Toxicological Risk Assessments of Iron Oxide Nanocluster- and Gadolinium-Based T1MRI Contrast Agents in Renal Failure Rats

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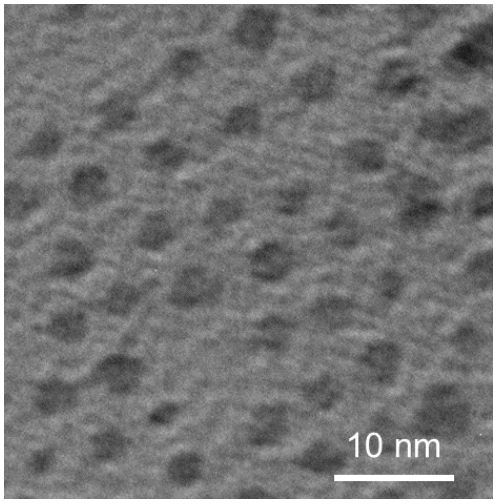

Figure S1. The high-resolution TEM image of IONCs in chloroform.

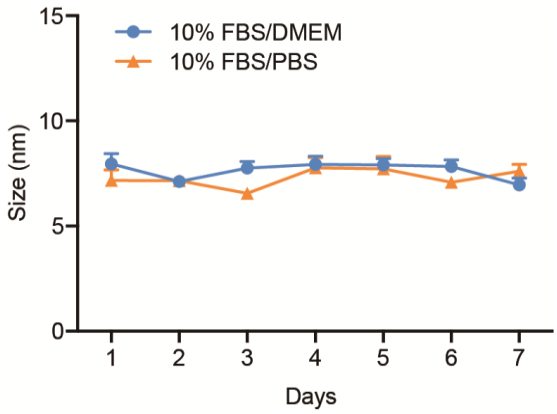

Figure S2. The colloidal stability of DSPE-PEG_{2000} modified IONCs in 10% FBS/DMEM and 10% FBS/PBS for 7 days (n = 3).

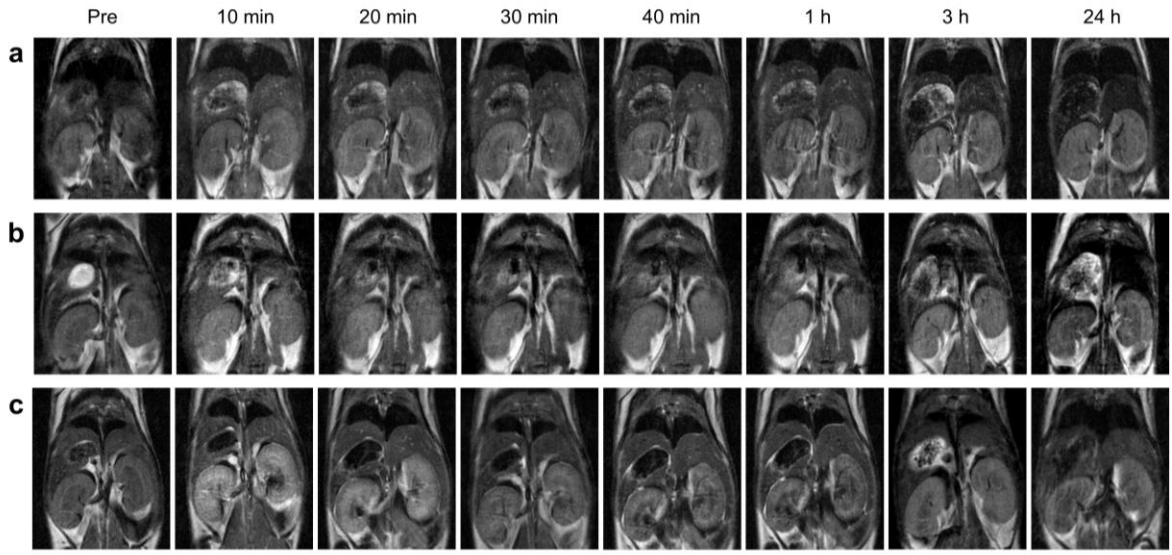

Figure S3. *In vivo* T1-weighted MRI of mice before and after i.v. injection of CAs: (a) IONCs (2.5 mg/kg), (b) IONCs (5 mg/kg), and (c) gadodiamide (0.75 mmol/kg).

