Supplementary Material

Safety evaluation of graphene oxide-based magnetic nanocomposites as MRI contrast agent and drug delivery vehicle

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S1: H&E and Prussian Blue stain standard protocol

Table S1

Operation of H&E and Prussian Blue staining

Procedure	Solution	Soak time (min)
1	Xylene	3
2	1/2 Xylene+1/2 Ethanol absolute	2
3	Ethanol absolute	2
4	95% Ethanol	2
5	83% Ethanol	2
6	70% Ethanol	2
7	50% Ethanol	2
8	35% Ethanol	2
9	Distilled water	2
10	Pearl solution	30
11	Distilled water	2
12	70% Eosin	2
13	70% Ethanol	0.5
14	83% Ethanol	0.1
15	95% Ethanol	2
16	100% Ethanol	2
17	1/2 Ethanol absolute + 1/2 Xylene	2
18	Xylene	2

Pearl solution: 10 mL of 8% potassium ferrocyanide was mixed with 30 mL 8% hydrochloric acid before use.

S2 Histopathological analysis

To further verify the uptake of uGO@Fe_3O_4 NSs and NPs in liver and spleen as described by MRI detection, Prussian blue staining of slides from two organs was achieved (Figure S2). In the liver tissue treated with the uGO@Fe_3O_4 NPs or NSs, the histological analysis revealed that iron particles were dispersedly distributed and persistently retained. In the case of uGO@Fe_3O_4 NSs, the iron staining of nanocrystals were obviously bigger than that of injecting NPs. The similar situations were also observed in the spleen. There was only a small amount of iron particle distribution in the case of uGO@Fe_3O_4 NPs, while there was denser particle distribution in the case of uGO@Fe_3O_4 NSs. It's worth noting that although the iron particles in the liver appeared less than that in the spleen, its absolute content in liver is higher because the liver is the bigger tissue. In addition to large amount of particle accumulations, there were apparent chronic toxicity effects occurring in the liver and spleen after intravenous injection. Take liver as example, histopathological observation indicated that liver of exposure to uGO@Fe_3O_4 NSs generated a time-dependent liver inflammatory response characterized by granulomas (G), swollen vacuolated bundles (S), and interstitial edema (E) (Figure S2 (A) and (B)). Over time, the toxicity reaction of the liver of mice becomes more and more severe. For example, the nanocomposites induced more swollen vacuolated bundles and interstitial edema. Some inflammation cells were infiltrated in liver interstitium. The hepatic architecture of mice showed some deformation.

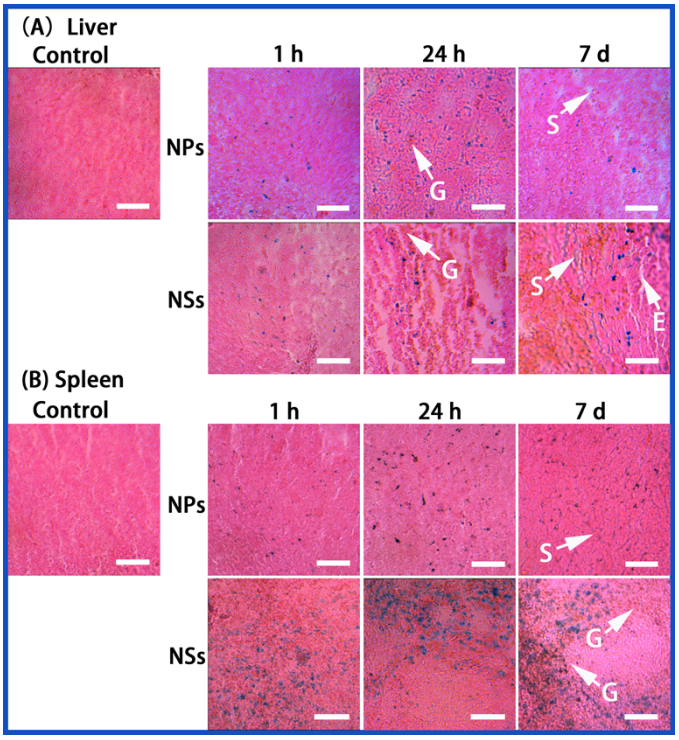


Figure S2. Representative histological images by iron Prussian blue staining in the liver (A) and spleen (B) after injecting uGO@Fe_3O_4 NPs and NSs formulations with a 5 mg Fe/kg of dose at 1 h, 24 h, and 7 days. Hepatic tissue from uGO@Fe_3O_4 NSs-treated group revealed granulomas (G), swollen vacuolated bundles (S), and interstitial edema (E). The control samples were derived from mouse tissues that were treated with PBS. The scale bar indicates 100 μm. H&E and Prussian Blue staining.

