (**a**) and fMIONs (**b**) were 99.99% and 47.66%, respectively.



**Supplementary Figure 5.** Magnetic responsiveness evaluation of fMIONs and eMIONs. Results of VSM analysis of eMIONs and fMIONs.



**Supplementary Figure 6.** Magnetic hyperthermia evaluation of fMIONs and eMIONs. **a** Temperature changes within different concentrations of eMIONs and

fMIONs in AMF (500 kHz, 20 KA/m). **b** Temperature changes within different AMF powers (eMIONs 0.5 mM; fMIONs 0.5 mM). Data are presented as mean  $\pm$  SD (n = 5 independent experiments).



**Supplementary Figure 7.** Cell uptake and cytotoxicity of NPs. **a** Cell MRI of eMIONs with different concentrations incubated 3 h with A549 cell line. **b** encABC incubated in different cell lines with different concentrations. **c** eMIONs incubated in different cell lines with different concentrations, p = 0.035. **d** Concentration of H<sub>2</sub>O<sub>2</sub> in different cell lines and after incubated with eMIONs and fMIONs. Data are presented as mean  $\pm$  SD (n = 5 independent samples), and statistical significance was calculated with two-tailed Student's *t* test, and \* indicated that p < 0.05.



**Supplementary Figure 8.** Cellular toxicity of LM3 cell line with treatment of eMIONs. Cell viabilities of LM3 cell line receiving different concentration of eMIONs with/without AMF. Data are presented as mean  $\pm$  SD (n = 5 biologically independent samples). Statistical significance was calculated with two-tailed Student's t test, and \* indicated that p < 0.05.



**Supplementary Figure 9.** Cellular toxicity of A549 and LM3 cell lines with treatment of fMIONs. Cell viabilities of A549 and LM3 cell lines receiving different concentration of fMIONs with/without AMF. Data are presented as mean  $\pm$  SD (n = 5 biologically independent samples).



**Supplementary Figure 10.** Biodistribution of eMIONs. Biodistribution of eMIONs *via* tail vein injection at different time points in main organs and tumor. Data are presented as mean  $\pm$  SD (n = 5 biologically independent mice).



**Supplementary Figure 11.** H&E staining, hemolytic activity test, and body weight changes of subcutaneous models after therapy. **a** H&E staining of A549 tumor regions at 7 d after different treatments, red solid line shows the regions of undergoing cell death. Scale bar of above pictures: 500  $\mu$ m; Scale bar of nether pictures: 200  $\mu$ m. **b** Hemolytic test to verify the biosafety of eMIONs. Data are presented as mean  $\pm$  SD (n = 3 biologically independent samples). **c** Body weight changing process of mice after therapy. **d** H&E stained major organs harvested from the A549 subcutaneous tumor mice after different therapy, Scale bar: 100  $\mu$ m. The tumor and all major organs of different groups (n = 5 biologically independent mice) were collected and a slice of each one was stained, the representative images are shown here for (**a**) and (**d**).



**Supplementary Figure 12.** H&E staining of orthotopic models after therapy. H&E stained major organs harvested from the orthotopic hepatocellular carcinoma mice after different therapy. Scale bar: 100  $\mu$ m. The major organs of PBS group (n = 3 biologically independent mice), eMIONs group (n = 3 biologically independent mice) and eMIONs + AMF group (n = 5 biologically independent mice) were collected and a slice of each one was stained, the representative images are shown here.