Electronic Supplementary Material (ESI) for RSC Advances.

Fusogenic Liposome-Enhanced Cytosolic Delivery of Magnetic Nanoparticles

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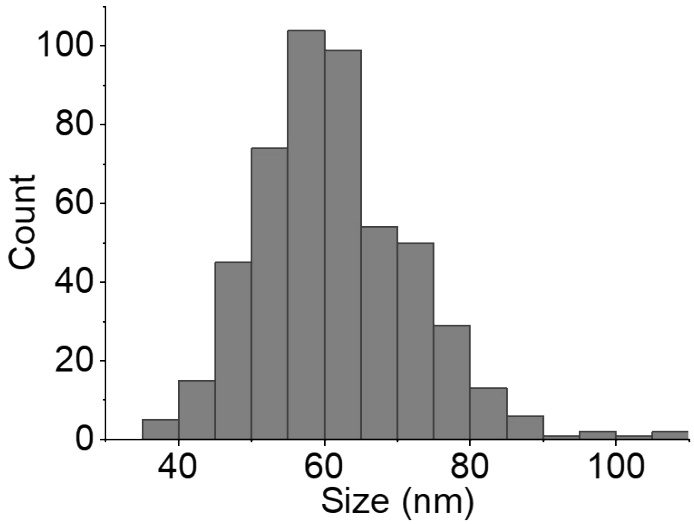

Figure S1. Size distribution (diameter) of the magnetic nanoparticles based on TEM measurements. The average diameter of the magnetic nanoparticles is 61 nm, and standard deviation is 11 nm (n=500).

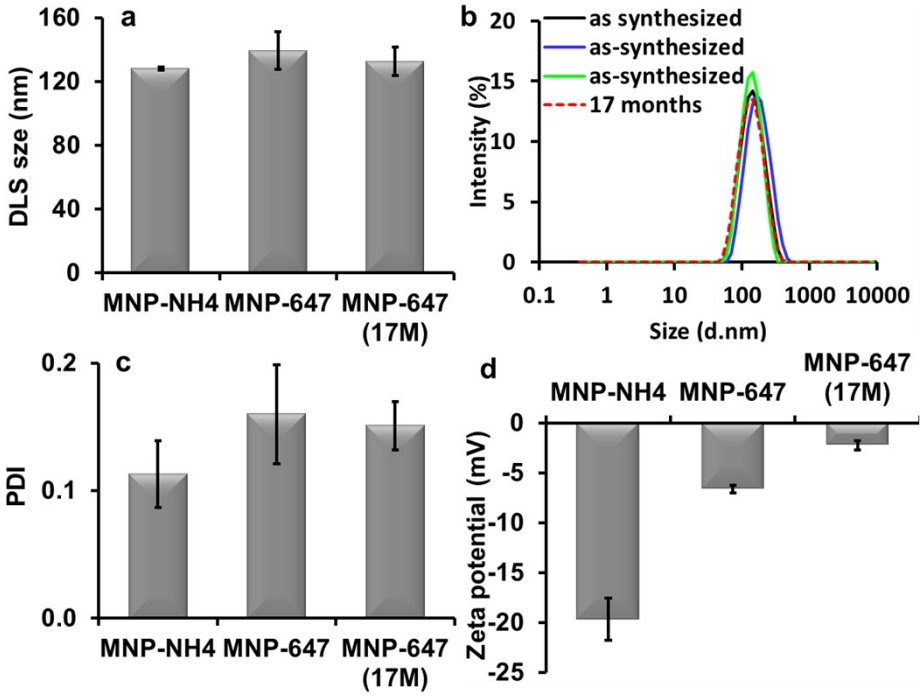

Figure S2. Dye conjugation and stability of magnetic nanoparticles. (a) Dynamic light scattering size of magnetic nanoparticles before (MNP-NH4) and after (MNP-647) dye conjugation, and dye-conjugated MNPs after storage in dark at 4°C for 17 months (MNP-647(17M)). (b) Size distribution of magnetic nanoparticles after dye conjugation and 17-month storage. (c) Polydispersity index of magnetic nanoparticles. (d) Surface zeta potential of magnetic nanoparticles.