S3 The ratio of absorbance values 260/280

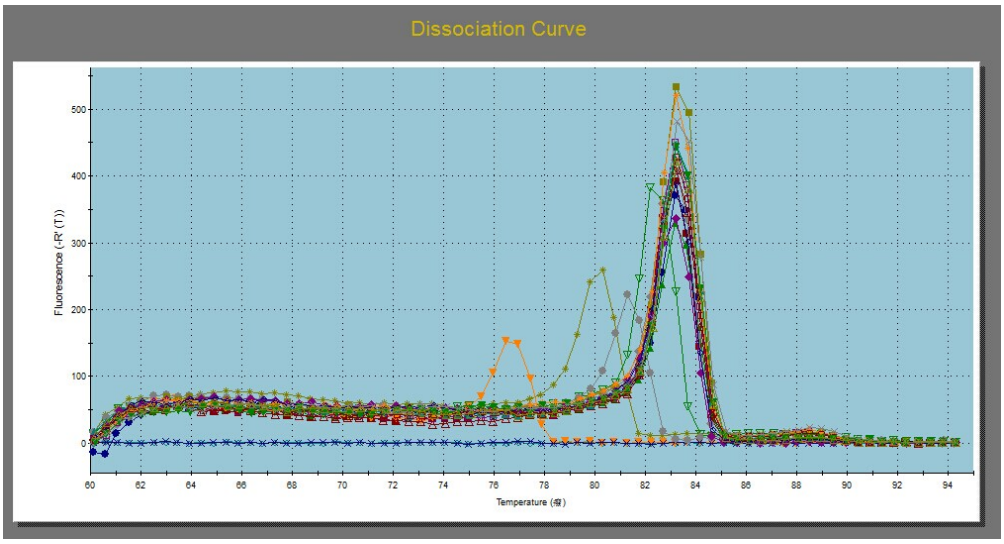
Table S3

The ratio of absorbance values 260/280 of total RNA extracted from mice liver of various administrations.

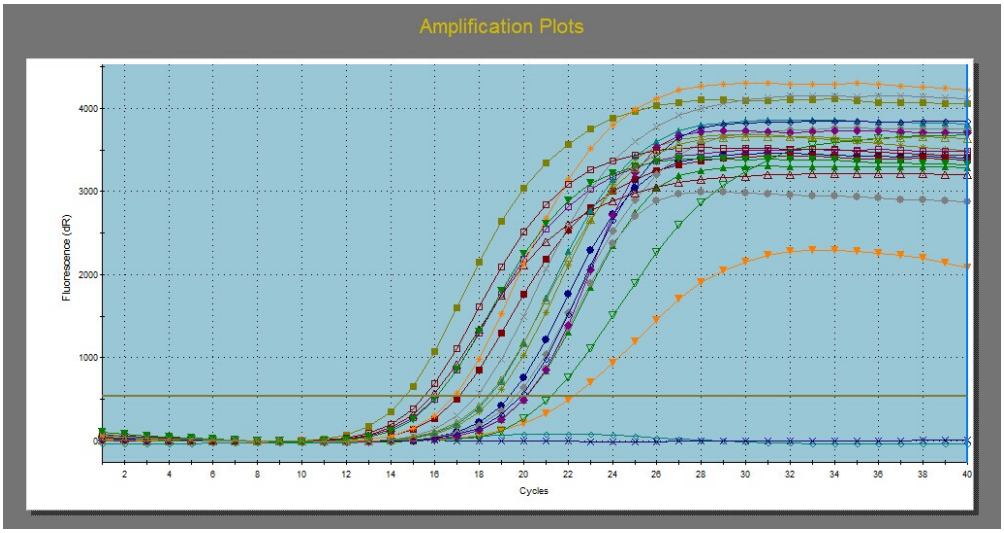
Sample number	Nanocomposites	Dosage (mg Fe/kg)	Ratio of Abs (260/280)
1	uGO@Fe_3O_4 NSs	5	1.87
2			1.88
3			1.86
4		7.5	1.80
5			1.86
6			1.93
7		10	1.96
8			1.82
9			1.85
10	uGO@Fe_3O_4 NPs	5	1.81
11			1.87
12			1.87
13		7.5	1.99
14			1.89
15			1.80
16		10	1.81
17			1.91
18			1.92
19	Control	PBS	1.82
20			1.90
21			1.88

The ratio of absorbance values 260/280 of obtained RNA samples were between 1.8~2.0.

