Supplementary information

Materials and methods

Determination of the amines on NH_2-PEG-QDs

The amines on NH_2-PEG-QDs were determined using fluorescamine reagent (Sigma) as previously described[1], with a minor modification. Briefly, a 5 mg mL^{-1} solution of fluorescamine in acetone was freshly prepared. Glycine standards (130 nM – 1.3 mM) and PEG-QDs samples (0.08 μM) were prepared in 50 mM borate buffer (pH8.4). The assay was initiated by mixing 100 μL sample or standard and 5 μL fluorescamine solution. After 5 minutes of reaction in the dark, the fluorescence intensity at 475 nm, with 390 nm excitation, was measured by a microplate reader (Bio-Tek Inc., Winooski, VT).

QD stability characterization

For QD stability characterization, all the QDs were incubated with PBS or DMEM medium up to 48 h. The hydrodynamic sizes of QDs at indicated time points were measured using a Zetasizer Nano ZS instrument as previously described.

Non-specific binding analysis of QDs

The non-specific binding of QDs was examined using agarose gel electrophoresis as previously described[2], with a minor modification. Briefly, 1 μL QDs (8 μM) diluted in 10 μL 0.5 × TBE buffer were incubated with or without 1 μL BSA (20 mg mL^{-1}) at 37°C for 1 h. After incubation, samples of QDs alone or QD/BSA mixture were separated by 1% agarose gel, respectively. The migration positions of QDs or BSA were revealed using UV light or coomassie blue staining, respectively.

By contrast with QDs alone, the migration retardation or acceleration of QDs mixed with BSA indicated the non-specific adsorption of BSA onto the QD surface. Accordingly, in the samples of QD/BSA mixture, the band of BSA overlapped with QDs represented BSA adsorption.

Supplementary Table 1. The sequences of primers for PCR analysis of human IL-1β, TNF-α, CCL5, SRA and β-actin.

target gene	sense primer (5'-3')	antisense primer (5'-3')
IL-1β	CCCAGAGAGTCCTGTGCTGAATG	GAGAGCTGACTGTCCTGGCTGAT
TNF-α	TGGAGAAGGGTGACCGACTCAG	GTTTGGGAAGGTTGGATGTTCG
CCL5	AACCCAGCAGTCGTCTTTGTC	GGACAAGAGCAAGCAGAAACAG
SRA	GGACGAAAGAAGTATGGAGCAGTG	CCAATGAGAGGGATGAGAACTGC
β-actin	CGCGAGAAGATGACCCAGATC	CATGAGGTAGTCAGTCAGGTCCC

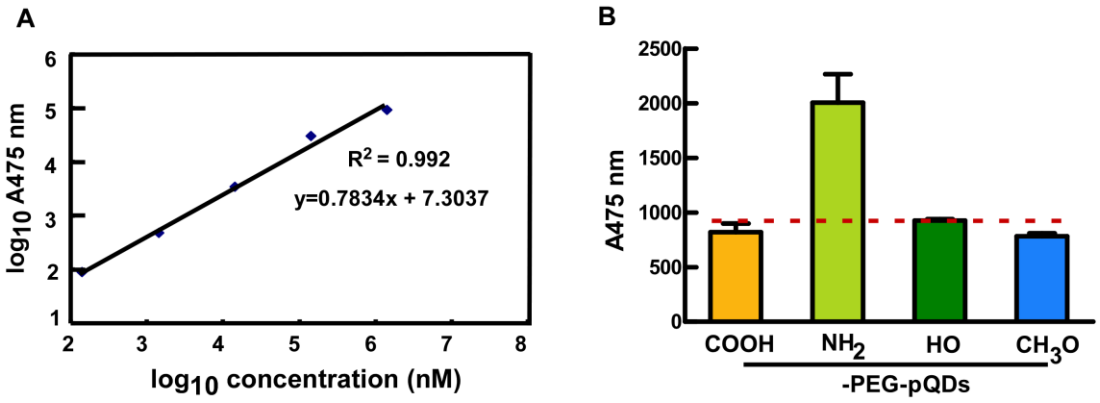

Figure S1. Determination of the amines on NH_2-PEG-pQDs. A, A glycine standard curve was prepared by plotting the absorbance of samples against the known concentrations. Both the values of absorbance and concentrations were $\log_{10}$ transformed. B, The fluorescence intensity of fluorescamine after reaction with PEG-pQDs was detected by a microplate reader. The dashed red line indicated the threshold value set by PEG-pQDs functionalized with carboxyl, hydroxyl and methoxyl groups.

