

Systematic Evaluation of Quantum Dot Cellular Uptake and Toxicity in the Developing Brain

Supporting Information

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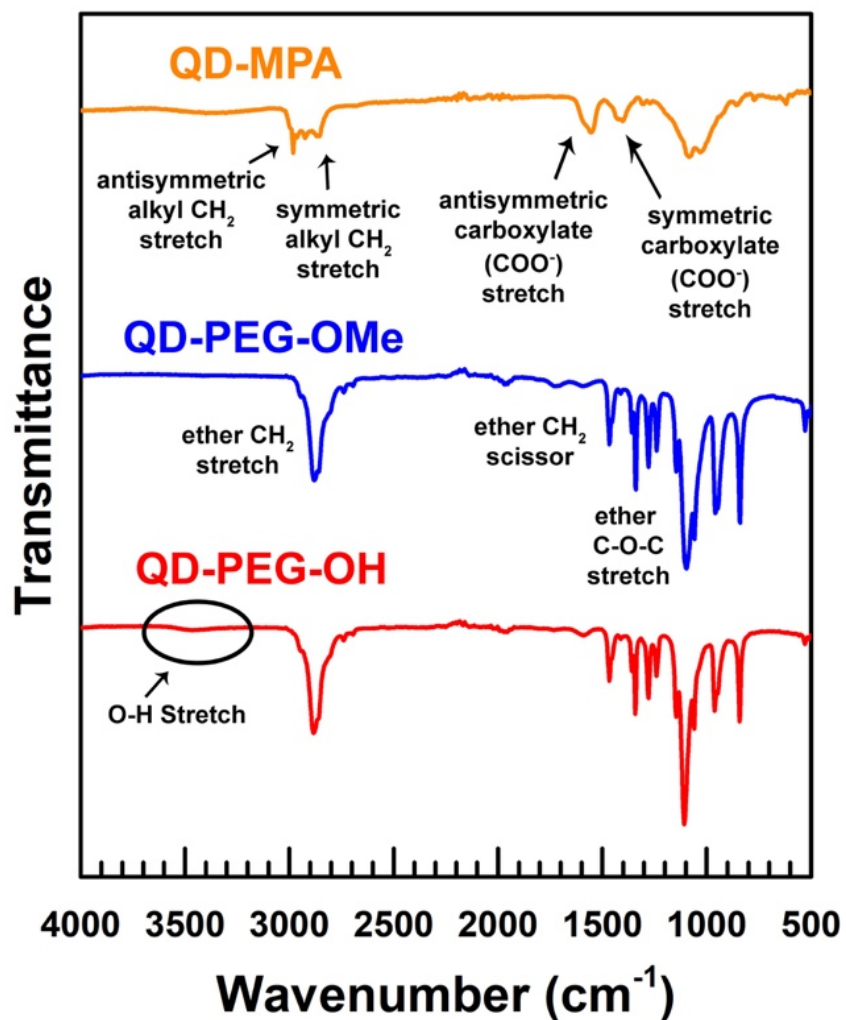
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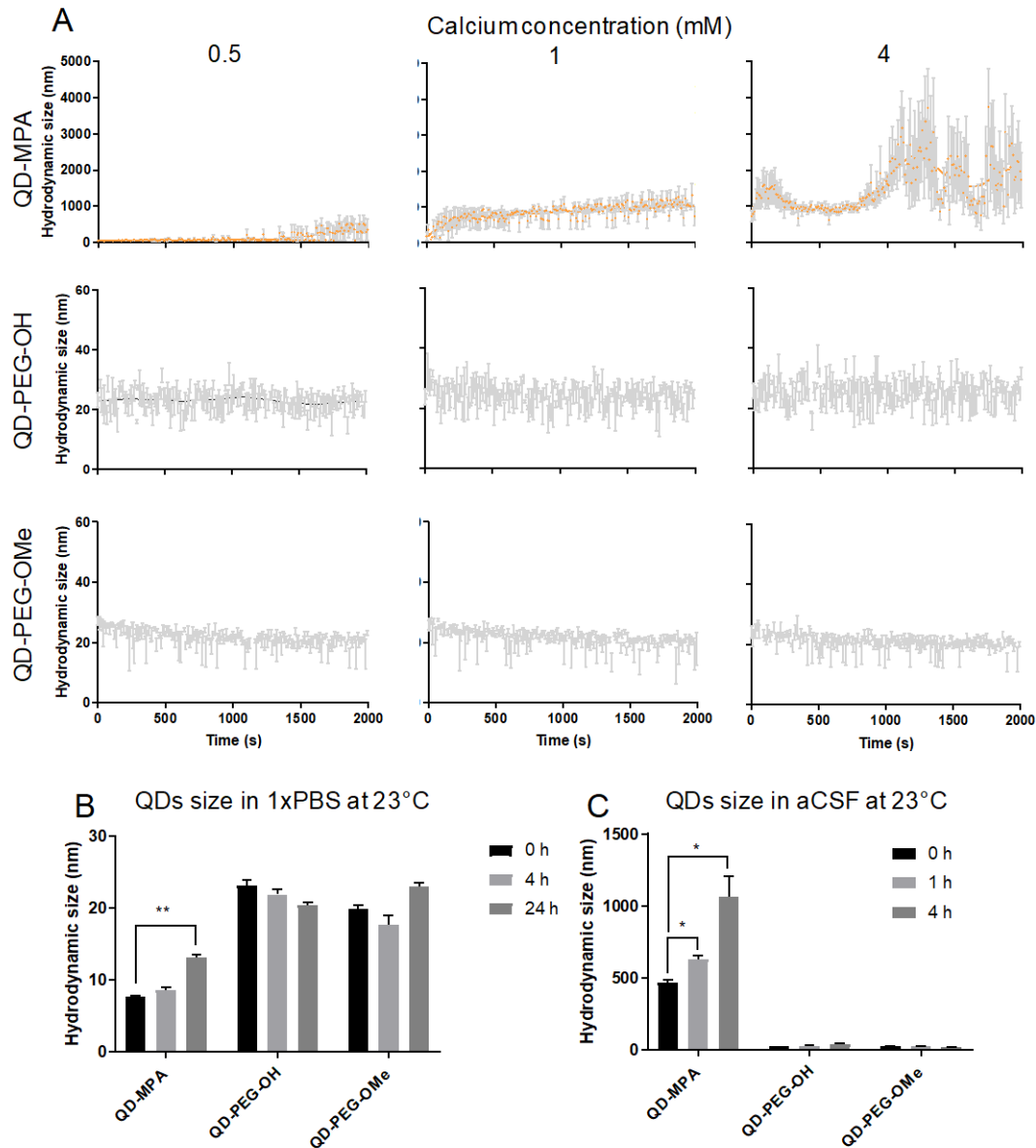
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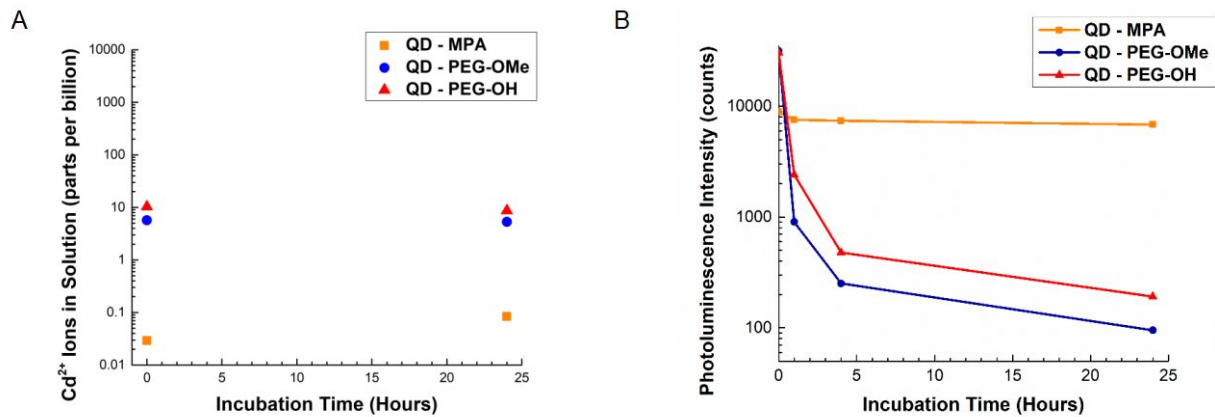


