**Supplemental Methods**

*Synthesis of IOPC-$NH_2$ fluorescent derivatives*

IOPC particles were synthesized as described.[1, 2] IOPC-$NH_2$ series particles were synthesized according to Figure 1, *A*. Briefly, 0.4 ml of IOPC particles (4.12 mg Fe/ml, carboxyl group concentration 35 mM) was resuspended in 10.0 ml of 2-(N-morpholino)ethanesulfonic acid (MES, 50 mM, pH 5.0) buffer. 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDAC) and sulfo-N-hydroxysuccinimide (Sulfo-NHS) were added to the particle suspension. The final concentrations of EDAC and NHS are 75 mM and 150 mM, respectively. The reaction mixture was stirred at room temperature for 30 min. The resulting NHS ester was purified by passing the above mixture through a centrifugal filter (molecular weight cut 30 kD, Millipore, Billerica, MA). 10 μL of ethylenediamine (10 folds excess of calculated carboxyl groups on the particles) was dissolved in 10 ml PBS and pH was adjusted to 8.0. The purified NHS ester was added to the ethylenediamine solution. The reaction mixture was allowed to stir for 4 hours at room temperature. The resulted IOPC-$NH_2$ particles were purified by using a 30 kD centrifugal filter (Millipore Corporation, Billerica, MA). Fluorescein isothiocyanate (FITC) and DyLight 649-NHS ester (Thermo Fisher Scientific Inc., Rockford, IL) were individually conjugated to IOPC-$NH_2$ particles, taking advantage of the amine groups (Figure 1, *A*). Extensive size filtration by centrifugation was used to remove excess dye molecules.

*Measurement of particle size*

The iron core size of particles was determined by TEM. The diameter of particles was measured by dynamic light scattering using a Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK).

*Measurement of zeta potential*

The zeta potential of the particles was measured in water at pH 7.2 using a Malvern Zetasizer Nano ZS instrument (Malvern Instruments).

*Magnetic resonance (MR) relaxometry*

The longitudinal relaxation time $T_1$ and transverse relaxation time $T_2$ were measured using a Bruker Minispec mq20 NMR Analyzer (20 MHz). Iron concentrations were as follows: 10-100 μg/ml USPIO, 10-100 μg/ml Feidex, 20-180 μg/ml MPIO, 5-20 μg/ml IOPC, 2.5-20 μg/ml IOPC-$NH_2$, 2.5-20 μg/ml IOPC-$NH_2$-FITC, and 2.5-20 μg/ml IOPC-$NH_2$-DyLight 649. The MR probe temperature was set at 25 °C. $T_1$ measurements were conducted by collecting 13 data points with inversion time from 2 ms. $T_2$ were measured with $\tau$ of 0.12 ms. Both $T_1$ and $T_2$ were fitted with monoexponential decay models. All $T_1$ and $T_2$ values were measured in triplicate and the averages were applied for future data fitting. The longitudinal ($r_1$) and transverse ($r_2$) relaxivities of the iron-oxide particles were obtained as the slopes of the fitting curves of the longitudinal relaxation rate $R_1$ ($1/T_1$) and transverse relaxation rate $R_2$ ($1/T_2$) against iron concentration, respectively.

*TEM*

TEM images of IOPC-$NH_2$ series particles and labeled-T-cells were acquired as described previously.[1, 3]

*Semi-quantitative RT-PCR assay for mRNA expression in IOPC-$NH_2$ labeled Jurkat T-cells*

