Supporting Information

Flower-like Mn-doped Magnetic Nanoparticles Functionalized with $\alpha_v\beta_3$-Integrin-Ligand to Efficiently Induce Intracellular Heat after Alternating Magnetic Field Exposition, Triggering Glioma Cell Death.

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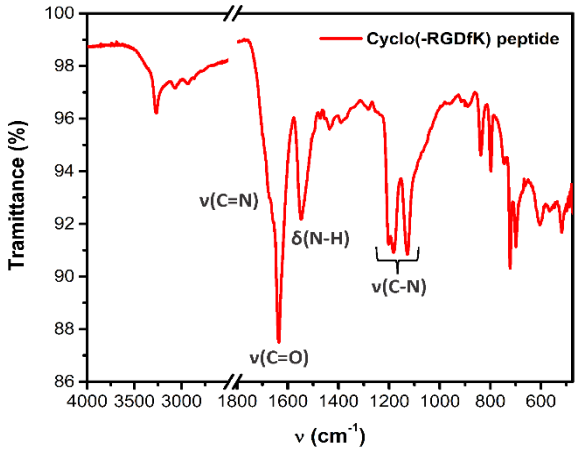

Figure S1. ATR spectra of Cyclo(-RGDfK) peptide.

S2. Estimation of the number of cRGD peptide molecules on NF-DMSA nanoparticles surface.

To estimate the amount of peptide molecules per nanoparticle, we consider the particle as a sphere to simplify the calculations, so its volume is given by:

$$V = \frac{4}{3}\pi r^3$$

where, the diameter from TEM is $D = 18.3$ nm, so the volume of each nanoparticle is:

$$V = 3591\ \times 10^{-21} cm^3.$$

The nanoparticle volume in one gram is obtained as follows:

$$V_{Fe_3O_4} = \frac{m}{\rho_{Fe_3O_4}} = \frac{1gr}{5.1\frac{gr}{cm^3}} = 0.196cm^3,$$

so, the total number of nanoparticles in one gram is:

$$Nº\ of\ NPs\ per\ gram = \frac{V_{Fe_3O_4}}{V} = 5.46 \times 10^{22}.$$

From the Micro BCA Protein Assay Reagent Kit analysis, we know that $1\frac{mg\ NPs}{mL}$ is equivalent to $1.47\frac{\mu g\ Peptide}{\mu L}$. To know the number of peptide molecules in $1.47\frac{\mu g\ Peptide}{\mu L}$, we divide by the molecular weight of it and multiply it by Avogadro's number $N_A = 6.022\ 141\ 29 \times\ 10^{23} mol^{-1}$, so:

$$Nº\ of\ peptide\ molecules\ per\ gram = \frac{1.47 \times 10^{-3} g}{603.7\frac{g}{mol}} \times N_A = 1.47 \times 10^{18}$$

Therefore, to know the number of peptide molecules per nanoparticle:

$$Nº\ of\ peptide\ molecules\ per\ particle = \frac{5.46 \times 10^{22}}{(1.47 \times 10^{15})(10^3)} = \mathbf{37.19}$$

(The factor 10^3 in the denominator is due to the equivalence mentioned above)

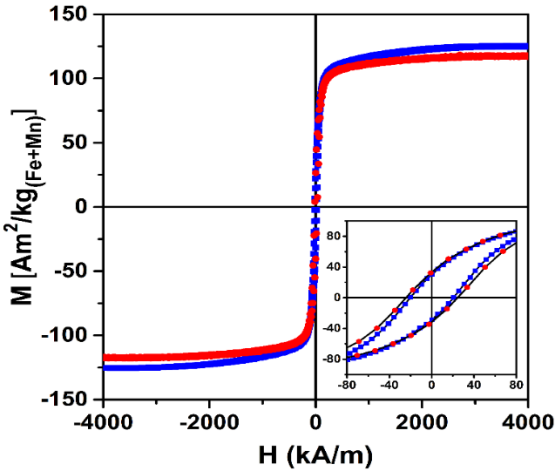

Figure S2. Hysteresis loops recorded at 5 K for NF-DMSA (blue) and NF-DMSA-PEP (red) nanoparticles. Inset: low field hysteresis loops for both nanoparticles.