Supplemental Figure 1. ATR-FTIR spectra of QD-MPA (orange), QD-PEG-OMe (blue), and QD-PEG-OH (red). The FTIR spectra of the PEGylated QDs show the distinct features of the polyethylene glycol polymer ligand. The peak at 1100 cm⁻¹ is characteristic of an ether C-O-C band, while the peaks at 2870 and 1465 cm⁻¹ correspond to CH₂ stretching and scissoring vibrations, respectively. The remaining peaks indicate the presence of a long-chain polyether. The peaks at 1340, 1280, and 1235 cm⁻¹ are indicative of CH₂ wagging, symmetric twisting, and antisymmetric twisting, respectively. The peak at 960 cm⁻¹ corresponds to a combination of coupled symmetric

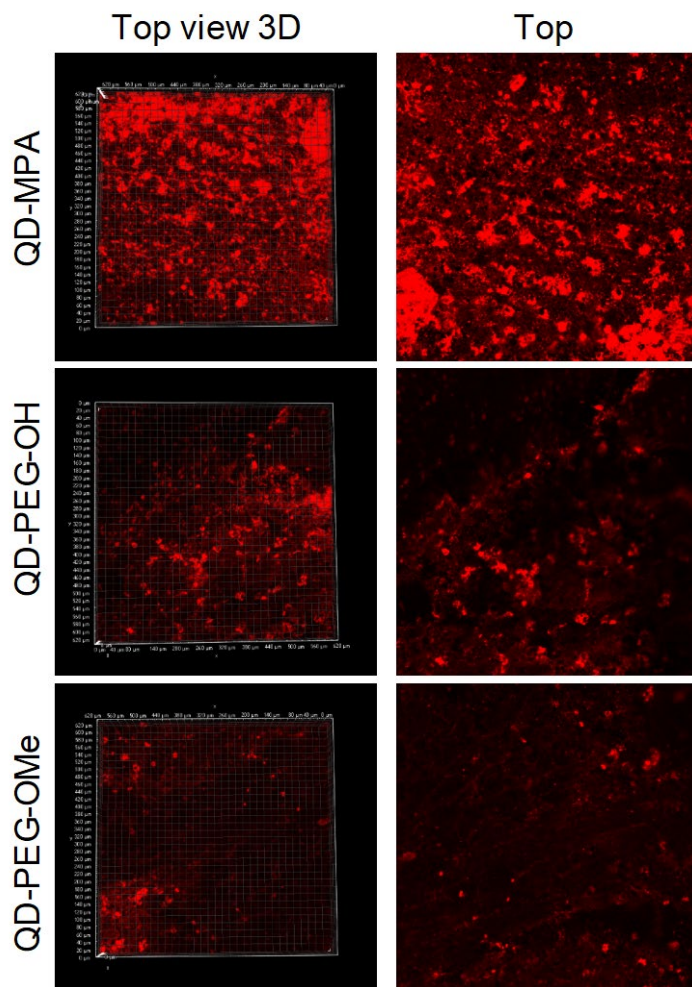
CH₂ rocking, C-C stretching, and C-O stretching, while the peak at 840 cm⁻¹ corresponds to coupled CH₂ rocking and C-O stretching. The QD-PEG-OH samples show an additional broad O-H stretching band extending from 3300 to 3560 cm⁻¹, which is indicative of the additional terminal O-H functional group attached to the PEG. The 3-mercaptopropionic acid-functionalized QDs exhibited characteristic antisymmetric and symmetric alkyl CH₂ stretching bands at 2920 and 2860 cm⁻¹, respectively, along with clear antisymmetric and symmetric carboxylate (COO⁻) stretching bands at 1560 and 1410 cm⁻¹, respectively. These observations, in combination with the absence of a strong carboxylic acid C=O stretch at 1710 cm⁻¹, indicate complete deprotonation of the carboxylic acid groups on the MPA ligands.