Jurkat Clone E6-1 cells (ATCC) were maintained in RPMI1640 (GibcoBRL, Grand Island, NY) medium containing 10% heat-inactivated foetal bovine serum (FCS), 2 mM glutamine, 100 g/mL streptomycin, and 100 U/mL penicillin. Jurkat T-cells were suspended at a concentration 0.5 x $10^6$ cells/mL in culture medium and grown for 24 hours without, or in the presence of IOPC-$NH_2$ (50 μg Fe/mL) or LPS (100 ng/mL). RT-PCR was performed to estimate expression of mRNAs for cytokines and chemokines as described previously.[4] Total cellular RNA was extracted from pooled T-cells by using RNeasy Mini Kits (Qiagen, Hilden, Germany) and converted to cDNA with SH-reverse transcriptase (Stratagene, La Jolla, CA, USA). The nucleotide sequences of sense and anti-sense primers for each assessed cytokine were described previously.[4] PCR amplification (GeneAmp PCR System 2400; PerkinElmer, Branchburg, NJ) was performed on a 1-μL cDNA sample. The PCR products were electrophoresed on 2% agarose gel and stained with ethidium bromide.

*ELISA for cytokine levels of Jurkat T-cells*

ELISA was performed on supernatants from challenged Jurkat T-cells to quantify TNF-α and IL-6 (Human IL-6 Duoset, RD and Human TNF DuoSet, RD system, USA) according to

the manufacturer's instructions. Briefly, Jurkat T-cells, with or without IOPC-$NH_2$ labeling, were stimulated with LPS (1 μg/mL) for 12 hours. The supernatants were collected by centrifugation and stored at -80°C until use.

## Supplemental Figures

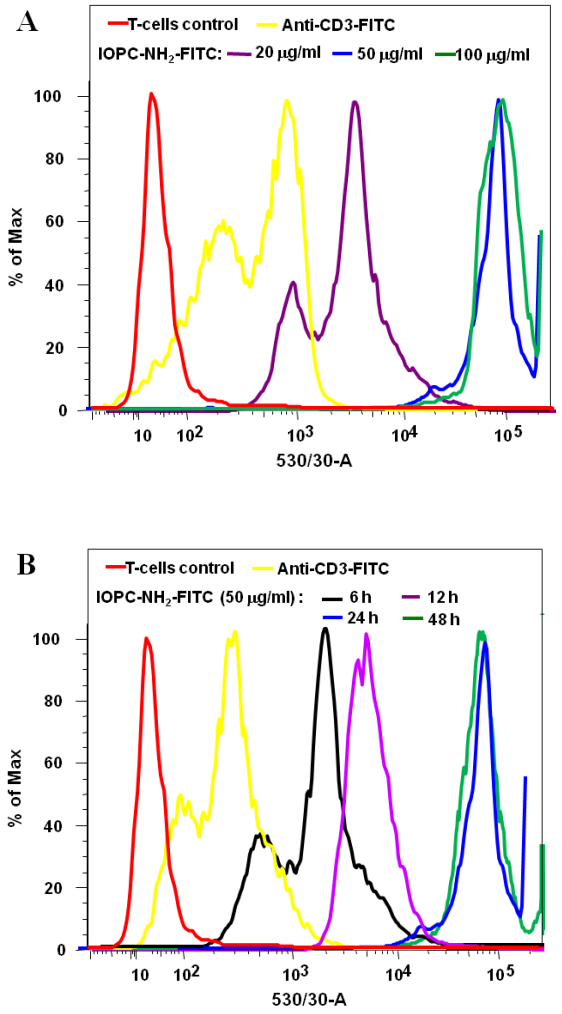


Figure S1. Optimization of T-cell labeling conditions: (**A**) flow cytometry analysis of T-cells labeled with IOPC-$NH_2$–FITC particles (20 µg Fe/mL, 50 µg Fe/mL, 100 µg Fe/mL) for 24 hours. IOPC-$NH_2$–FITC labeled cells in a dose dependent manner. Internalization reached a plateau at 50 µg Fe/mL; and (**B**) flow cytometry analysis of T-cells labeled with IOPC-$NH_2$–FITC particles (50 µg Fe/mL) for 6, 12, 24, and 48 hours. T-cells are labeled in a time dependent manner. Internalization reached a plateau at 24 hours.