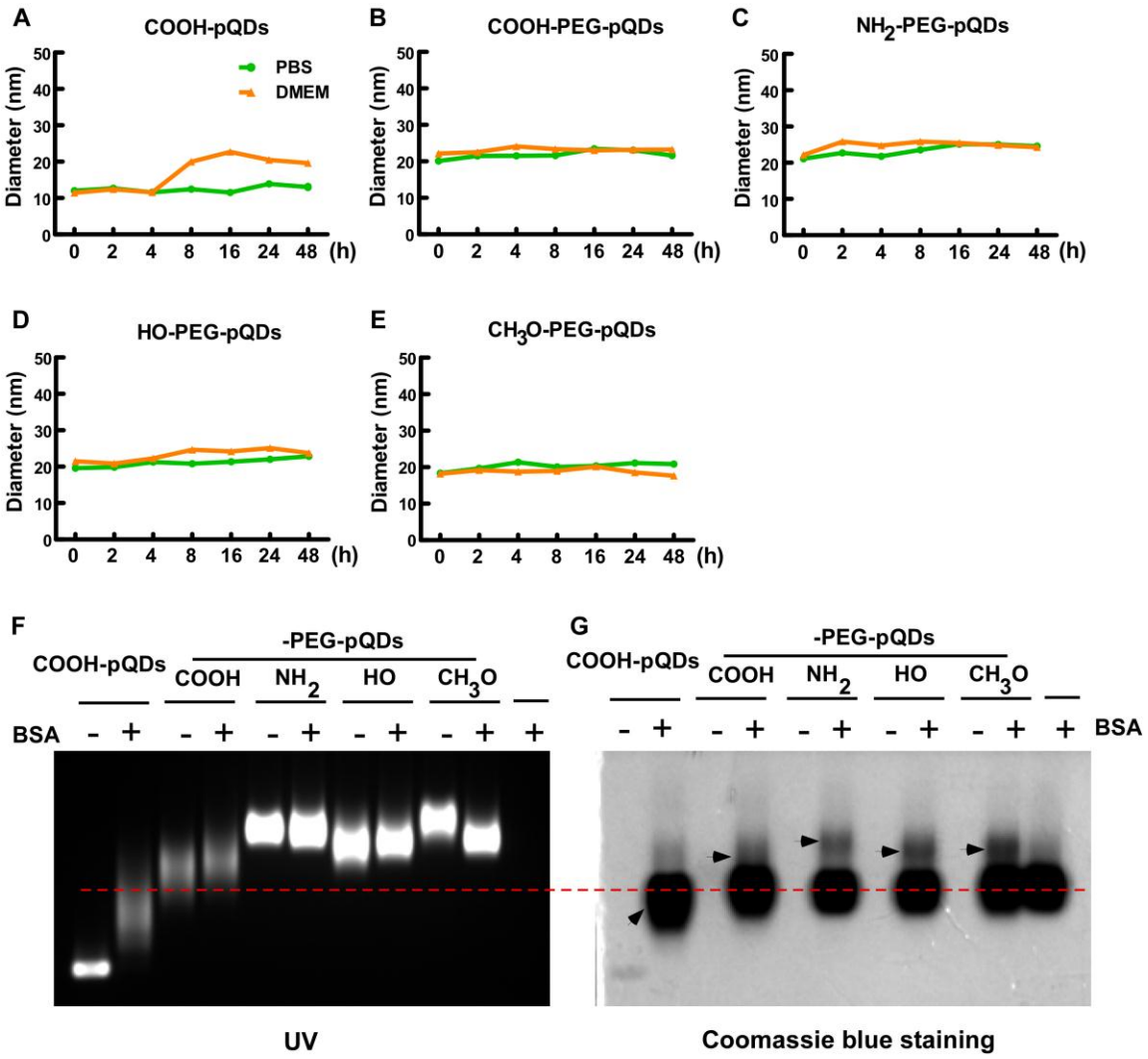

Figure S2. Characterization of QD stability and non-specific binding. A-E, The hydrodynamic sizes of QDs incubated in PBS or DMEM medium was measured at indicated time points, using a Zetasizer Nano ZS instrument. F and G, after QDs were incubated with BSA at 37^{o}C for 1 h, the non-specific binding of BSA onto the surface of QDs was analyzed using agarose gel electrophoresis as described above. The migration positions of QDs or BSA were revealed using UV light (F) or coomassie blue staining (G), respectively. The red dashed line indicated the migration position of BSA alone. The arrows indicated the band of BSA overlapped with QDs in the same sample. As shown in F, compared with QDs alone, the migration of COOH-pQDs,

COOH-PEG-pQDs and HO-PEG-pQDs with BSA incubation was retarded by 23%, 4% and 2%, respectively. In addition, the migration of CH_3O-PEG-pQDs with BSA incubation was accelerated by 12%, whereas NH_2-PEG-pQDs migration was not affected by BSA at all.