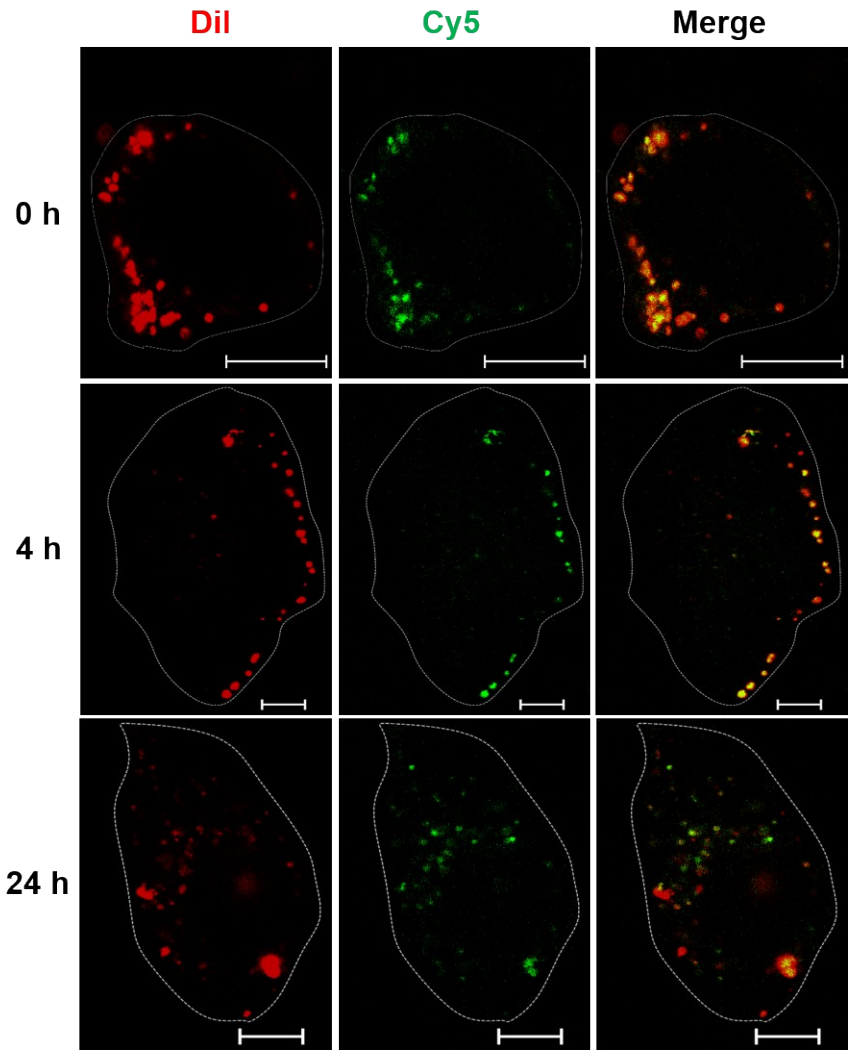

Figure S3. Co-localization of DiI and Cy5 signals from liposomes after incubation with ARPE-19 cells. Liposomes labeled with 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000]-N-(Cyanine 5) (DSPE-PEG-CY5) and DiI were incubated with ARPE-19 cells for 10 min. Cells were fixed with 4% paraformaldehyde (PFA) for 10 min at room temperature at 0 h, 4 h and 24 h after liposome incubation. DiI and Cy5 signals were acquired by confocal microscopy. Scale bar = 10 µm.