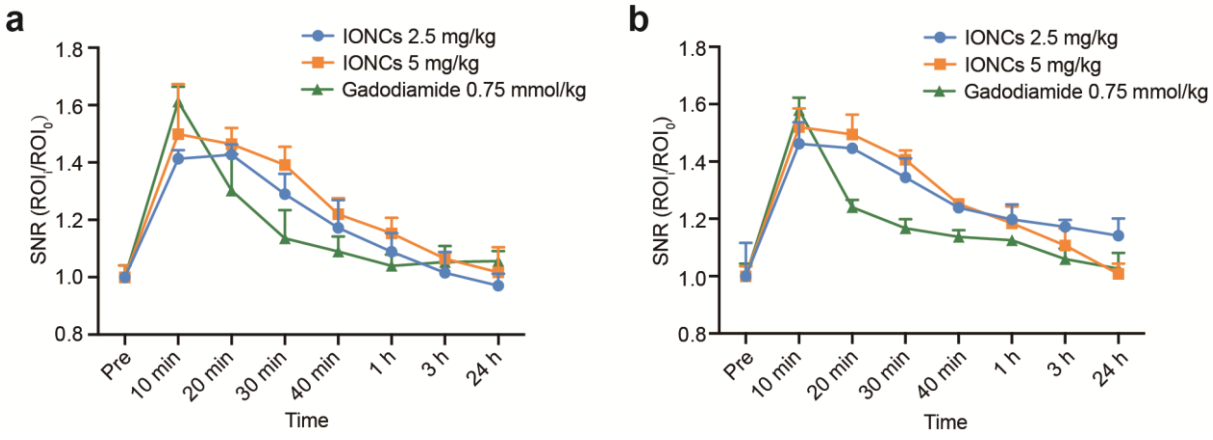

Figure S4. Temporal signal to noise (SNR) changes of the regions of interest (ROI): (a) liver and (b) kidney before and after i.v. injection of CAs.

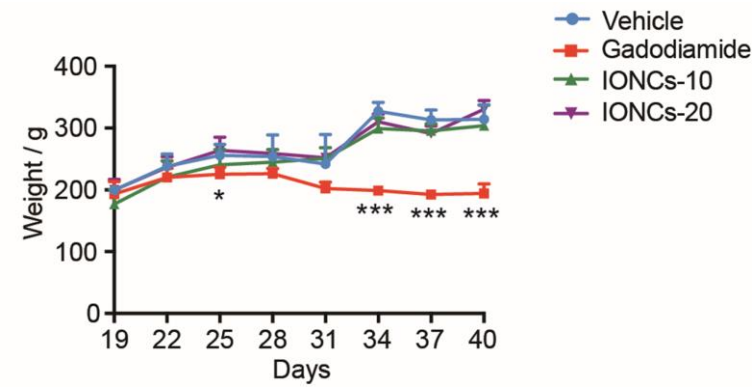

Figure S5. Weight changes of renal failure rats after the i.v. injection of CAs (n = 4/group). Data are presented as mean ± SEM; $^{*}p < 0.05$, $^{***}p < 0.001$ *versus* vehicle-treated renal failure group.