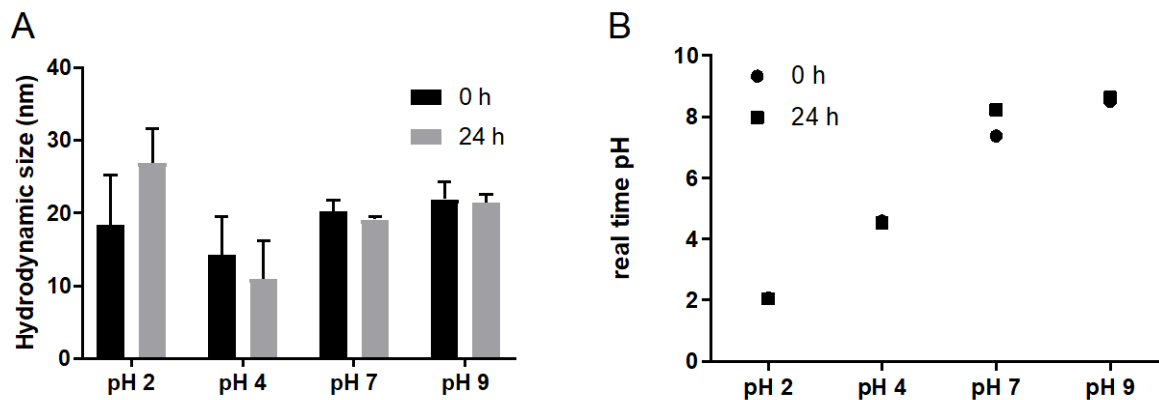
Supplementary Figure 2. Colloidal stability of QDs at 23°C in neurophysiological fluids. (A) Initial aggregation kinetics of QD-MPA, QD-PEG-OH and QD-PEG-OMe at 23°C in CaCl₂ solution with 0.5, 1, and 4 mM Ca²⁺ over a period of 2000 sec (n=3 measurements per particle type). Trendlines showing initial QD-MPA aggregation were generated using a LOWESS regression in GraphPad. (B) QD hydrodynamic size at 23°C in 1xPBS at 0, 4, 24 h (n=3 measurements per particle type). (C) QD hydrodynamic size at 23°C in aCSF at 0, 4, 24 h (n=3 measurements per particle type).



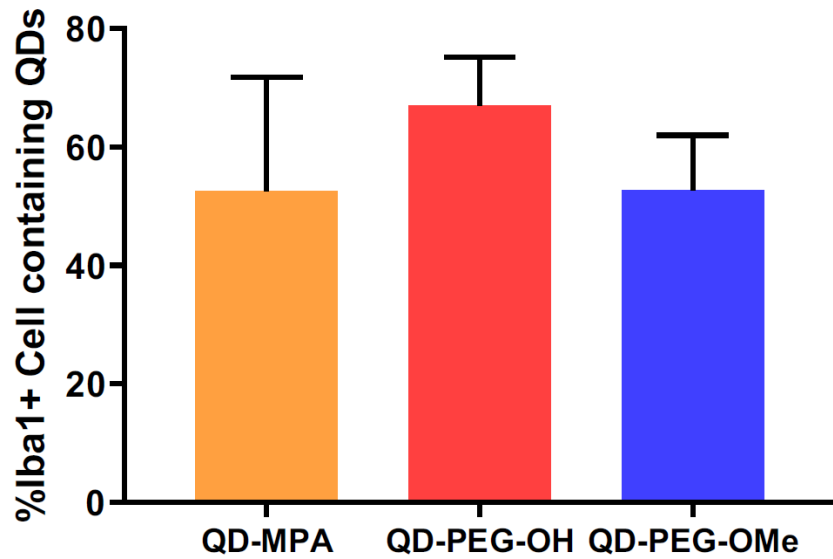
Supplementary Figure 3. (A) Cadmium ion release of 0.1 μM CdSe/CdS QDs with different surface functionalities in aCSF at 37°C after 0 h and 24 h incubation. The level of free Cd²⁺ ions in all groups tested was less than 10 parts per billion (ppb). Note that the initial 0-h baseline levels of Cd²⁺ are due to a small subset of CdSe particles that pass through the 50-kD filter and are digested to Cd²⁺ during ICP-MS sample preparation. When a lower molecular weight cutoff filter (3 kD) was used on identical PEGylated samples, a baseline Cd²⁺ ion level of 1 ppb was achieved. The concentration of free Cd²⁺ ions remained virtually unchanged (less than 1 ppb change in concentration) for all samples over the 24-hour measurement period. (B) Photoluminescence intensity of QDs incubated in aCSF under physiological conditions as a function of time.