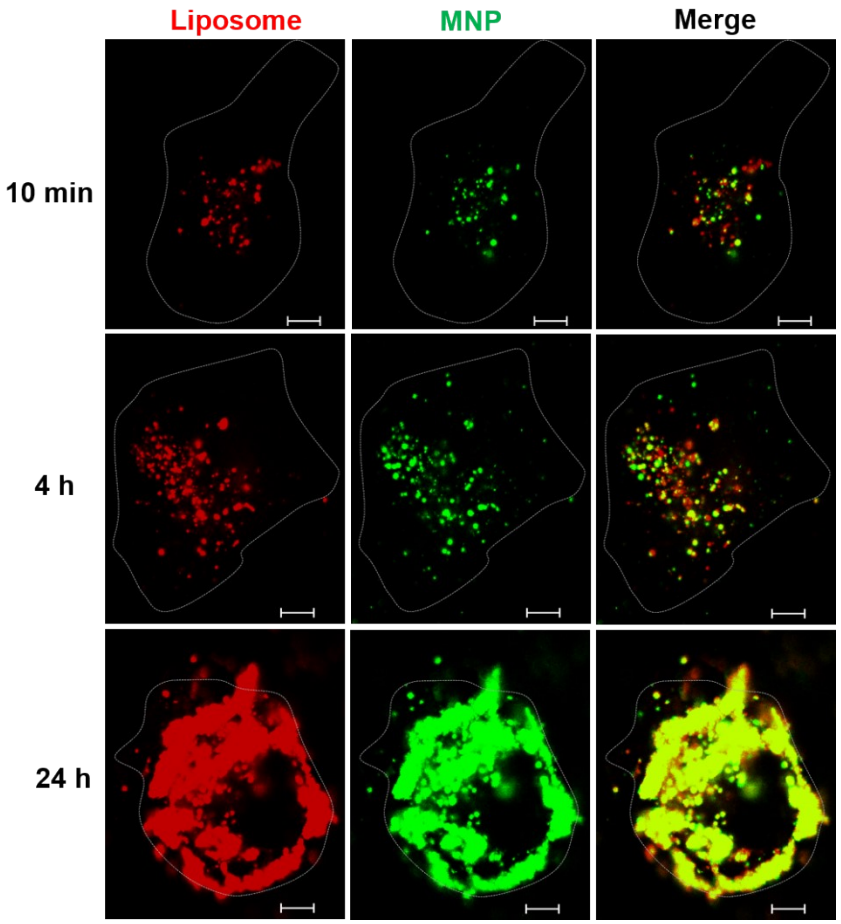

Figure S4. Administration of liposome-coated MNPs with different incubation times in ARPE-19 cells. Liposome-coated MNPs suspended in cell culture medium with MNPs at a concentration of 100 µg/ml were administrated to ARPE-19 cells for 10 min, 4 h or 24 h. Cells were washed with PBS and fixed with 4% PFA for 10 min at room temperature at 24 h after liposome application. DiI and Alexa Fluor 647 signals were acquired by confocal microscopy. Scale bar = 10 µm.