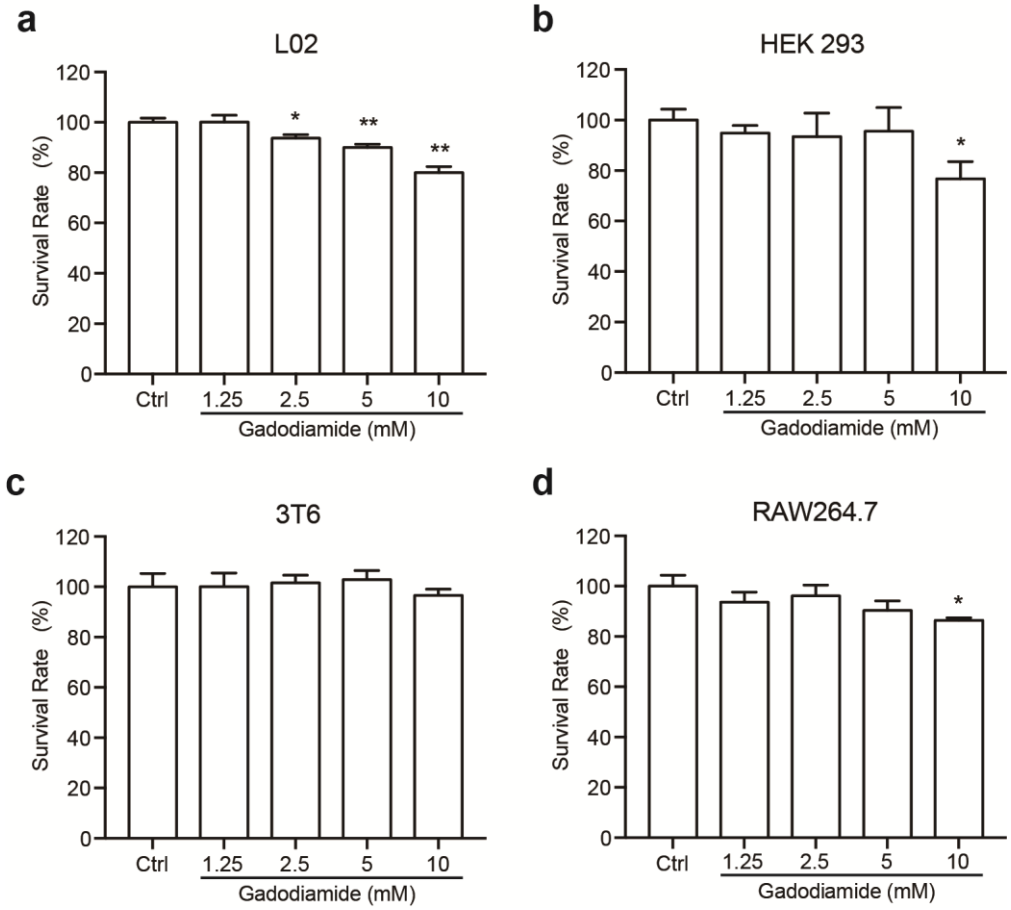

Figure S6. The cytotoxicity of gadodiamide to (a) L02 liver cells, (b) HEK 293 embryonic kidney cells, (c) 3T6 mouse fibroblast cells, and (d) RAW264.7 mouse macrophage cells (n = 3). Data are presented as mean ± SEM; $^{*}p < 0.05$, $^{**}p < 0.01$ *versus* Ctrl.