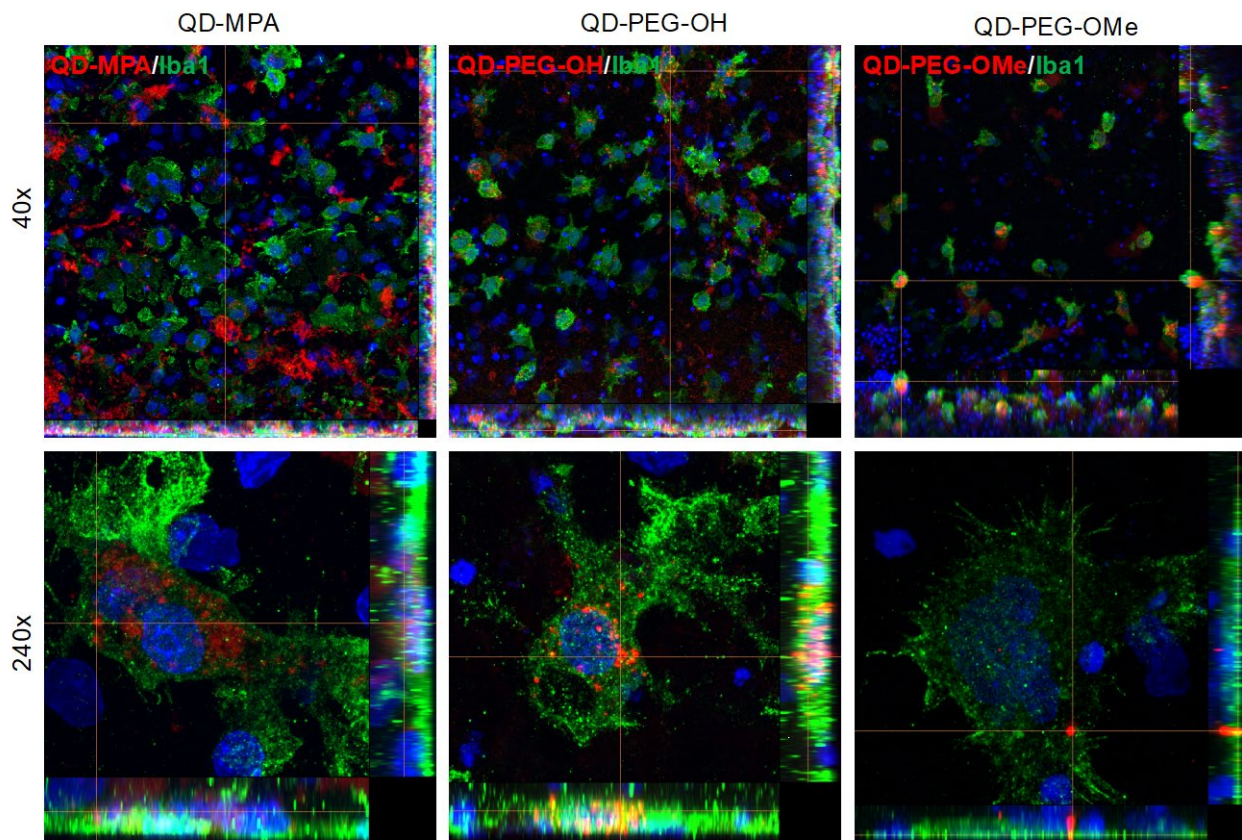
Supplementary Figure 4. QD Penetration in OWH slices. QD distribution and penetration is shown at 20X magnification in 3D view (left panel) and at a single-z top view (right panel).