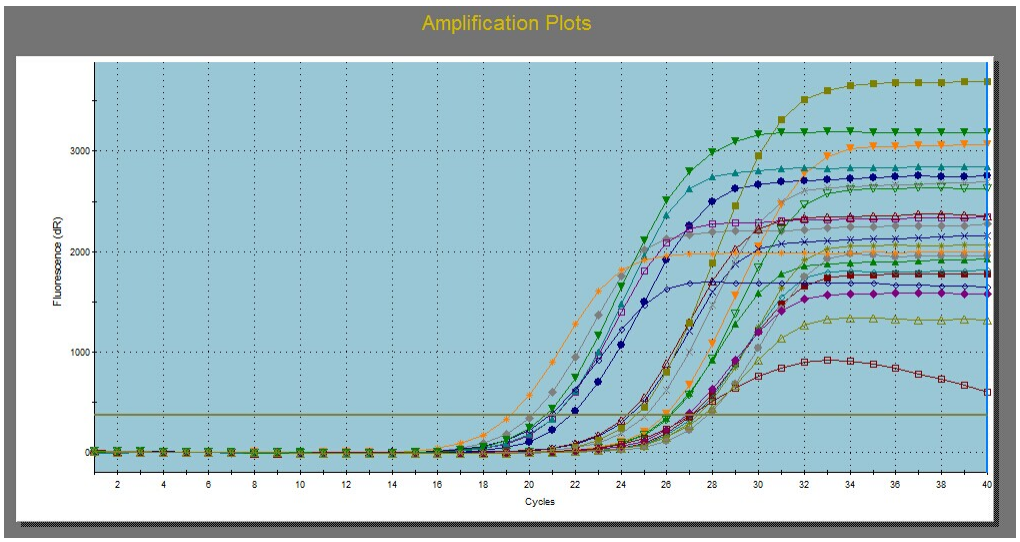
S4: Amplification plots and dissociation curve of SOD, CAT, and GPx