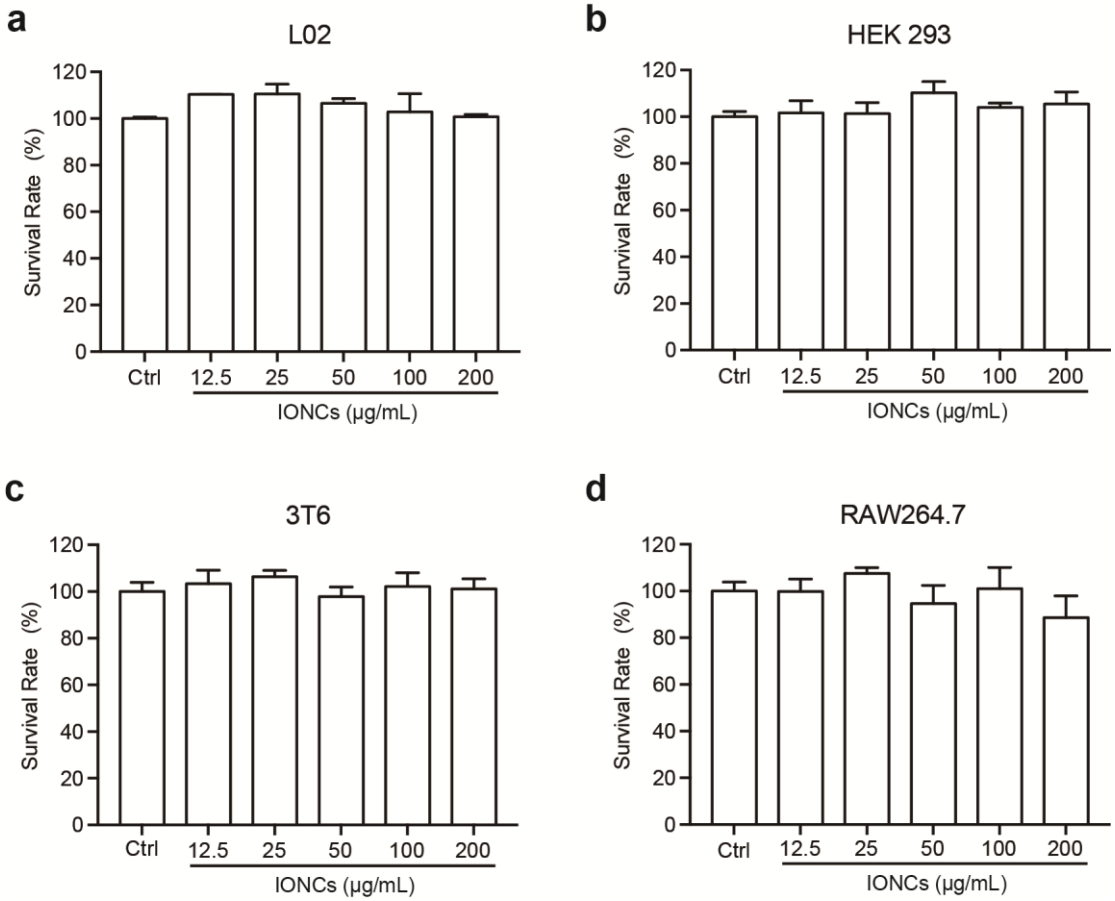

Figure S7. The cytotoxicity of IONCs to (a) L02 liver cells, (b) HEK 293 embryonic kidney cells, (c) 3T6 mouse fibroblast cells, and (d) RAW264.7 mouse macrophage cells (n = 3). Data are presented as mean ± SEM.