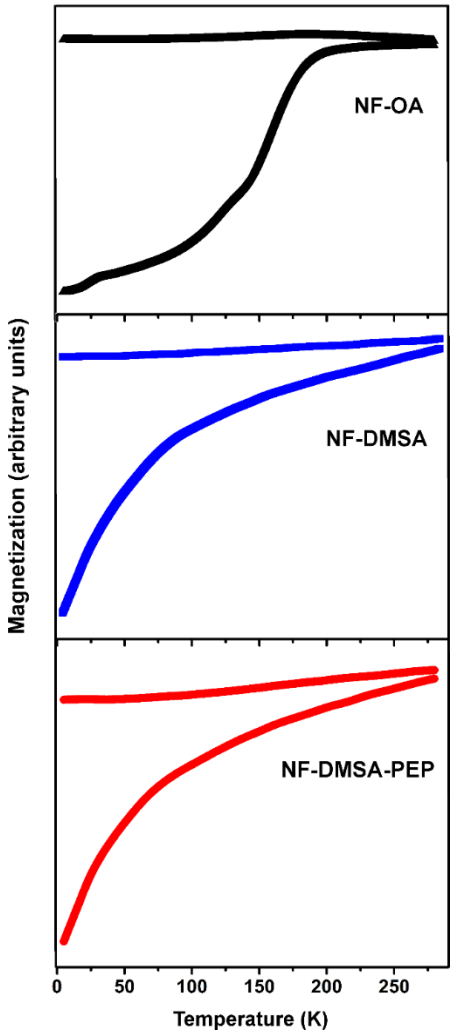

Figure S3. ZFC-FC of NF-OA, NF-DMSA and NF-DMSA-PEP nanoparticles under 100 Oe field.

Table S1. Magnetic properties of NF-DMSA and NF-DMSA-PEP nanoparticles at 5 and 300 K.

Samples	M_S ($Am^2/kg(Fe+Mn)$)		M_r ($Am^2/kg(Fe+Mn)$)		H_C (kA/m)	
	5 K	**300 K**	**5 K**	**300 K**	**5 K**	**300 K**
NF-DMSA	125	99	29.4	2.7	20.4	1.25
NF-DMSA-PEP	117	97	30.5	5.2	24.4	2.42

