**Supplementary Data**

**Cyclooxygenase-2 modulates ER-mitochondria crosstalk to mediate superparamagnetic iron oxide nanoparticles induced hepatotoxicity: *an in vitro and in vivo study***

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**Supplementary Figures**

**Figure S1:**



Figure S1. SPIO-NPs suspension characteristics in cell culture medium (RPMI-1640). (A) Representative TEM image of SPIO-NPs when suspended in RPMI-1640 medium. (B-C) The hydrodynamic size and zeta potential of SPIO-NPs were measured in RPMI-1640 medium with a Zetasizer instrument.

**Figure S2:**

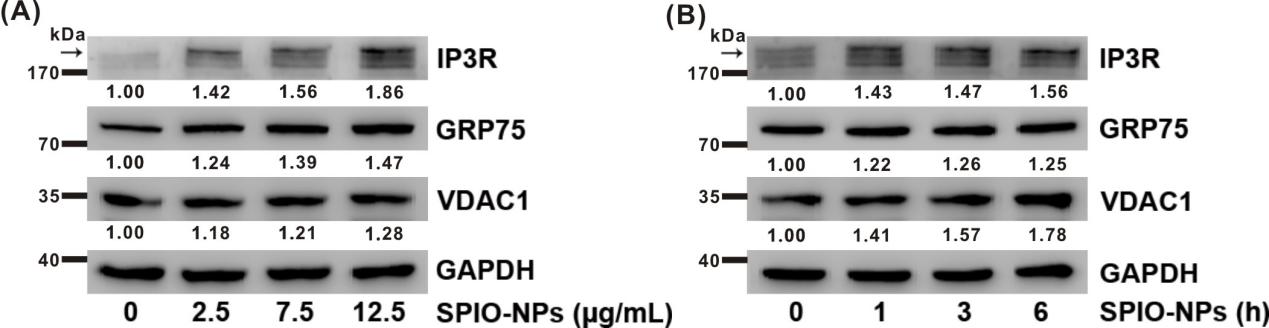
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Figure S2. Effects of SPIO-NPs on MAM-related proteins in L02 cells.(A) L02 cells were treated with different concentrations of SPIO-NPs for 6 h. (B) L02 cells were treated with SPIO-NPs (12.5 μg/mL) for different duration. The levels of MAM-related proteins were detected by Western blot. Arrow indicates the specific band.

**Figure S3:**



Figure S3. Knockdown of GRP75 (si*HSPA9*) on the SPIO-NPs-induced apoptosis in L02 cells. L02 Cells were treated with or without 12.5 μg/mL SPIO-NPs for 6 h after transfected with si*HSPA9* or siNC (50 nmol/L) for 8 h. siNC, siRNA negative control. Apoptosis was determined by Annexin V-FITC staining. The representative fluorescence images (A) and quantification of apoptotic cells (B) with Annexin V positive (green) were shown. Scale bars are 10 μm. Data were expressed as means ± SD. \* *P*<0.05, vs corresponding control; # *P*<0.05, vs cells with siNC transfection and SPIO-NPs treatment group.

**Figure S4:**

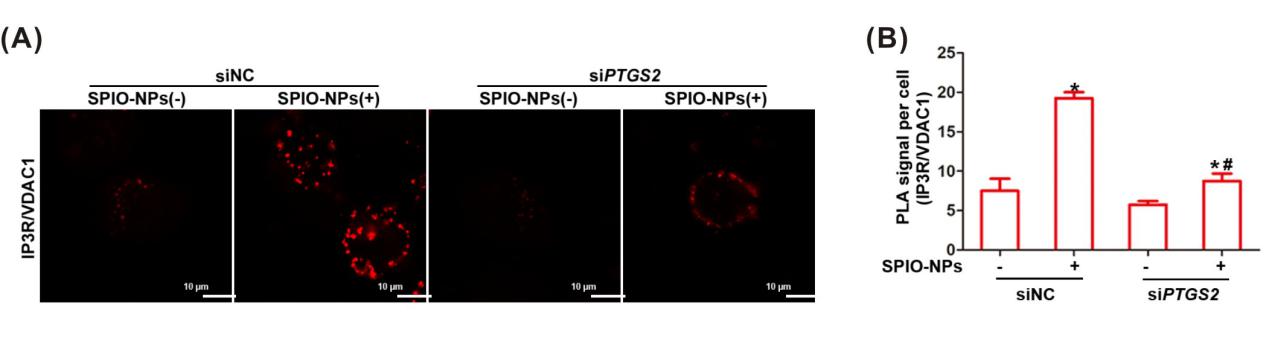


Figure S4. Inhibition of COX-2 (si*PTGS2*) reduced the physical interaction of MAMs in SPIO-NPs-treated L02 cells. L02 cells were treated with or without 12.5 μg/mL SPIO-NPs for 6 h after transfected with si*PTGS2* or siNC (50 nmol/L) for 8 h. siNC, siRNA negative control. (A) PLA showed the interaction between IP3R and VDAC1. Interaction events were shown as red fluorescent PLA foci. (B) Quantification of IP3R/VDAC1 PLA foci was shown in the bar graph. Data were expressed as means ± SD. \* *P*<0.05, vs corresponding control; # *P*<0.05, vs cells with siNC transfection and SPIO-NPs treatment group.

**Figure S5:**



Figure S5. Effect of inhibition or overexpression of COX-2 on the SPIO-NPs-induced apoptosis in L02 cells. L02 cells were treated with or without 12.5 μg/mL SPIO-NPs after COX-2-intervention. Apoptosis was determined by Annexin V-FITC staining, while cells were immunostained with anti-Annexin V antibody (green) and DAPI. (A-B) L02 cells were treated with or without 12.5 μg/mL SPIO-NPs for 6 h after transfected with si*PTGS2* or siNC (50 nmol/L) for 8 h to knockdown COX-2. siNC, siRNA negative control. (C-F) L02 cells were pretreated with or without 100 μM COX-2 inhibitors, aspirin (C-D) and celecoxib (E-F) for 6 h and exposed to SPIO-NPs for another 24 h. (G-H) L02-pB-*PTGS2* cell was constructed for the overexpression of COX-2. L02-pBabe cell was as control cell. Cells were treated with SPIO-NPs (12.5 μg/mL) for 6 h. The representative confocal microscope images (A, C, E, G) and corresponding quantification of apoptotic cells (B, D, F, H) with Annexin V positive (green) were shown. Scale bars are 10 μm. Data were expressed as means ± SD. \* *P*<0.05, vs corresponding control; # *P*<0.05, vs cells with SPIO-NPs treatment group.

**Figure S6:**

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Figure S6. Inhibition of COX-2 (si*PTGS2*) reduced the interactions of COX-2 with IP3R in SPIO-NPs-treated L02 cells. L02 cells were transfected with si*PTGS2* or siNC for 8 h and then treated with 12.5 μg/mL SPIO-NPs for another 6 h. (A) PLA images showed the interactions of COX-2 with IP3R. (B)Quantification of COX-2/IP3R PLA foci was shown in the bar graph. Data were expressed as means ± SD. \* *P*<0.05, vs corresponding control; # *P*<0.05, vs cells with siNC transfection and SPIO-NPs treatment group.