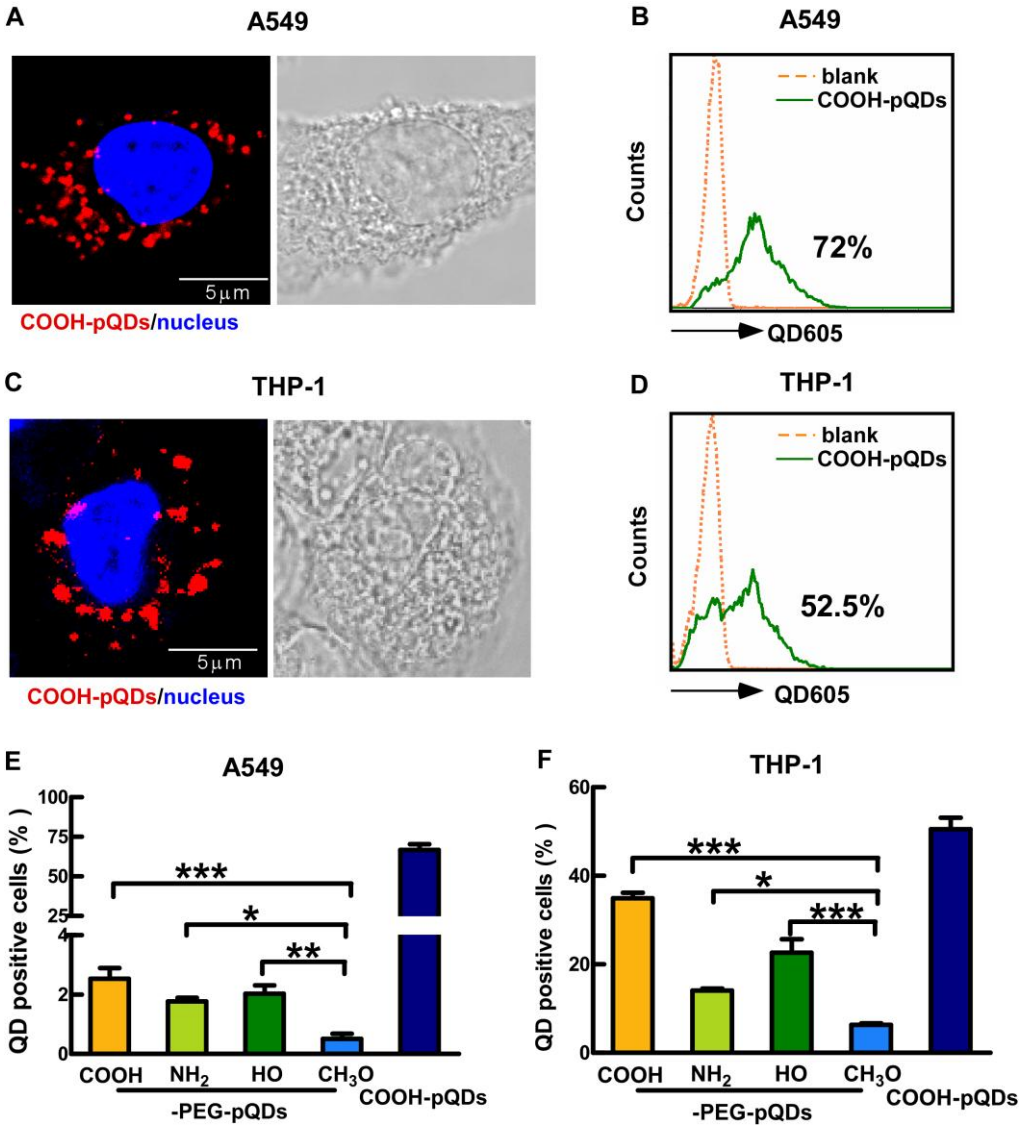

Figure S3. Cellular uptake analysis of QDs by A549 cells and THP-1 derived macrophages. A-D, A549 cells (A and B) or THP-1 derived macrophages (C and D) were exposed to 10 nM COOH-pQDs for 6 h or 1 h, respectively. The cell nucleus

was stained with Hoechst 33258 (blue). The QD fluorescence (red) in A549 cells was detected by confocal microscopy (A and C) or flow cytometry (B and D). E and F, A549 cells (E) or THP-1 derived macrophages (F) were exposed to 10 nM QDs for 6 h or 1 h, respectively. The QD-internalized cells were detected by flow cytometry. Data shown represent mean±SEM of three independent experiments. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