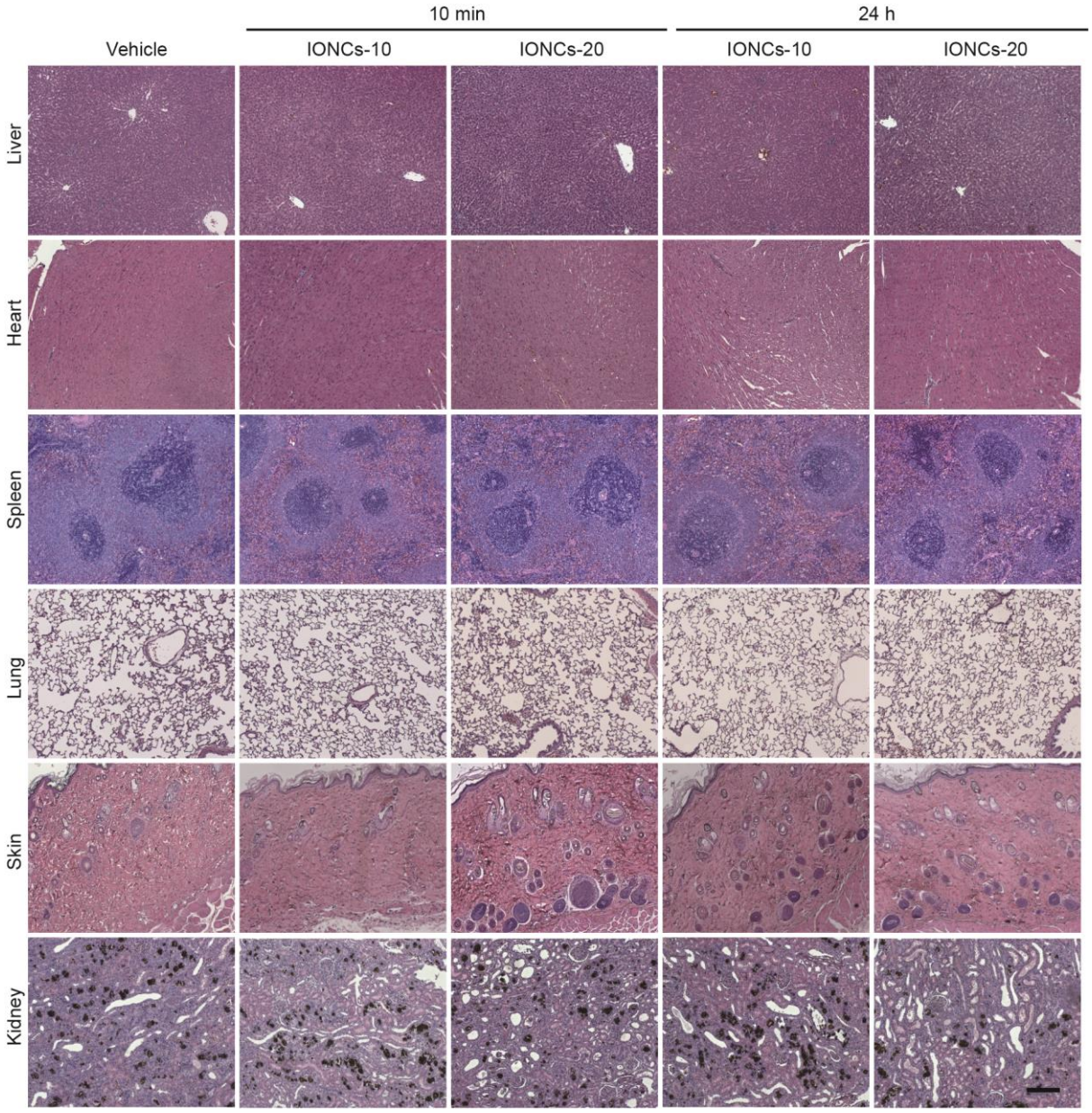

Figure S8. Histopathological changes in renal failure rat tissues after exposure to IONCs for 10 min and 24 h for the evaluation of the acute inflammatory responses (n = 3/group). Scale bar: 200 μm.

