Supporting Information

AMPK mediates the neurotoxicity of iron oxide nanoparticles retained in mitochondria or lysosomes

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Supplementary methods

Preparation of nanoparticles

Synthesis of iron-oleate precursor complex: iron-oleate precursor complex was synthesized using a modified method.[1] Briefly, 10.8 g of iron (III) chloride and 36.5 g of sodium oleate were dissolved in a solvent of 80 mL of ethanol, 60 mL of distilled water and 140 mL of hexane. The resulting solution was heated to 60 °C and kept for four hours. Afterward, the upper organic layer was washed three times with 30 mL of distilled water. After washing, hexane was evaporated off to obtain iron-oleate precursor complex in a waxy solid form.

Synthesis of oleic acid-stabilized magnetic nanoparticles (OA-MNPs): 3.6 g of the iron-oleate precursor complex and 0.57 g of oleic acid were dissolved in 20 g of 1-octadecene at room temperature. The mixture was heated to 320 °C with a constant heating rate of 3.3 °C/minute and kept for 60 min. The resulting solution was then cooled to room temperature. OA-MNPs were washed three times by using hexane with acetone.

Synthesis of magnetic nanoparticles (MNPs):[2] 50 mg of caffeic acid was dissolved in 6 mL of THF in a three-neck flask. 20 mg of OA-MNPs dispersed in 1 mL of THF was added dropwise. The solution was heated to 50 °C under argon protection and kept for three hours. After the reaction was cooled to room temperature, 500 μL of NaOH (0.5 M) was added to the solution to precipitate MNPs. MNPs were collected by centrifugation at 3000 rpm/min for 5 min and re-dispersed in 2 mL of water.

Synthesis of different IONPs: The nontargeting IONP, lysosome-targeted IONP-APM and mitochondrion-targeted IONP-TPP were synthesized as following. The carboxyl groups in the synthesized MNPs reacted with amino groups in mPEG-NH_2, NH_2-PEG-COOH and 8-Arm PEG-NH_2 to prepare IONP, IONP-PEG-COOH and IONP-PEG-NH_2, respectively, by EDC/NHS reaction. Afterward, 3-morpholinopropylamine and (4-carboxybutyl)triphenylphosphonium bromide were conjugated to IONP-PEG-COOH and IONP-PEG-NH_2 to form IONP-APM and IONP-TPP, respectively.

Synthesis of Cy5-labeled nanoparticles: Cy5-NHS was added to the nanoparticles

(mass ratio = 1:1000) and then stirred at room temperature overnight. The resultant of reaction was dialyzed against distilled water for 24 h (molecular weight cut-off = 3500 Da) to remove free Cy5-NHS.