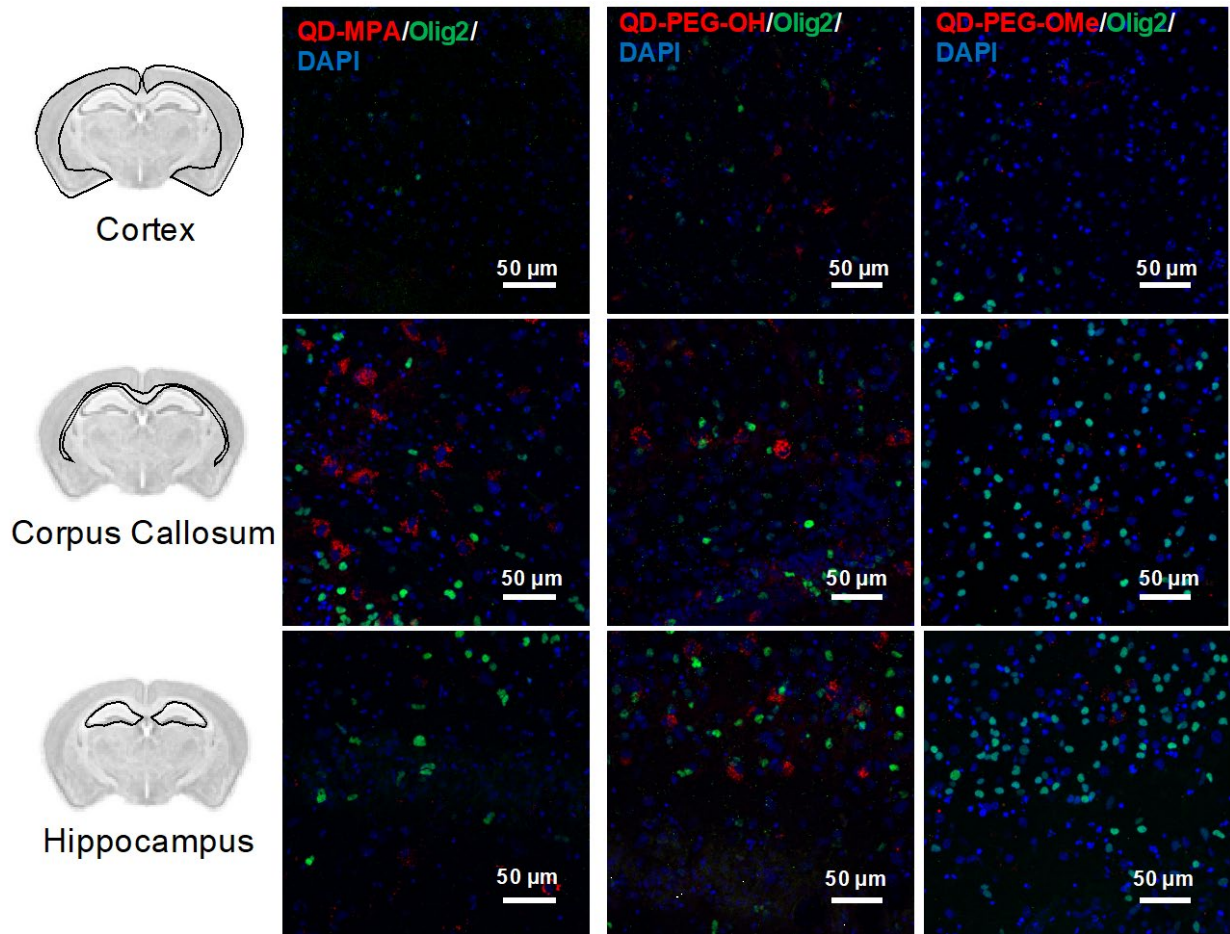
Supplementary Figure 5. pH influence on QD-PEG-OMe stability in aCSF media. (A) QD-PEG-OMe hydrodynamic size remains constant from 0 h to 24 h after incubation in aCSF at pH 2, pH 4, pH 6 and pH 9 ($n = 3$ measurements per condition). (B) Across the full range of pH values evaluated in this study, the pH stays constant during the experimental window (24 h incubation), even in the presence of QD addition.



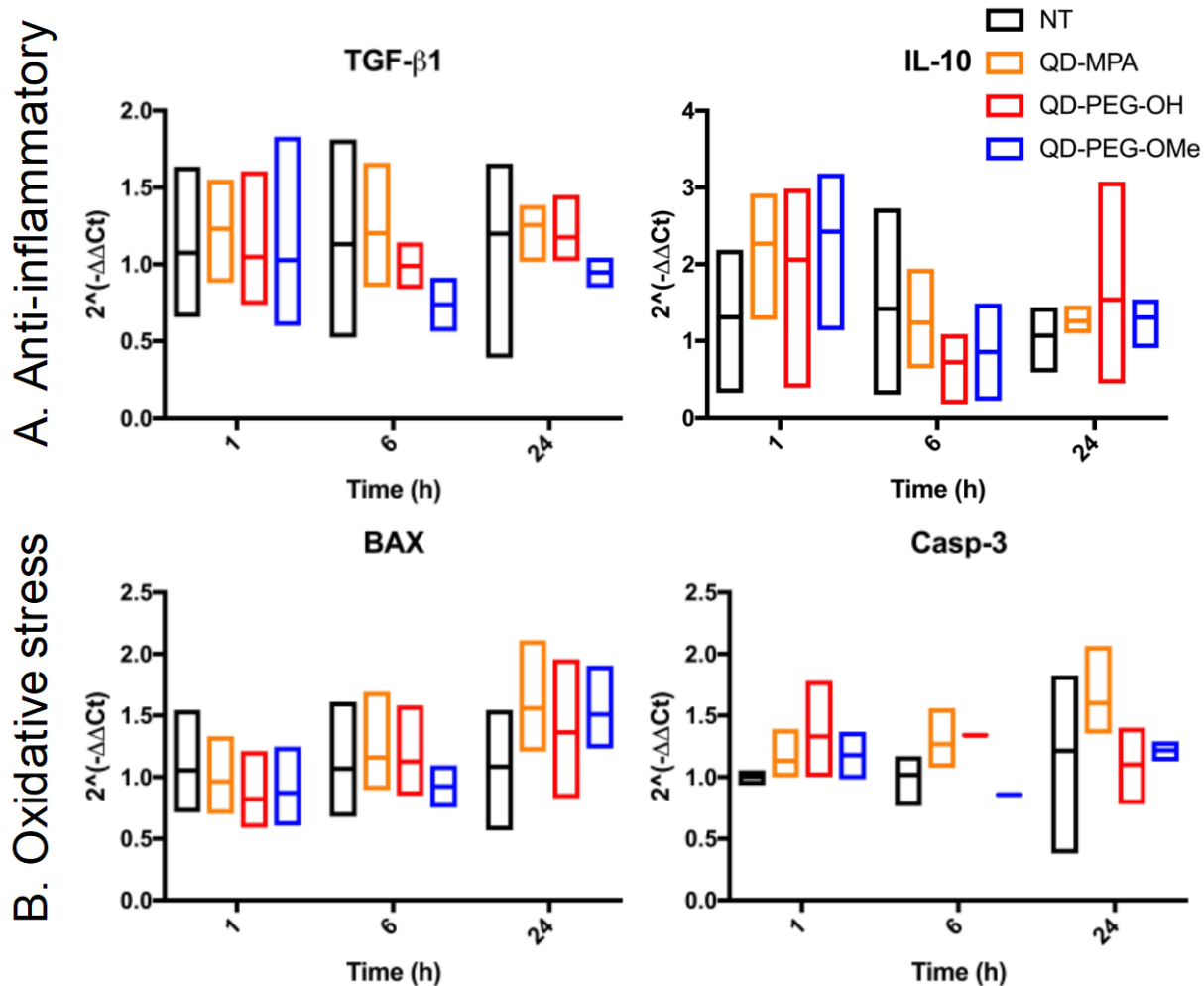
Supplementary Figure 6. Fraction of Iba1+ cells containing QDs in the corpus callosum region in OWH slices. Iba1+ microglia cells containing QDs were counted in the corpus callosum region in OWH slices and divided by the total number of Iba1+ microglia cells in the same region. $n = 3$ slices per QD surface functionality were evaluated. For each slice, $n = 3$ regions in corpus callosum were imaged for cell counting. The 3 regions evaluated were located in the same positions across all 3 slices. The percentage of Iba1+ cells containing QDs was calculated using all 3 regions of each slice, and the mean and SEM values were calculated based on the 3 slices.



