

Supporting Information

IL-6-targeted Ultrasmall Superparamagnetic Iron Oxide Nanoparticles for Optimized MRI Detection of Atherosclerotic Vulnerable Plaques in Rabbits

Huaqiang Mo, ‡^{a, b} Chenxing Fu, ‡^{a, b} Zhiye Wu,^{a, b} Peng Liu,^{a, b} Zhibo Wen,^c Qingqing Hong,^{a, b} Yanbin Cai,^{*a, b} and Gongxin Li^{*a, b}

^a Department of Cardiology, Zhujiang Hospital, Southern Medical University, Guangzhou 510280, People's Republic of China; E-mail: ligxin@163.com.

^b Laboratory of Heart Center, Zhujiang Hospital, Sino-Japanese Cooperation Platform for Translational Research in Heart Failure, Guangdong Provincial Biomedical Engineering Technology Research Center for Cardiovascular Diseases, Guangzhou 510280, People's Republic of China; E-mail: skyer1@smu.edu.cn.

^c Department of Radiology, Zhujiang Hospital, Southern Medical University, Guangzhou 510280, People's Republic of China.

‡ These authors equally contributed to this work.

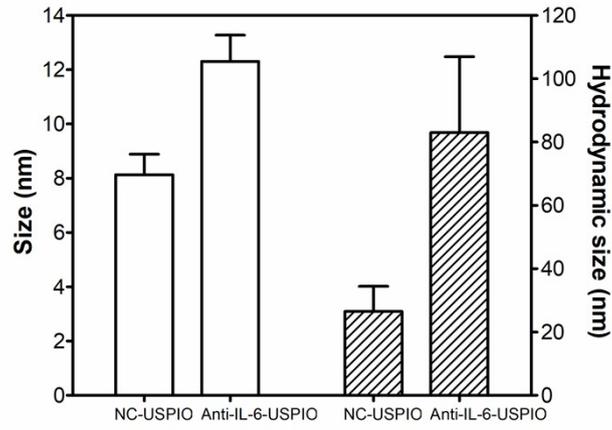


Fig. S1 TEM size (left), hydrodynamic size (right) of NC-USPIO and anti-IL-6-USPIO.

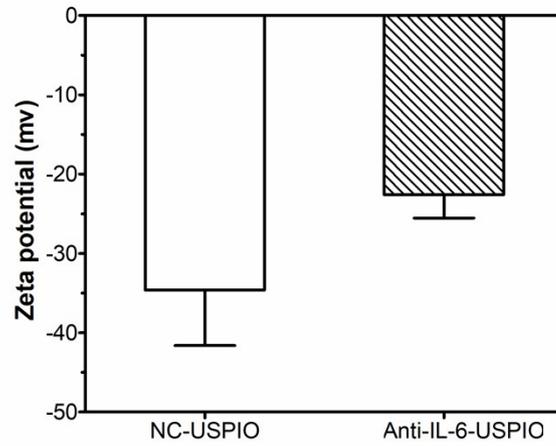


Fig. S2 Zeta potential of NC-USPIO and anti-IL-6-USPIO.

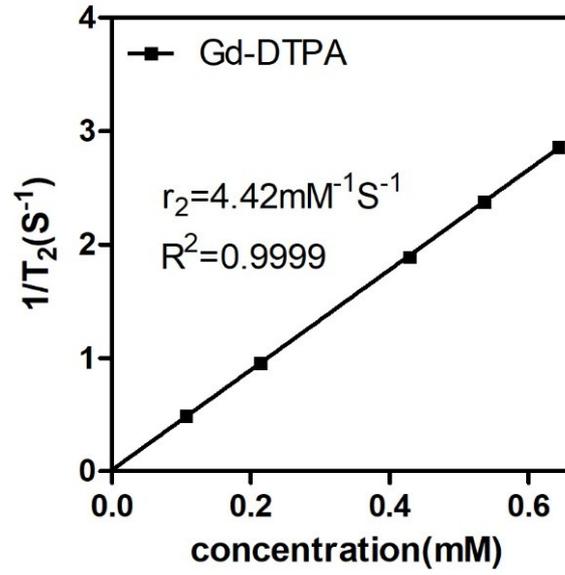


Fig.S3 The correlation between different concentrations and $1/T_2$ in Gd-DTPA.