Supplementary figures

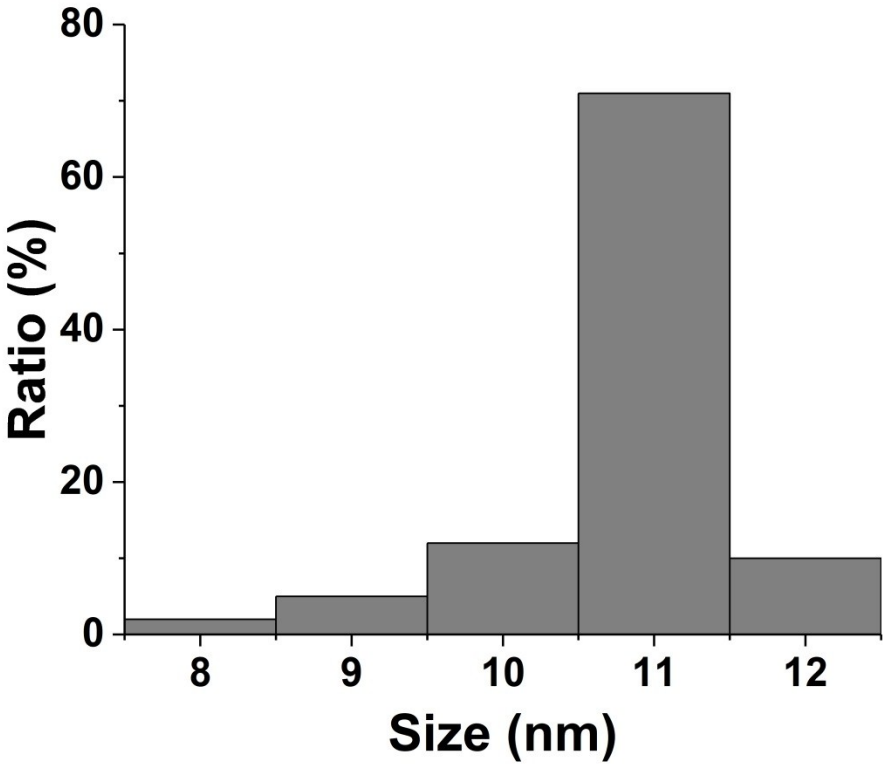

Fig. S1 A size distribution histogram of iron nanoparticles.

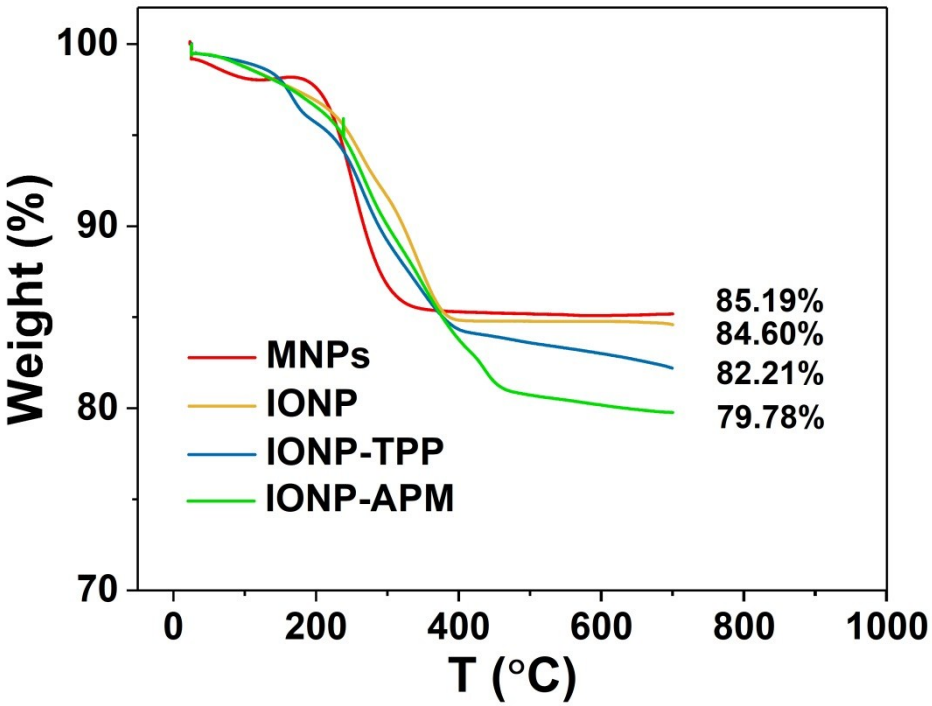

Fig. S2 TGA curves of MNPs, IONP, IONP-TPP and IONP-APM.