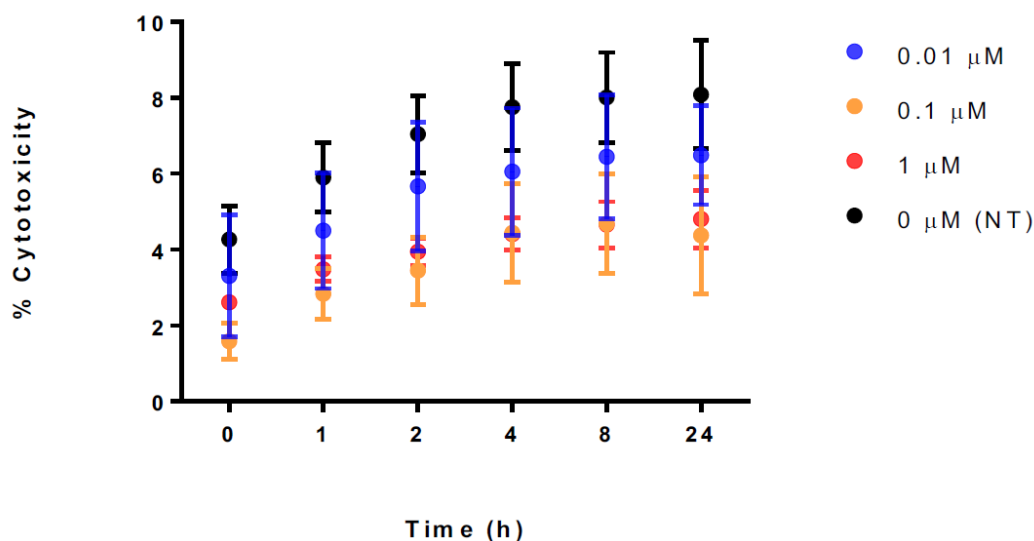
Supplementary Figure 7. Cross-sectional views of z-stack confocal images of QD (red, all images) colocalization with Iba1+ microglia (green, all images) following 24 h incubation on *ex vivo* OWH slices. This colocalization analysis was performed using Nikon software. For each individual image, the line intersection in the 40x maximum intensity projected image represents the QDs selected for colocalization analysis at 240x. The right side-view images represent a “front” view and side view of the 3D z-stack image. Yellow color indicates colocalization of QDs and microglia.



Supplementary Figure 8. Olig2⁺ oligodendrocyte uptake of QDs in OWH slices. 40X magnification images of QD-MPA, QD-PEG-OH and QD-PEG-OMe (red, all images) distribution and interaction with Olig2⁺ oligodendrocytes (green, all images) in the cortex, corpus callosum, and hippocampus in P14 SD rat pup brain slices (300-μm thickness). Cell nuclei were stained with DAPI (blue).