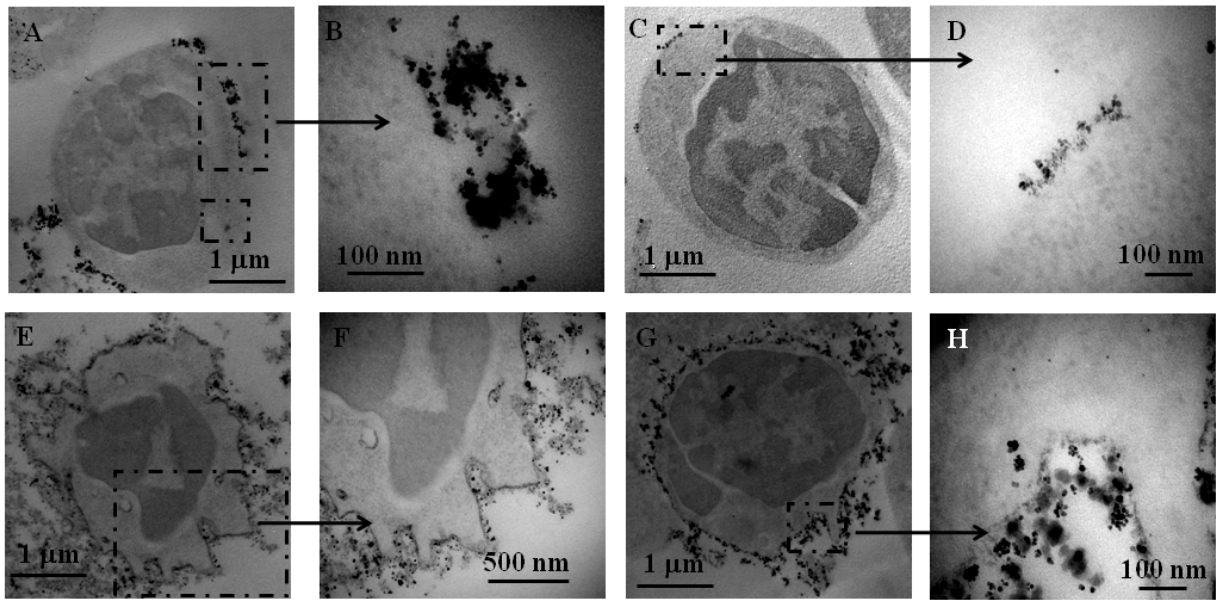


Figure S2. Representative TEM images of rat T-cells labeled with, (**A-B**) IOPC-$NH_2$ –protamine sulfate complexes (50:3 µg/mL), (**C-D**) IOPC-$NH_2$ –protamine sulfate complexes (50:5 µg/mL), (**E-F**) IOPC-$NH_2$ by electroporation (pulse strength = 100 V; *N* pulses = 5; pulse duration = 5 ms; and pulse interval = 120 ms), and (**G-H**) IOPC-$NH_2$ by electroporation (pulse strength = 250 V; *N* pulses = 5; pulse duration = 5 ms; and pulse interval = 120 ms). (*B, D, F* and *H*) are enlarged views of particles found in (*A, C, E* and *G*).