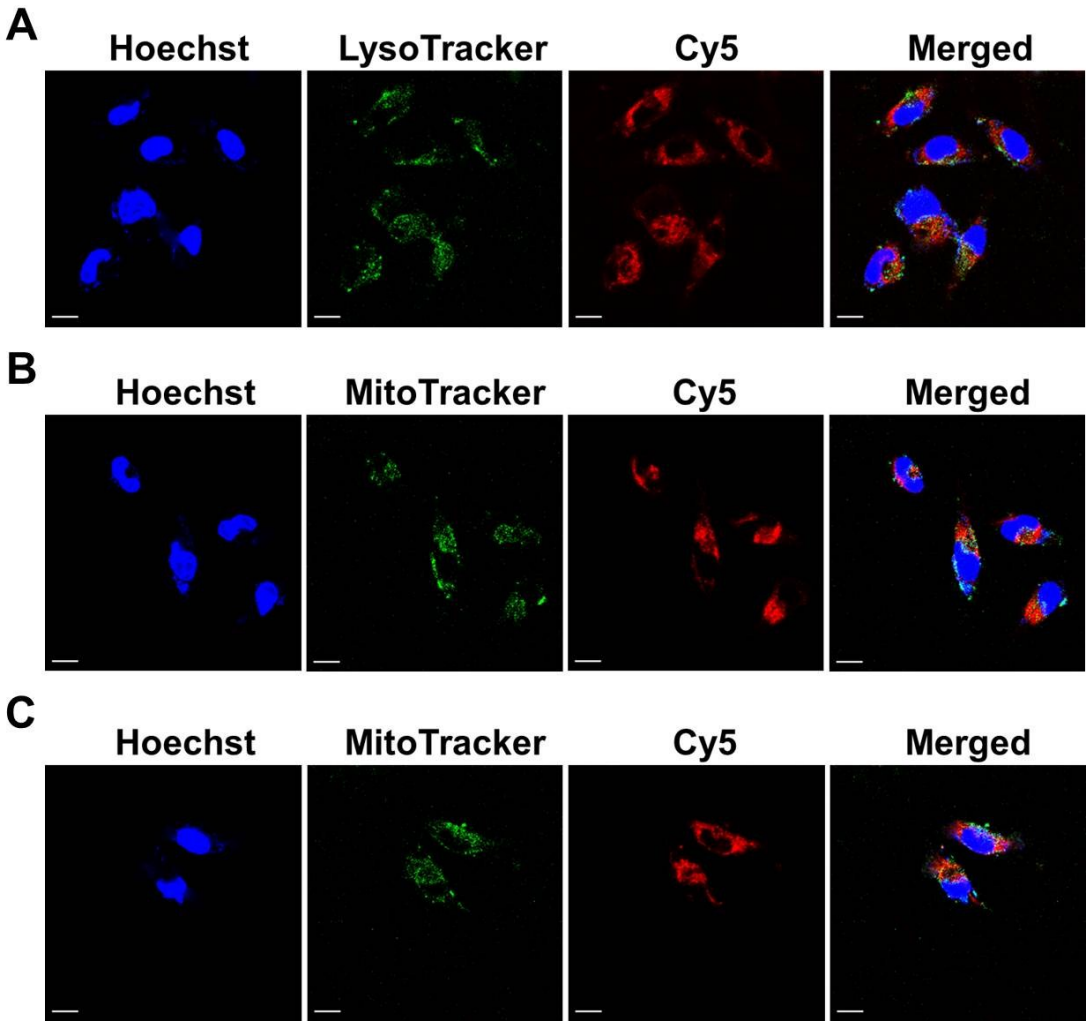

Fig. S3 (A) Images of Cy5-IONP-TPP (red) and lysosomes (green) in SH-SY5Y cells. (B) Images of Cy5-IONP-APM (red) and mitochondria (green) in SH-SY5Y cells. (C) Images of Cy5-IONP (red) and mitochondria (green) in SH-SY5Y cells. Scale bar = 10 μm.

References:

1. J. Park, K. An, Y. Hwang, J. G. Park, H. J. Noh, J. Y. Kim, J. H. Park, N. M. Hwang and T. Hyeon, *Nat. Mater.*, 2004, **3**, 891-895.
2. Y. Liu, T. Chen, C. Wu, L. Qiu, R. Hu, J. Li, S. Cansiz, L. Zhang, C. Cui, G. Zhu, M. You, T. Zhang and W. Tan, *J. Am. Chem. Soc.*, 2014, **136**, 12552-12555.