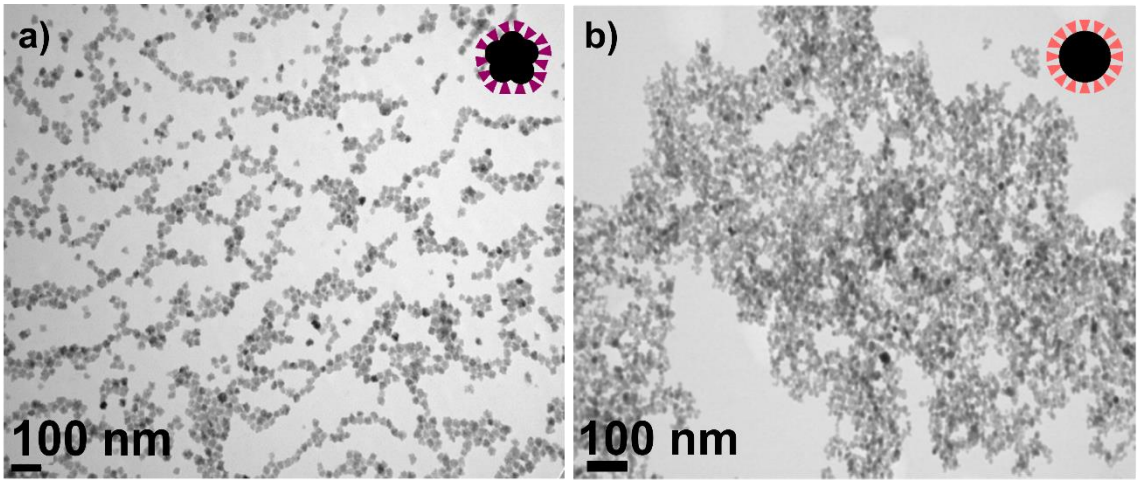

Figure S4. Transmission electron microscope (TEM) images of nanoparticles used as reference samples. (**a**) 20.8±0.1 nm Flower-like Fe_3O_4 nanoparticle coated with citric acid (NF-REF) and (**b**) 12.2±2.3 nm spherical Fe_3O_4 nanoparticle coated with dimercaptosuccinic acid (NP-REF). Inset: Carton images corresponding to NF-REF and NP-REF, respectively.

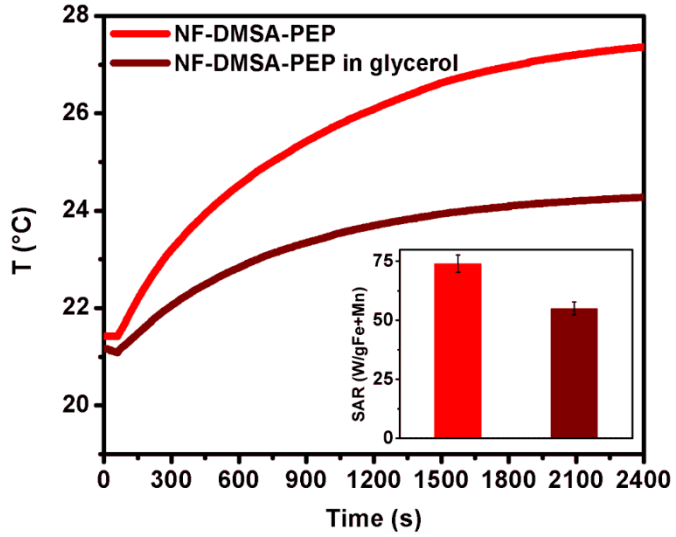

Figure S5. Heating curves of NF-DMSA-PEP in water and NF-DMSA-PEP in a water/glycerol mixture (50/50, v/v) under the experimental condition of (f = 96 kHz, H = 47 kA/m), (V_F = 1ml for NF-DMSA-PEP in water and V_F = 500µl NF-DMSA-PEP in a water/glycerol mixture) for 40 minutes. The content of the magnetic ions for the sample was ([Fe + Mn] = 0.45 mg/m L). Inset: SAR values of both samples. The SAR values were calculated using the first 30 s of the initial slopes.