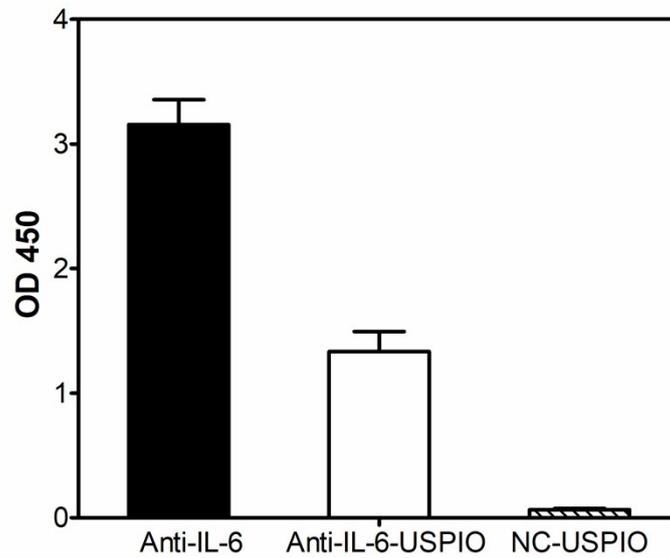


Fig.S4 ELISA results show the activity of anti-IL-6-USPIO on OD450 value.

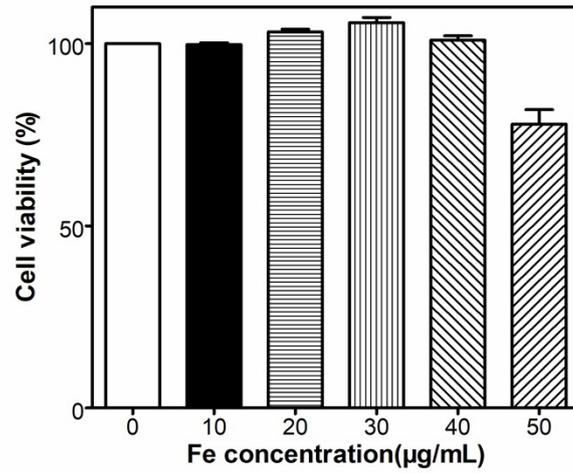


Fig. S5 In vitro cytotoxicity test of anti-IL-6-USPIO against HUVECs. CCK-8 assay results for evaluating the viability of the cells after incubation with different concentrations of Fe for 48 h.

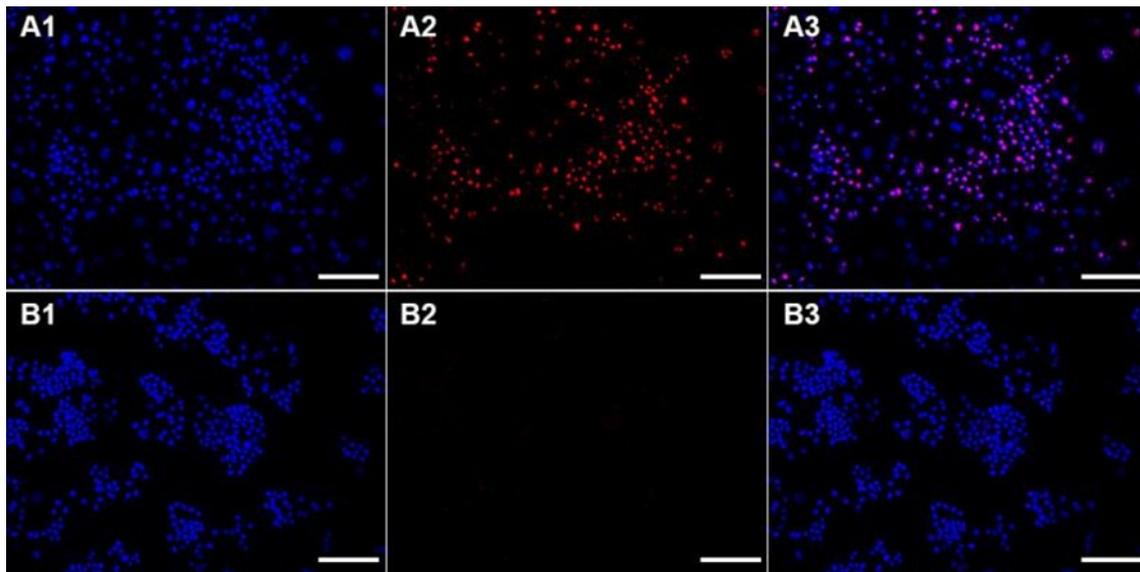


Fig. S6 Confocal microscopy images of macrophages after 24 h incubation with LPS (A) and without LPS (B).

