Supplementary Information

Biocompatibility of graphene oxide post intravenously administrated in mice—effects of dose, size and exposure protocol

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S1. Preparation of graphene oxide (GO)

Graphite (3.0 g) was mixed with $K_2S_2O_8$ (2.5 g) and P_2O_5 (2.5 g). The mixture was added into 12 mL H_2SO_4 and stirred at 80 °C for 4.5 h. After cooling to room temperature, the suspension was poured into 500 mL deionized water. The residues were filtered and washed with deionized water to remove excess acid. The residues

were dried and added into 120 mL H_2SO_4. Then, 15 g $KMnO_4$ was added slowly under vigorous stirring. The reaction was performed at 35 °C for 2 h. With the addition of 250 mL deionized water, the mixture was stirred for another 2 h. To the mixture, 500 mL deionzed water and 20 mL H_2O_2 (30% v/v) were added. After filtration, the residues were washed by HCl (10% v/v) and then deionized water. The residues were dispersed in water and sonicated (180 W) for 60 min to generate homogeneous solution.

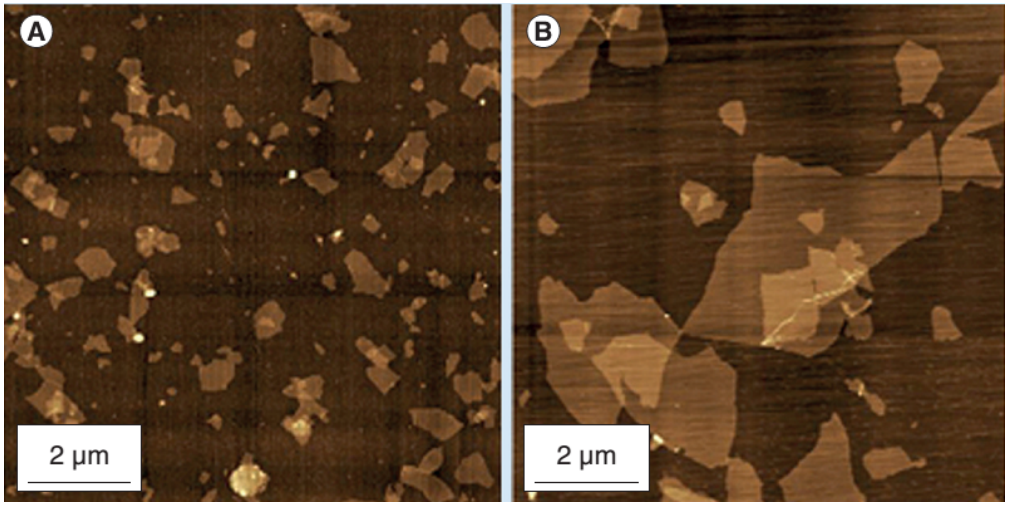

Fig. S1 Representative atomic force microscopy images of GO. (A) s-GO. (B) l-GO. (Reproduced from Nanomedicine. (2012) 7(12), 1801-1812 with permission of Future Medicine Ltd.)