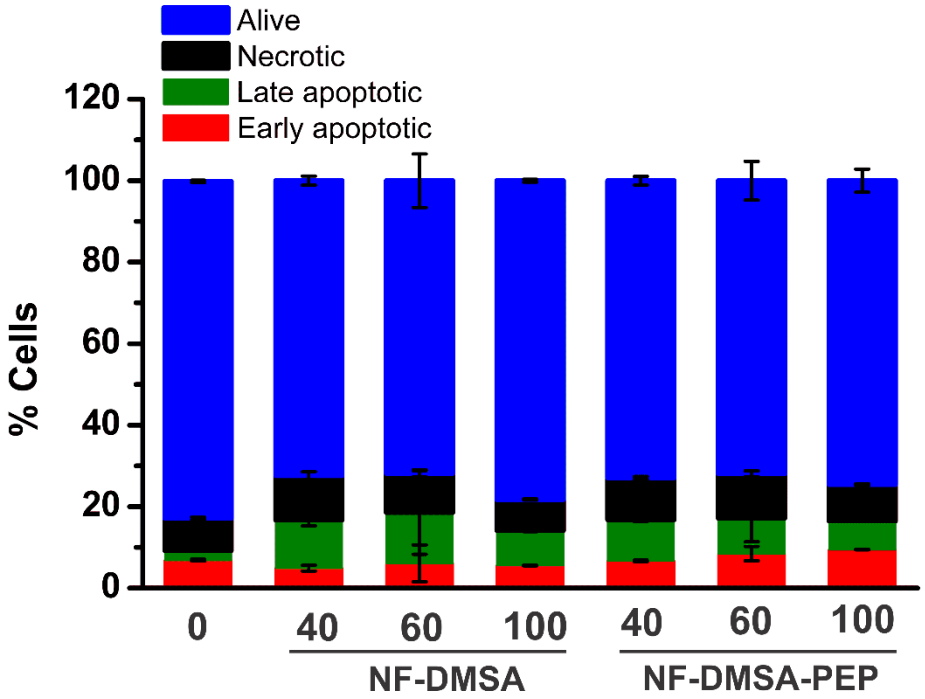

Figure S6. Annexin-V/PI analysis by flow cytometry of U87MG cells after incubation with NF-DMSA or NF-DMSA-PEP at 24 h. Concentration was varied between 0 -100 µg Fe/mL concentration of NPs. Data (mean ±SD) are representative of three independent

experiments. Cell survival was analyzed for FITC-annexin V/propidium iodide staining (Ex/Em = 495/519 nm). The samples were analyzed by flow cytometry, with the FC500 flow cytometer (Beckman Coulter).

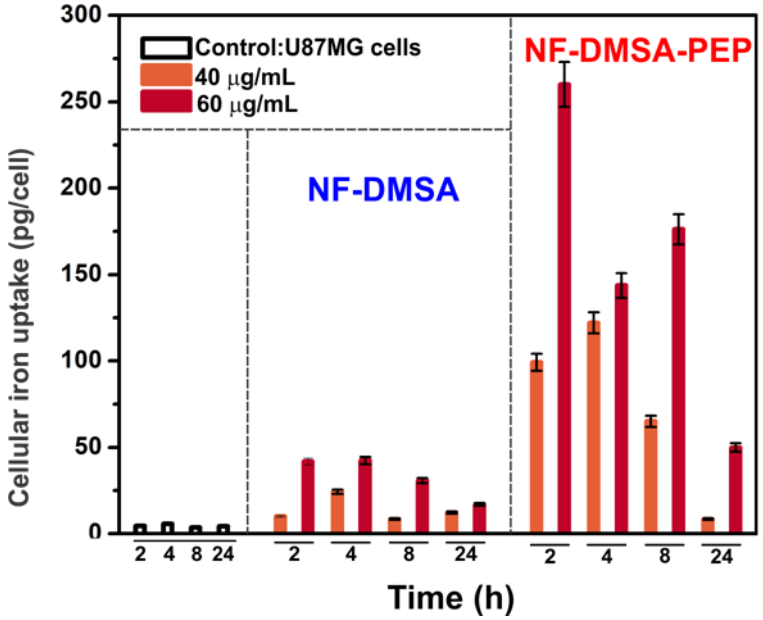

Figure S7. Uptake kinetics for NF-DMSA and NF-DMSA-PEP nanoparticles at (0, 40 and 60 μg Fe/mL concentration of NPs in U87MG cells determined by ICP-OES. Data (mean ±SD) are representative of three independent experiments.