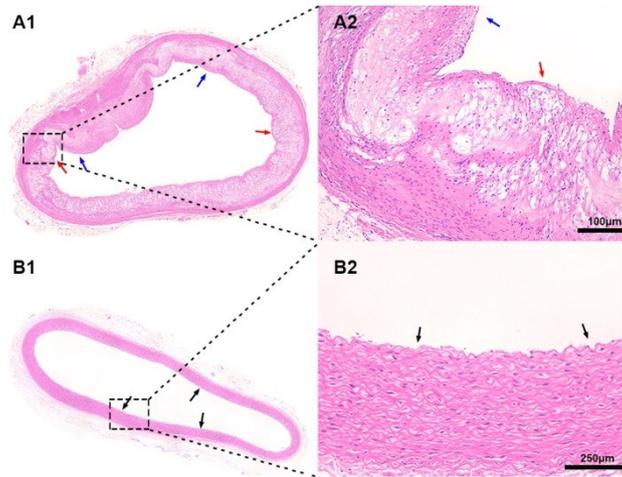


Fig. S7 HE staining in control (B2) and experimental (A2) group with pathological section of signal variation (Original magnification: $\times 200$ or $\times 100$), the two groups are respectively showed in Figure B1 and A1 (Original magnification: $\times 50$). Red and blue arrows in A show vulnerable plaque and stable plaque respectively.

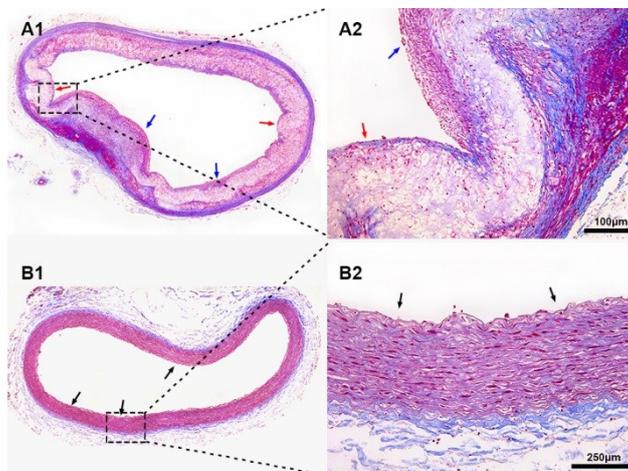


Fig. S8 Masson staining in control (B2) and experimental (A2) group with pathological section of signal variation (Original magnification: $\times 200$ or $\times 100$), the two groups are respectively showed in Figure B1 and A1 (Original magnification: $\times 50$). Red and blue arrows in A show vulnerable plaque and stable plaque respectively.

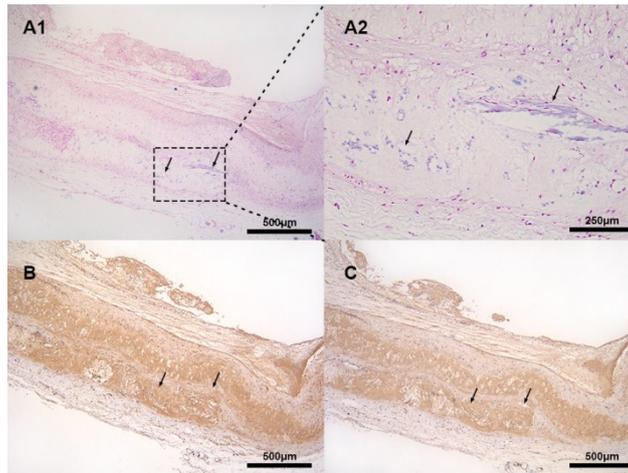


Fig. S9 Prussian blue staining (A1×50, A2×200), Immunohistochemistry staining of IL-6 (B×50) and CD68 (C×50) in experimental group. black arrows in A show the iron deposition in atherosclerotic plaques.