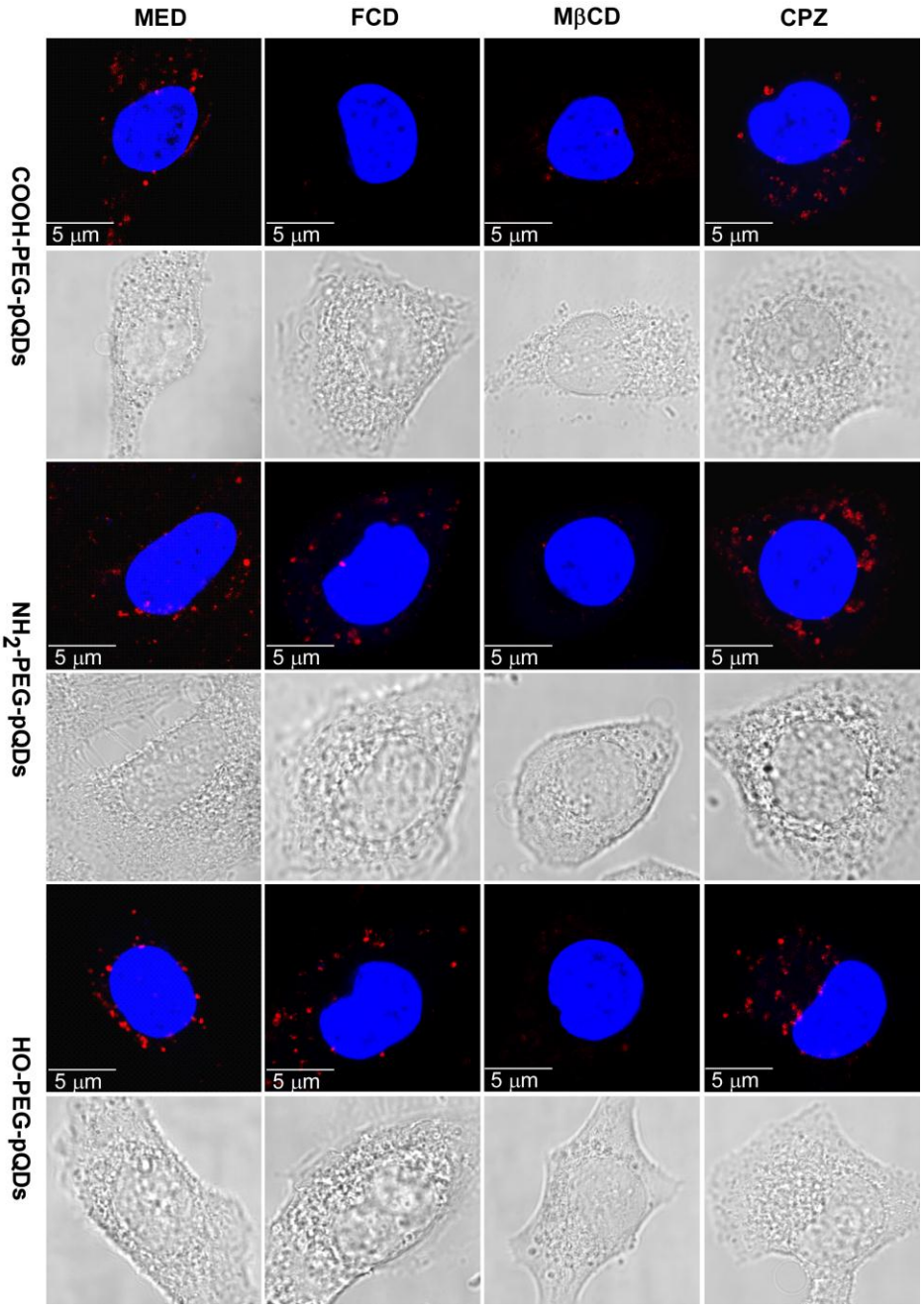

Figure S4. The endocytic pathways of PEG-pQDs in A549 cells. A549 cells were pre-treated with FCD, MβCD or CPZ prior to exposure to 10 nM PEG-pQDs for 6 h. The cell nucleus was stained with Hoechst 33258 (blue). The QD fluorescence (605 nm, red) in cells were detected using confocal microscopy. The bright field image was showed below the fluorescent image.

References

1. A. M. Smith and S. Nie, *Journal of the American Chemical Society*, 2008, **130**, 11278.
2. R. E. Anderson and W. C. Chan, *ACS Nano*, 2008, **2**, 1341-1352.