Iron Quantification in supernatants by ICP-OES. The cells have been seeded into a 6-well plate with a density of 2 x 10^4 cells per well (24h, 37ºC) in Dulbecco's modified Eagle's medium (DMEM). Then, NF-DMSA-PEP sample at concentration of 60 μg/mL has been added into the plate at different time intervals (0, 2, and 4 h). After incubation, the supernatants have been removed (named in the Figure S8 as SN1), and the cells have been harvested with fresh DMEM. After that, the cells loaded NF-DMSA-PEP have been exposed to an AMF of 96 kHz and 47 kA/m for 1 h. Immediately, after MHT, the new supernatants have been collected and reserved in an Eppendorf tube. The samples (25 μL) have been digested in aqua regia at 60ºC overnight and diluted up a volume of 25 mL with deionized water. The amount of iron in each supernatant has been measured by ICP-OES (Perkin Elmer-2400).

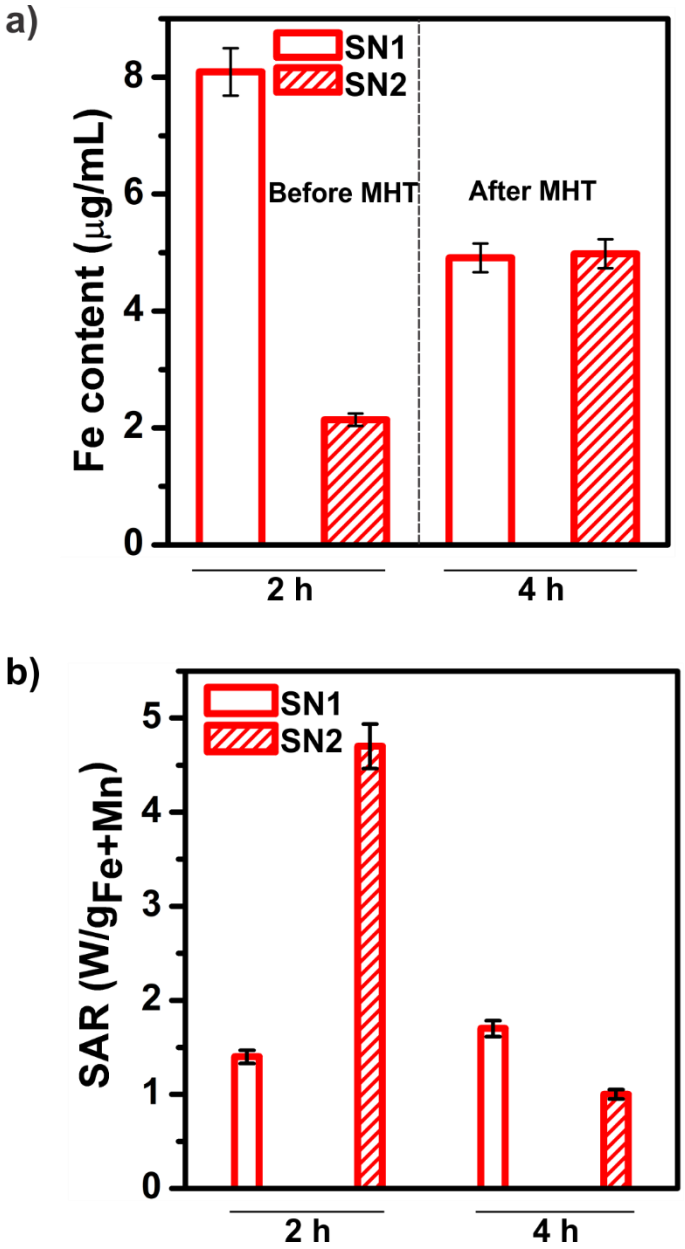

Figure S8. (a) Quantification of iron concentration in 1mL of the supernatant before (SN1) and after (SN2) MHT with NF-DMSA-PEP loaded U87MG cells at 2 and 4 h by ICP-OES. Data are shown as mean ± SD (n = 3) and (b) SAR values of SN1 and SN2 under the same experimental condition (f = 96 kHz, H = 47 kA/m) at 2 h and 4 h. The heating behavior of each supernatant to determine de SAR values have been performed in an Eppendorf tube, after 20 min of ultrasound sonication of the samples.