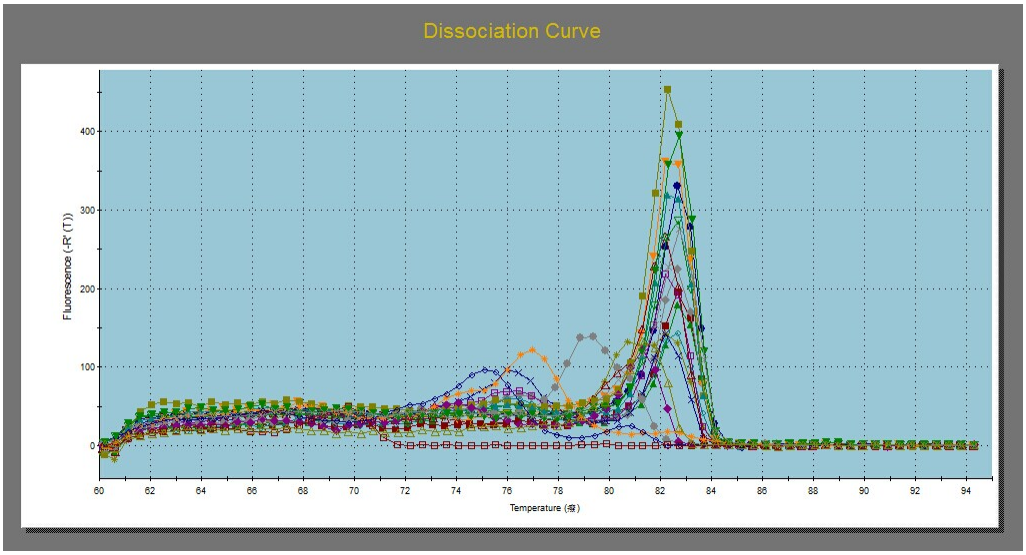
SOD



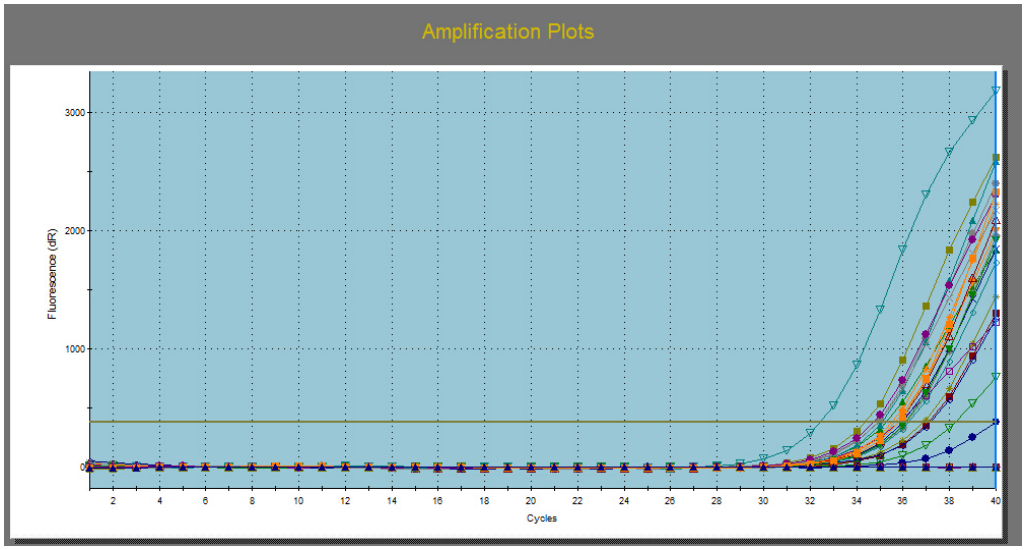
SOD



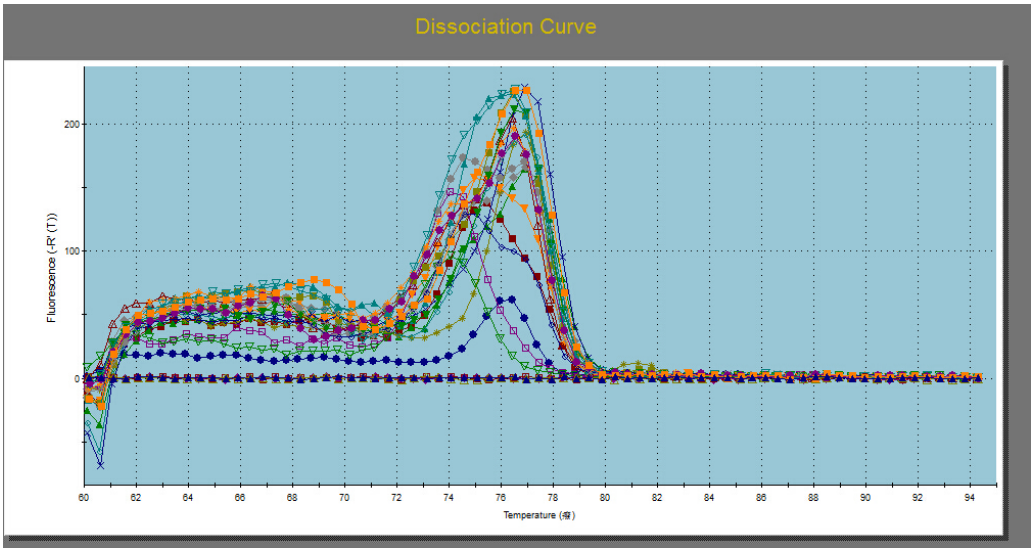
CAT


Dissociation Curve
Fluorescence (-R' (T))
Temperature (°C)