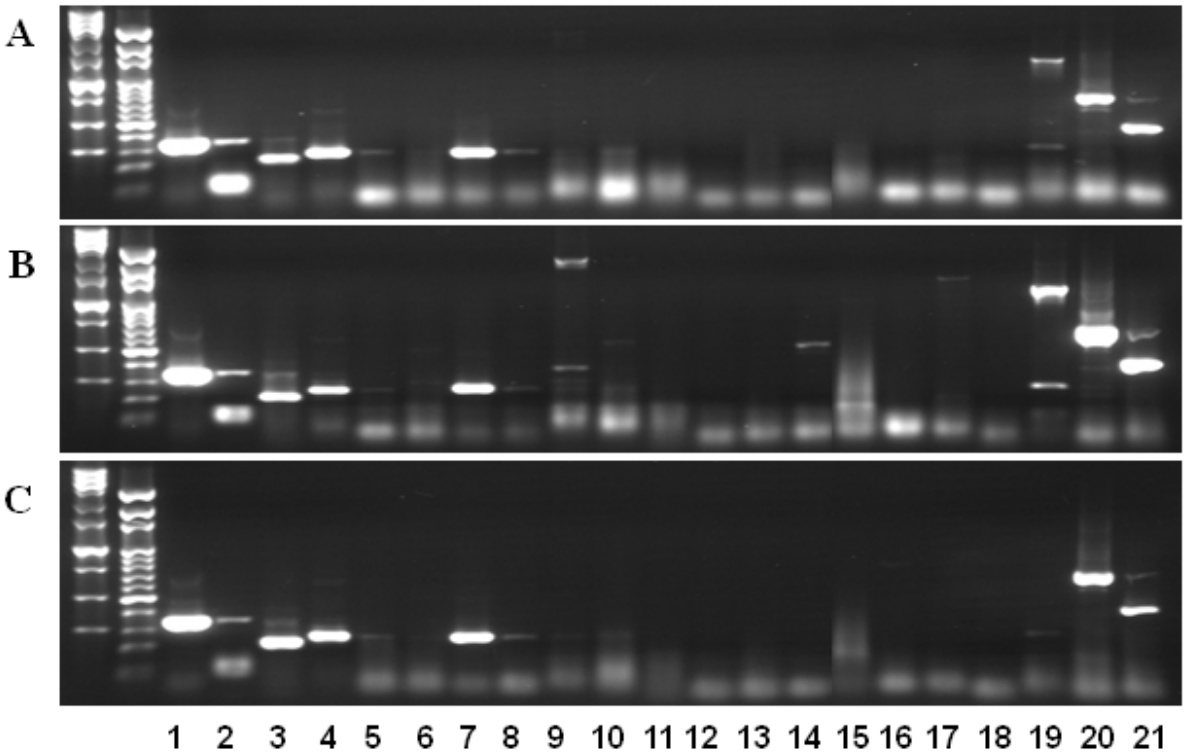


Figure S3. Gene expression profile analyzed by semi-quantitative RT-PCR: (**A**) Jurkat T-cells cultured in medium; (**B**) Jurkat cells after LPS stimulation; (**C**) Jurkat cells after IOPC-$NH_2$ labeling. Lane 1, β-actin; lane 2, non-specific GAPDH; lane 3, GAPDH; lane 4, AF113795; lane 5, AF185284; lane 6, Eotaxin; lane 7, GATA-3; lane 8, iNOS; lane 9, IL-1; lane 10, IL-6; lane 11, IL-9; lane 12, IL-12; lane 13, IL-13; lane 14, IP-10; lane 15, MIP-2; lane 16, MCP-2; lane 17, MCP-3; lane 18, CCL5; lane 19, TGF-β1; lane 20, TNF-α; lane 21, T-bet.

**References for Supplemental Material**


1. Chen CL, Zhang H, Ye Q, Hsieh WY, Hitchens TK, Shen HH, Liu L, Wu YJ, Foley LM, Wang SJ, Ho C. A new nano-sized iron oxide particle with high sensitivity for cellular magnetic resonance imaging. *Mol Imaging Biol*. 2010;13:825-839
2. Chang w.H., hsieh w.Y., huang h.H., ling c.I., wang s.J. Biocompatible polymer and magnetic nanoparticles with biocompatibility. Patent application number: P54960092wo, data of filling: April 23, 2008.
3. Ye Q, Wu YL, Foley LM, Hitchens TK, Eytan DF, Shirwan H, Ho C. Longitudinal tracking of recipient macrophages in a rat chronic cardiac allograft rejection model with noninvasive magnetic resonance imaging using micrometer-sized paramagnetic iron oxide particles. *Circulation*. 2008;118:149-156
4. Chen CL, Lee CT, Liu YC, Wang JY, Lei HY, Yu CK. House dust mite dermatophagoides farinae augments proinflammatory mediator productions and accessory function of alveolar macrophages: Implications for allergic sensitization and inflammation. *J Immunol*. 2003;170:528-536