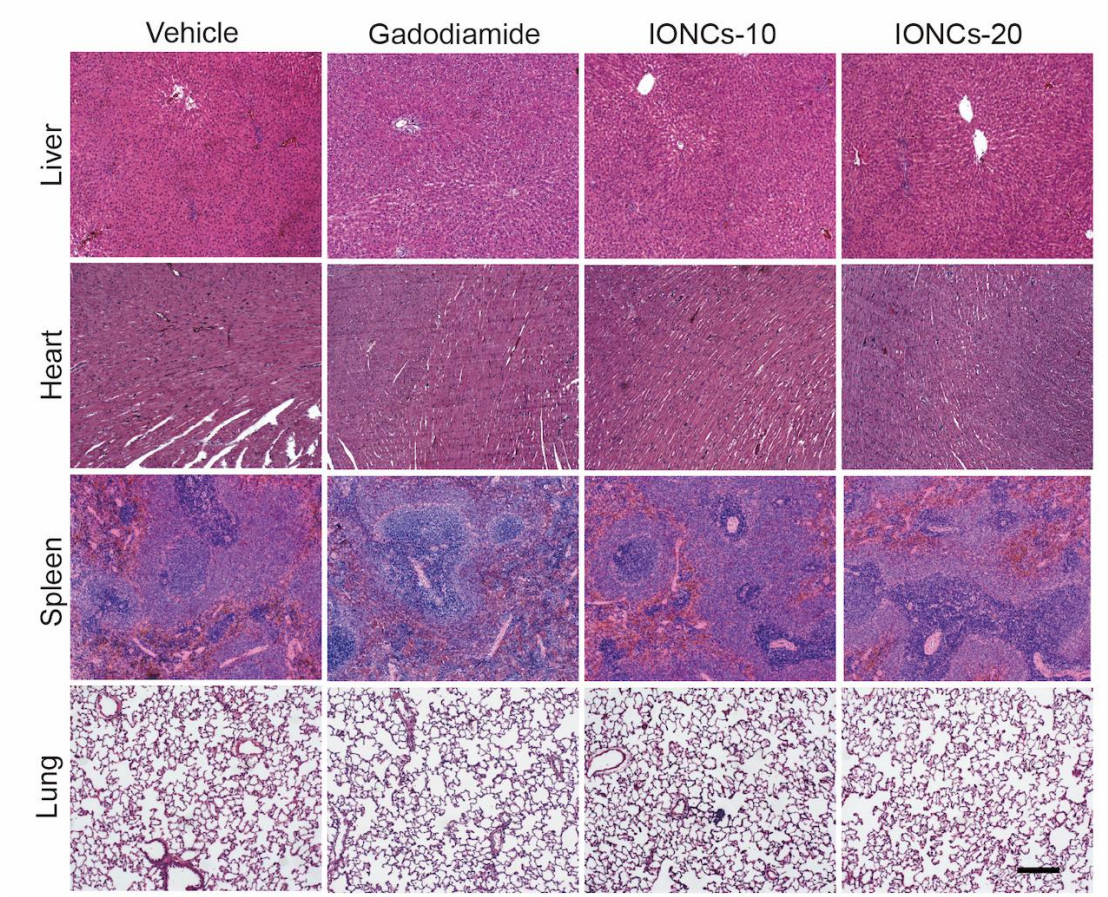

Figure S9. Histopathological changes in renal failure rat tissues after exposure to CAs (n = 4/group). Scale bar: 200 μm.

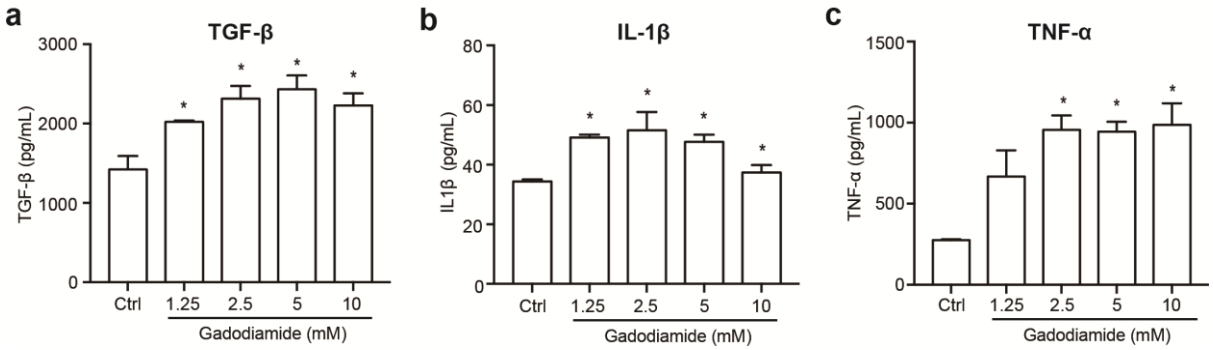

Figure S10. The secretion of (a) TGF-β, (b) IL-1β, and (c) TNF-α in RAW264.7 macrophage cells after treatment with control (Ctrl) or gadodiamide (1.25, 2.5, 5, 10 mM) for 24 h (n = 3). Data are presented as mean ± SEM; $^{*}p < 0.05$ *versus* Ctrl.