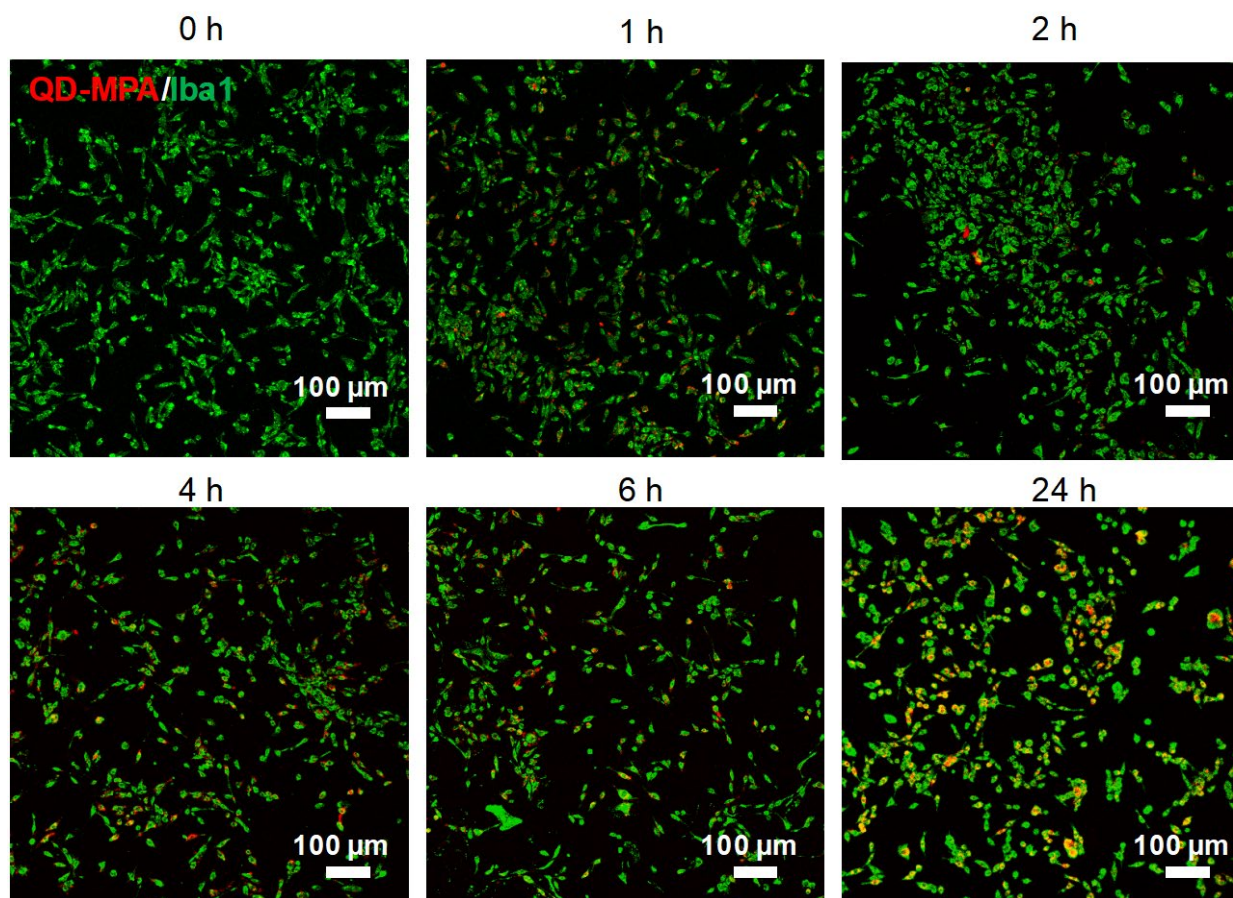


Supplementary Figure 9. Time-dependent mRNA profiles of QD-induced anti-inflammatory and oxidative stress markers in OWH slices. Fold-changes in mRNA expression were measured at 1 h, 6 h, and 24 h of QD-MPA, QD-PEG-OH, and QD-PEG-OMe exposure at 0.1 μ M concentration in OWH slices. The fold-changes were measured for (A) anti-inflammatory markers TGF β and IL-10, and (B) oxidative stress markers BAX and Casp-3. For 1 h, 6 h and 24 h time points, n = 3 groups and n = 3-6 slices per group were evaluated for each experimental sample (except for the QD-PEG-OMe 6h proinflammatory sample, where n=2 groups with n = 6 slices in total were evaluated).

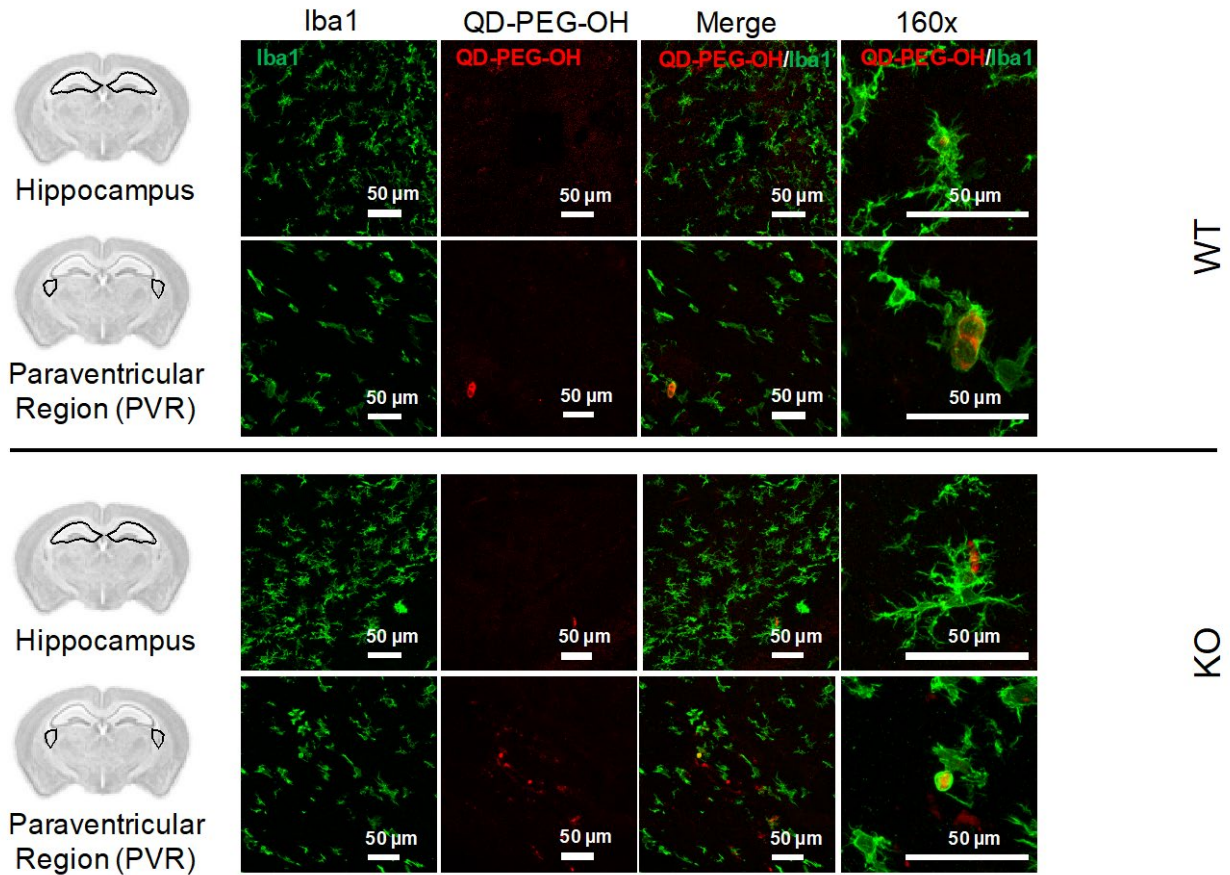


Supplementary Figure 10. Dose-dependent toxicity of QD-MPA in OWH slices by LDH assay.