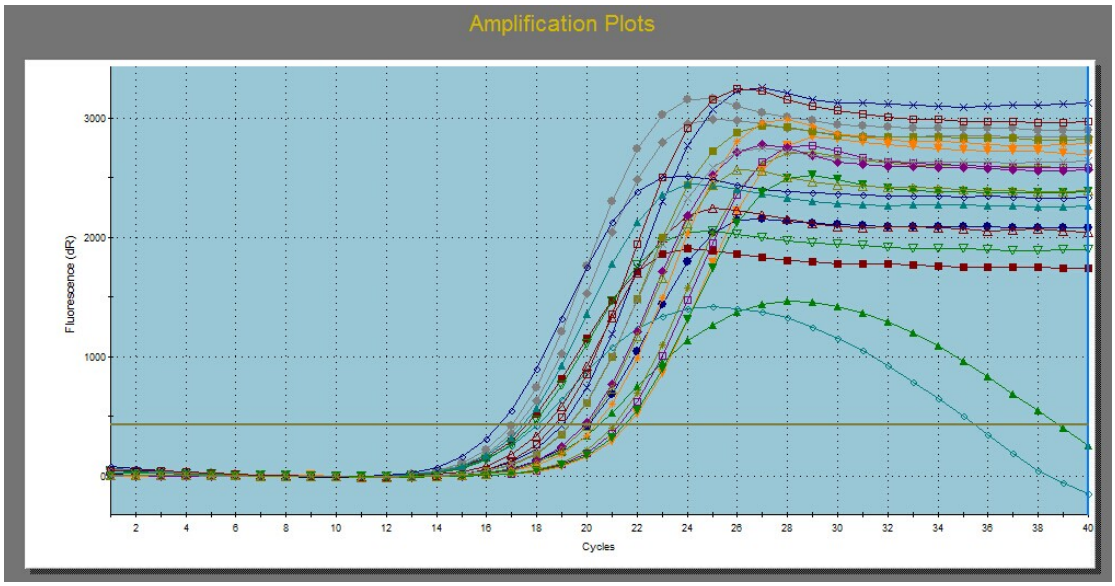
CAT


Amplification Plots
Fluorescence (dR)
Cycles

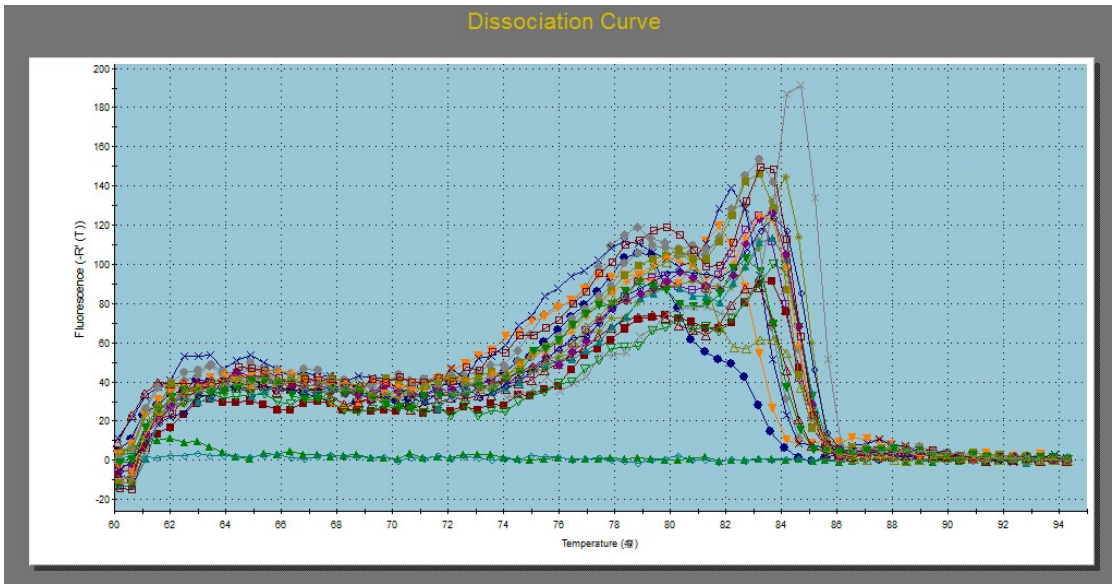
GPx


Dissociation Curve
Fluorescence (-R' (T))
Temperature (°C)

GPx



GAPDH (Internal standard primer)



GAPDH (Internal standard primer)

Figure S4. Amplification plots and dissociation curve of SOD, CAT, and GPx.

S5 The identification of PCR products

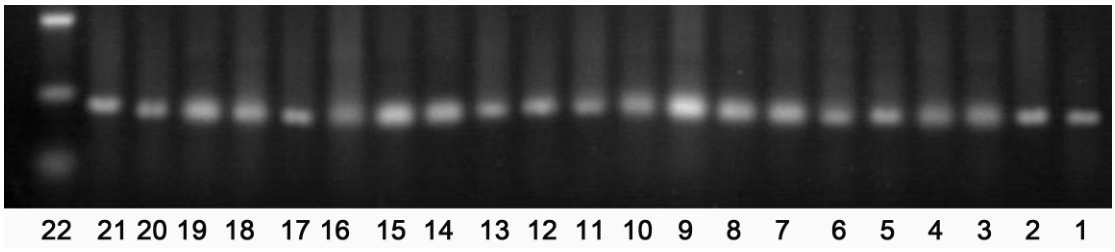


Figure S5. The agarose gel electrophoresis patterns of PCR amplification of mRNA isolated from livers of various administration mice. Lane 1～21 correspond to sample numbers in Table S3. Lane 22 is standard sample.

Only one single band was visualized on a 0.2% agarose gel under UV exposure (Fig. S5), confirming that each designed primer is specifically hybridized to the target sequence.