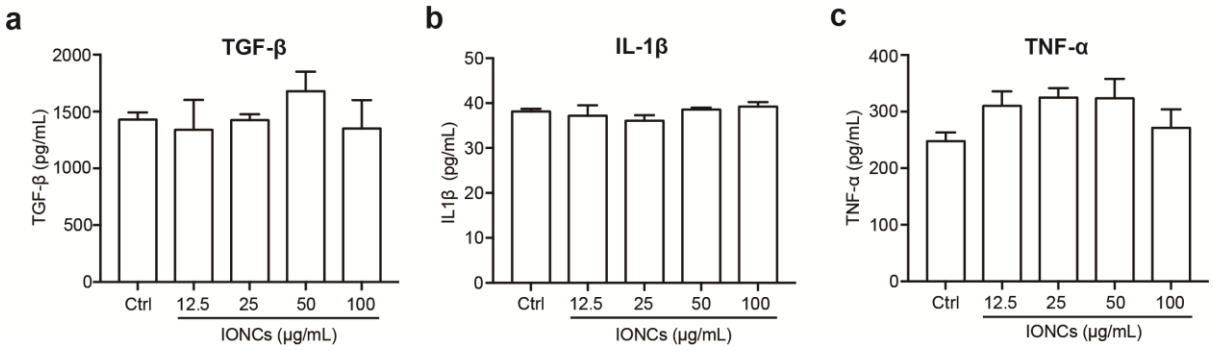

Figure S11. The secretion of (a) TGF-*β*, (b) IL-1*β*, and (c) TNF-*α* in RAW264.7 macrophage cells after treatment with Ctrl or IONCs (12.5, 25, 50, 100 μg/mL) for 24 h (n = 3).

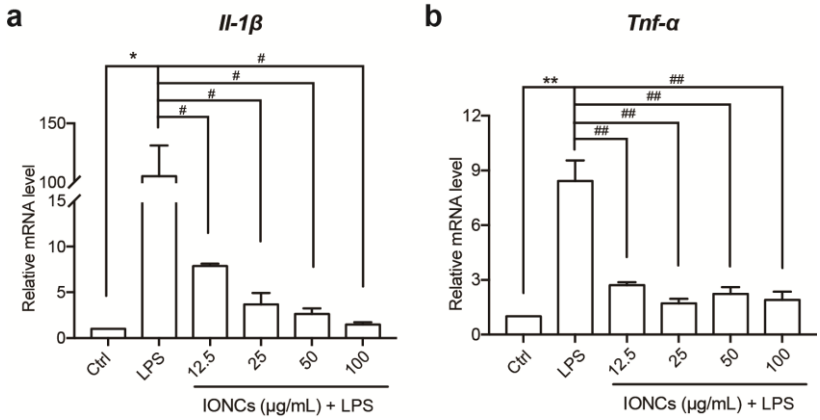

Figure S12. The mRNA levels of (a) *Il-1β* and (b) *Tnf-α* in RAW264.7 macrophage cells after treatment with LPS (50 ng/mL) and IONCs (12.5, 25, 50, 100 μg/mL) for 24 h (n = 3). Data are presented as mean ± SEM; $^{*}p < 0.05$, $^{**}p < 0.01$ *versus* Ctrl, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *versus* LPS-treated group.

Table S1. The primer sequences used in qRT-PCR analysis.

Gene	Species	Forward (5'-3')	Reverse (5'-3')	Application
Ccr2	Rat	AGCATACTTGTGGCCCTTATT	CTGAGTAGCAGATGACCATGAC	qRT-PCR
Ccr7	Rat	GACTGAAGACCATGACGGATAC	CACAGGTAGGCACCAAAGAT	qRT-PCR
Nox4	Rat	TTCTGGACCTTTGTGCCTATAC	CCATGACATCTGAGGGATGATT	qRT-PCR
Ccl2	Rat	GTCTCAGCCAGATGCAGTTAAT	CTGCTGGTGATTCTCTTGTAGTT	qRT-PCR
Il-1β	Rat	TGCAGGCTTCGAGATGAAC	GGGATTTTGTCGTTGCTTGTC	qRT-PCR
Tnf-α	Rat	CTTCTCATTCCTGCTCGTGG	TGATCTGAGTGTGAGGGTCTG	qRT-PCR
Tgf-β	Rat	CTCCCGTGGCTTCTAGTGC	GCCTTAGTTTGGACAGGATCTG	qRT-PCR
Gapdh	Rat	ACTCCCATTCTTCCACCTTTG	CCCTGTTGCTGTAGCCATATT	qRT-PCR
Tgf-β	Mouse	GGTGGTATACTGAGACACCTTG	CCCAAGGAAAGGTAGGTGATAG	qRT-PCR
Il-1β	Mouse	CTCCACCTCAATGGACAGAATATC	GGGTGTGCCGTCTTTCATTA	qRT-PCR
Tnf-α	Mouse	CGATGGGTTGTACCTTGTCTAC	GAGGTTGACTTTCTCCTGGTATG	qRT-PCR
Gapdh	Mouse	GGAGAAACCTGCCAAGTATGA	GAAGAGTGGGAGTTGCTGTT	qRT-PCR