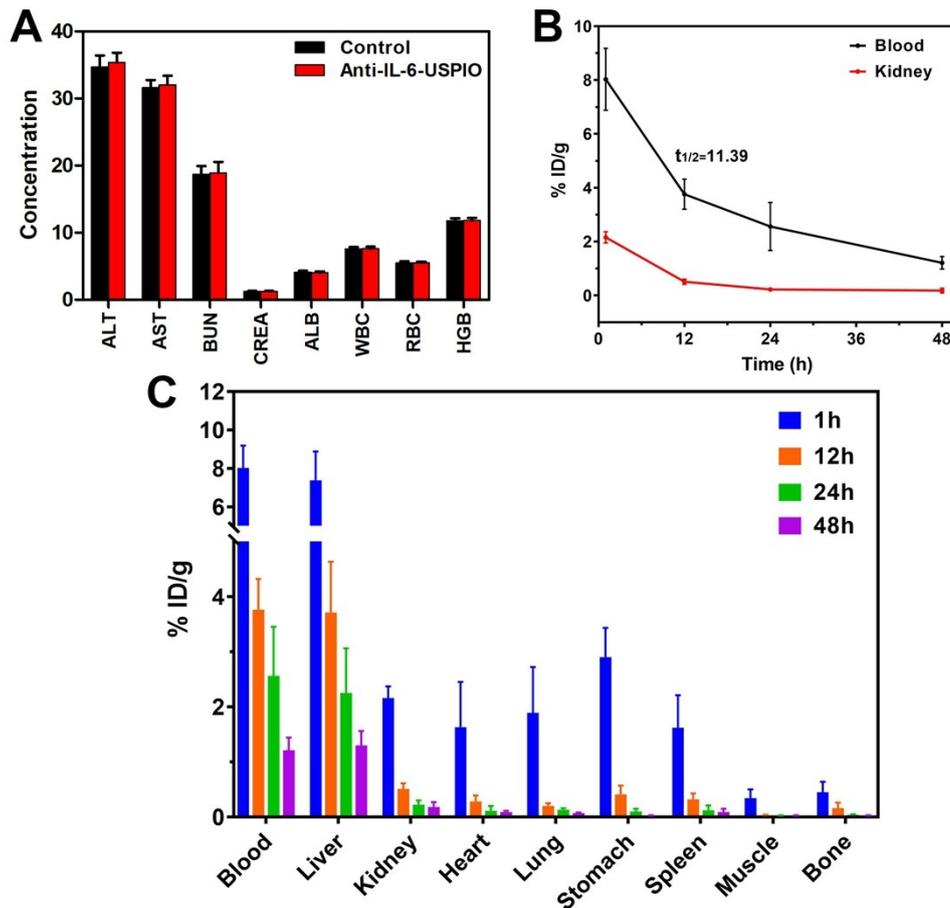


Fig. S10 A) Blood analysis of Anti-IL-6-USPIO with different blood index. B) The time-activity curve of ¹²⁵I-Anti-IL-6-USPIO in kidney and blood, respectively. C) The biodistribution of ¹²⁵I-Anti-IL-6-USPIO measured at 1, 12, 24, and 48 h post injection.

Tab. S1 Comparison of SNR in blood vessel walls before and after injection of NC-USPIO. (Mean \pm SD, * P <0.01 vs. Plain scan)

Group	Plain scan	Time after NC-USPIO enhancement scan (h)	
		24	48
Experiment (n=45)	40.59 \pm 1.25	28.09 \pm 1.58*	23.98 \pm 2.53*
Control(n=15)	39.70 \pm 1.60	39.53 \pm 1.92	39.26 \pm 1.89

Tab. S2 Comparison of SNR in blood vessel walls before and after injection of anti-IL-6-USPIO. (Mean \pm SD, * P <0.01 vs. Plain scan)

Group	Plain scan	Time after anti-IL-6-USPIO enhancement scan (h)	
		24	48
Experiment (n=45)	40.59 \pm 1.25	21.94 \pm 2.47*	16.88 \pm 2.47*
Control(n=15)	39.70 \pm 1.60	39.23 \pm 2.19	39.05 \pm 2.25

Tab. S3 Comparison of plaque detection rate in three contrast agents. (* P =0.007 vs. Gd-DTPA, χ^2 =7.252; # P =0.039 vs. Gd-DTPA, χ^2 =4.270; ^ P =0.714 (Fisher probabilities) vs. NC-USPIO)

Contrast agent	Positive	Negative
Anti-IL-6-USPIO	42 (93.3) *^	3 (6.7)
NC-USPIO	40 (88.9) #	5 (11.1)
Gd-DTPA	31 (68.9)	14 (31.1)

Tab. S4 Comparison of vulnerable plaque detection rate in three contrast agents. (* P =0.014 vs. Gd-DTPA, χ^2 =6.067; # P =0.200 vs. Gd-DTPA, χ^2 =1.641; ^ P =0.350 (Fisher probabilities) vs. NC-USPIO)

Contrast agent	Positive	Negative
Anti-IL-6-USPIO	25 (96.2) *^	1 (3.8)
NC-USPIO	22 (84.6) #	4 (15.4)
Gd-DTPA	17 (65.4)	9 (34.6)