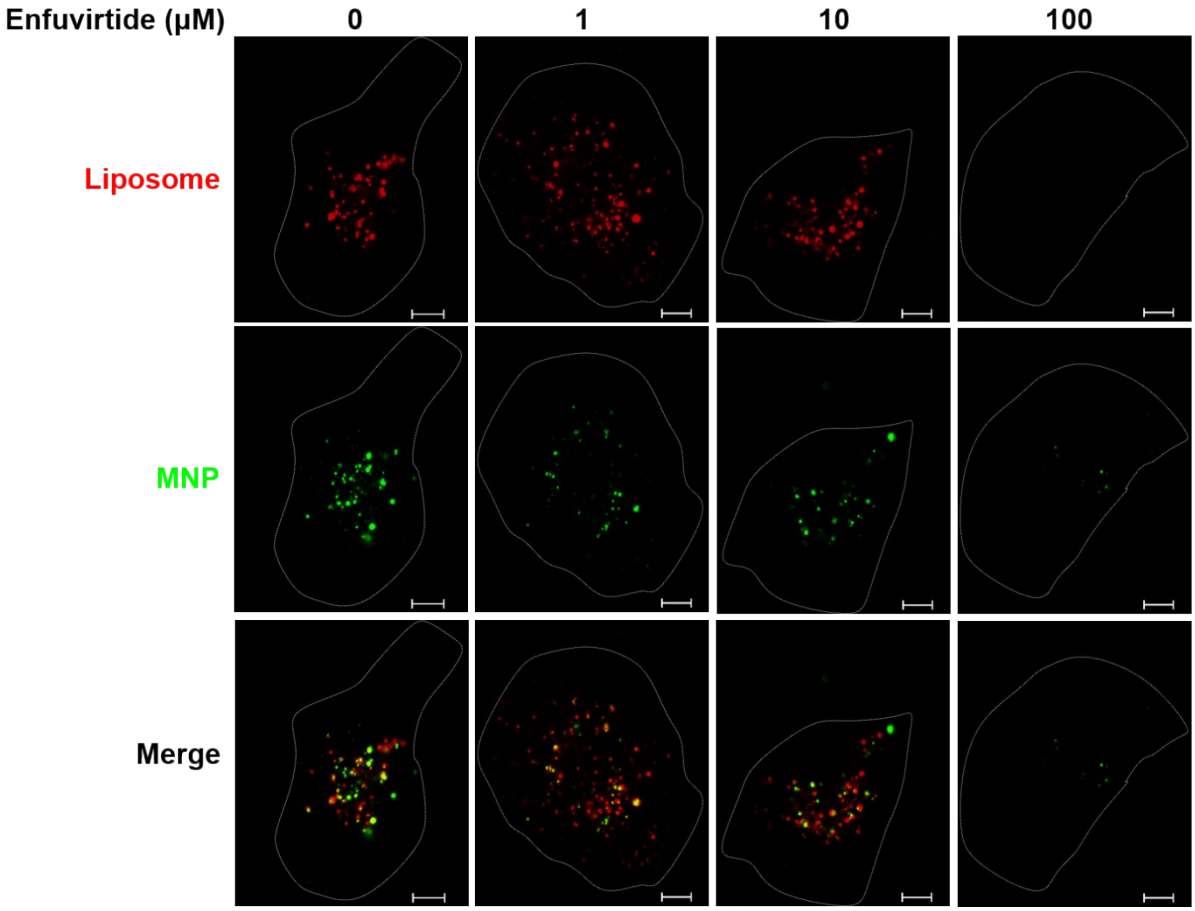

Figure S5. The effect of membrane fusion inhibitor on the uptake of liposome-coated MNPs in ARPE-19 cells. ARPE-19 cells were treated with membrane fusion inhibitor enfuvirtide at 1 μM, 10 μM and 100 μM for 1 h prior to 10 min incubation of liposome coated MNPs administration. Cells were washed with PBS and fixed with 4% PFA for 10 min at room temperature at 24 h after liposome incubation. DiI and Alexa Fluor 647 signals were acquired by confocal microscopy. Scale bar = 10 μm.