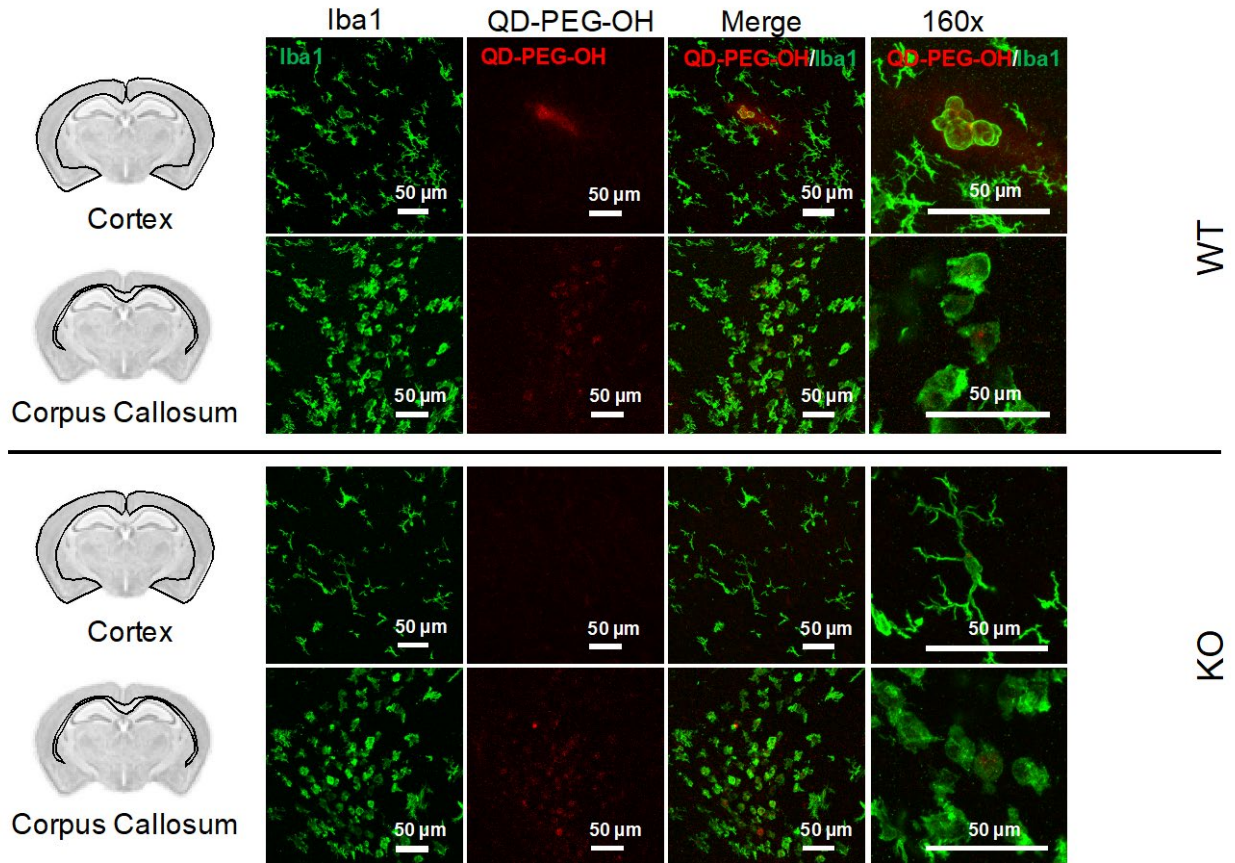
Treatment of QD-MPA at 0.01, 0.1, and 1 μM concentrations on P14 rat organotypic brain slices for 24 h. QD toxicity was determined by LDH assay at 0, 1, 2, 4, 8, and 24 h as follows: %Cytotoxicity = %LDH release = $\frac{\text{Experimental release}}{\text{Maximum release}}$. For each QD concentration, n=3 slices per quantum dot type per concentration condition were evaluated; for NT group (black), n=5 slices.



Supplementary Figure 11. Cellular uptake of QD-MPA on *in vitro* BV-2 cells. Application of QD-MPA (red, all images) at 0.01 μM concentration onto *in vitro* BV-2 cells for 1, 2, 4, 6, and 24 h. QD-MPA were taken-up into microglia (green, all images)) starting at 1 h.



Supplementary Figure 12. QD cellular uptake in P7 rats, 4 h post-administration. Representative images of QD-PEG-OH (red, all images) colocalization with Iba1+ microglia cells (green, all images) in the hippocampus and PVR regions in P7 *mgIur5* WT and KO pup brains, 4 hours after i.p. administration. 40X magnification images with 4-fold zoom (160X) are presented in the rightmost column to show QD-PEG-OH internalization in cells.



Supplementary Figure 13. QD cellular uptake in P7 rats, 24 h post-administration. Representative images of QD-PEG-OH (red, all images) colocalization with Iba1+ microglia cells (green, all images) in the cortex and corpus callosum regions in P7 *mglur5* WT and KO pup brains, 24 h after i.p. administration. 40X magnification images with 4-fold zoom (160X) are presented in the rightmost column to show QD-PEG-OH internalization in cells.