S2. **Histological changes of the spleen of the mice exposed to GO**

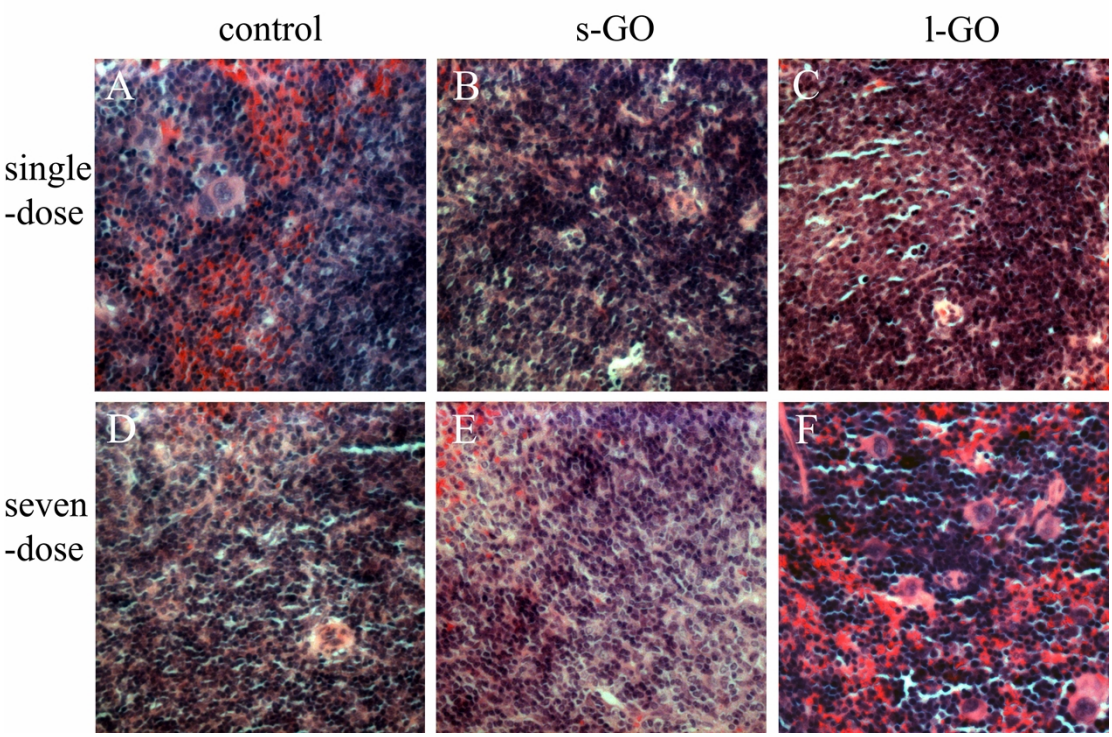

Fig. S2. Representative histological changes of the spleen of the mice exposed to GO. The dose of s-GO and l-GO is 2.1 mg/kg for single-dose groups, and 0.3 mg/kg (every other day, totally seven injections) for multiple-dose groups.

S3. **Determination of GO contents in lungs by the spectrometric method**

The lung tissue (~0.2 g) was minced and homogenized in 5 mL water. The homogenates were centrifuged at 2000 rpm for 10 min to remove ions. The deposit was digested in 5 mL HNO_3 by heating to boiling. Then 30% H_2O_2 was added by drops to make the solution transparent. The obtained solution was diluted to 5 mL with water for the UV-Vis spectrum measurement.

The concentration of GO in digested solution was calculated from the absorbance at 450 nm according to a standard calibration curve. The curve was made according to the absorbance of different concentration of GOs after being digested together with lung tissues. In brief, different aliquots of 1 mg/kg GO suspension were added into the beaker and then digested together with 0.2 g of lung tissue using the same method as that for the above samples.

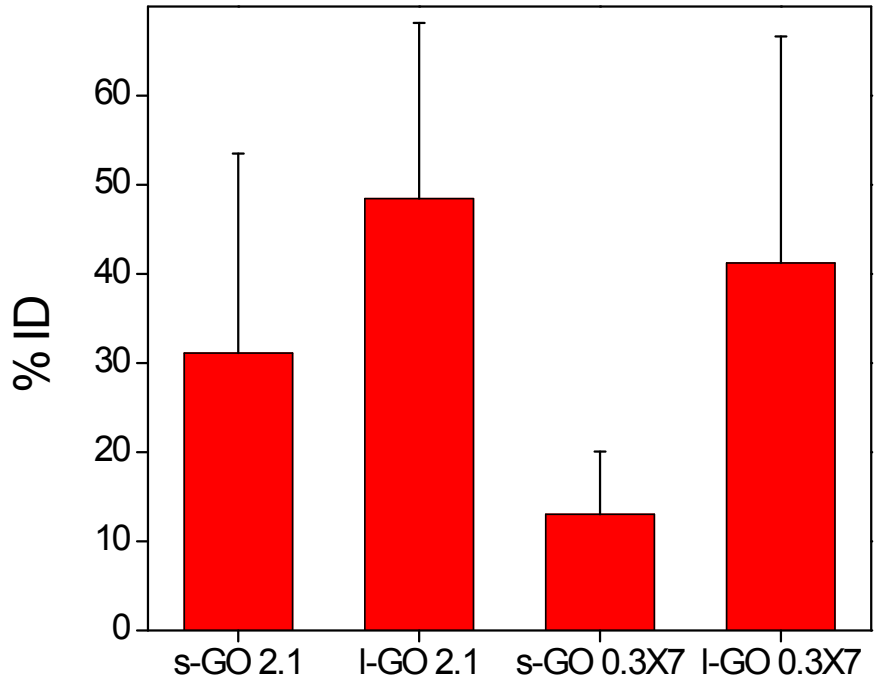

Fig. S3. Accumulation of GO in mouse lung over 14 days. Data are presented as mean±SD (n=4). s-GO 2.1 and l-GO 2.1 mean the single-dose groups that mice exposed to 2.1 mg/kg s-GO and 2.1 mg/kg l-GO, respectively. S-GO 0.3X7 and l-GO 0.3X7 mean the multiple-dose (every other day, totally seven injections) groups that mice exposed to 0.3 mg/kg s-GO and 0.3 mg/kg l-GO, respectively.