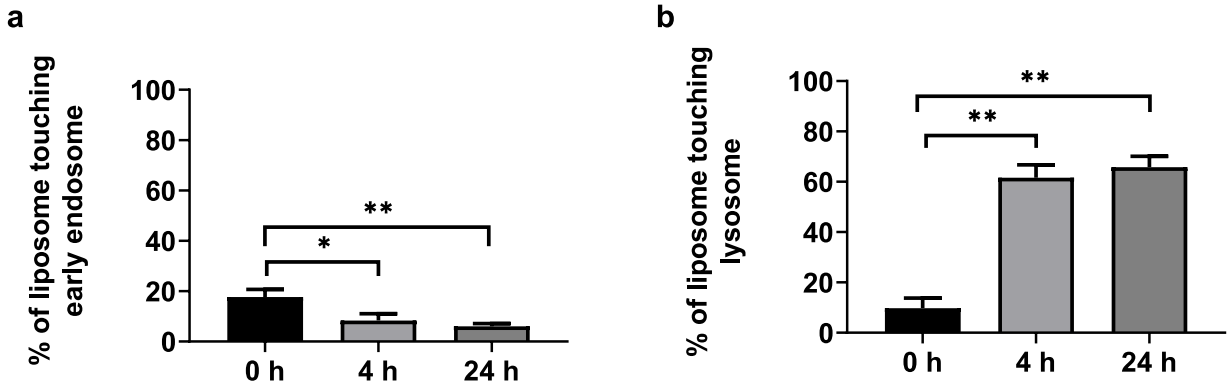

Figure S6. Intracellular distribution of liposome after incubation with ARPE-19 cells. CellLight® early endosome-GFP targeting Rab5a or CellLight® lysosome-GFP targeting Lamp1 were incubated with ARPE-19 for 24 h, DiI labeled liposome-coated MNPs were then administrated to ARPE-19 cells for 10 min respectively. Cells were washed with PBS and fixed with 4% PFA for 10 min at room temperature at 0 h, 4 h and 24 h after incubation with liposome-coated MNPs. Early endosome-GFP and DiI or lysosome-GFP and DiI signals were acquired by confocal microscopy; 10-15 cells from each group were recorded. Measurement of identified early endosome-GFP and DiI or lysosome-GFP and DiI positive objects were collected by Volocity, and the fractions of DiI signals touching early endosome-GFP(a) or lysosome-GFP(b) were calculated and analyzed by one-way ANOVA followed by Tukey's multiple comparisons test (* $p<0.05$, ** $p<0.01$, n = 10-15 cells).

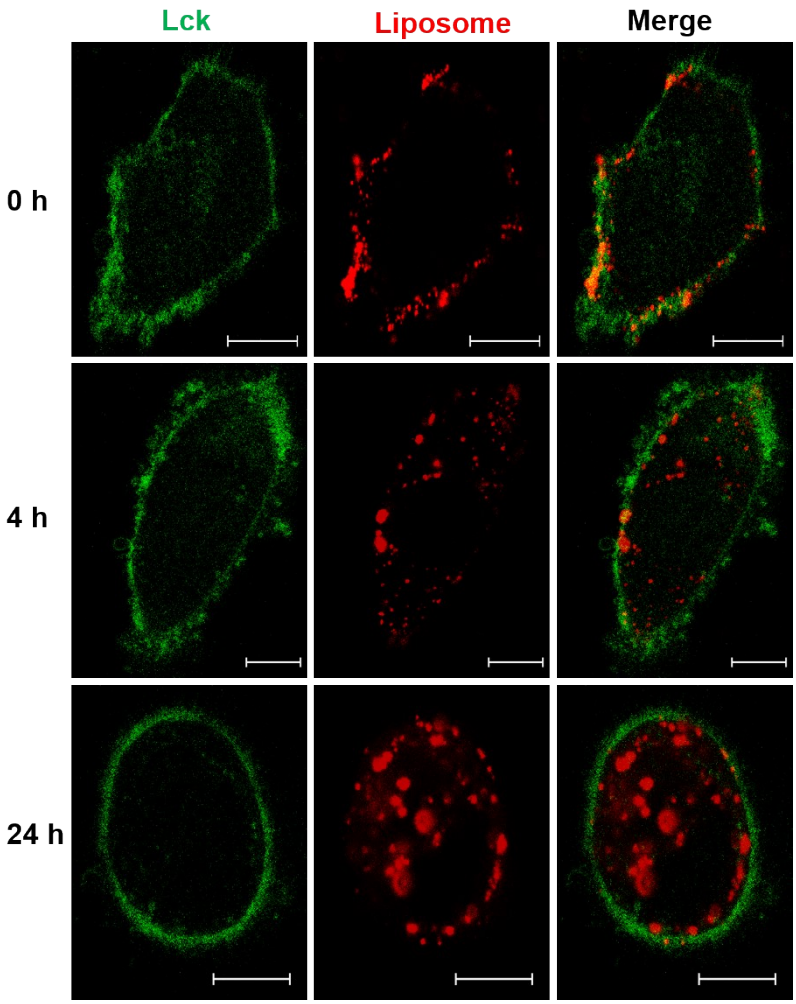

Figure S7. Co-localization of liposomes with Lck after incubation with ARPE-19. CellLight® plasma membrane-GFP targeting the myristolyation/palmitoylation sequence from Lck tyrosine kinase was incubated with ARPE-19 for 24 h, DiI labeled liposomes were then administrated to ARPE-19 for 10 min. Cells were washed with PBS and fixed with 4% PFA for 10 min at room temperature at 0 h, 4 h and 24 h after liposome incubation. Lck and DiI signals were acquired by confocal microscopy. Scale bar = 10 µm.