Supporting Information

Riboflavin Terminated, Multivalent Quantum Dot as Fluorescent Cell Imaging Probe

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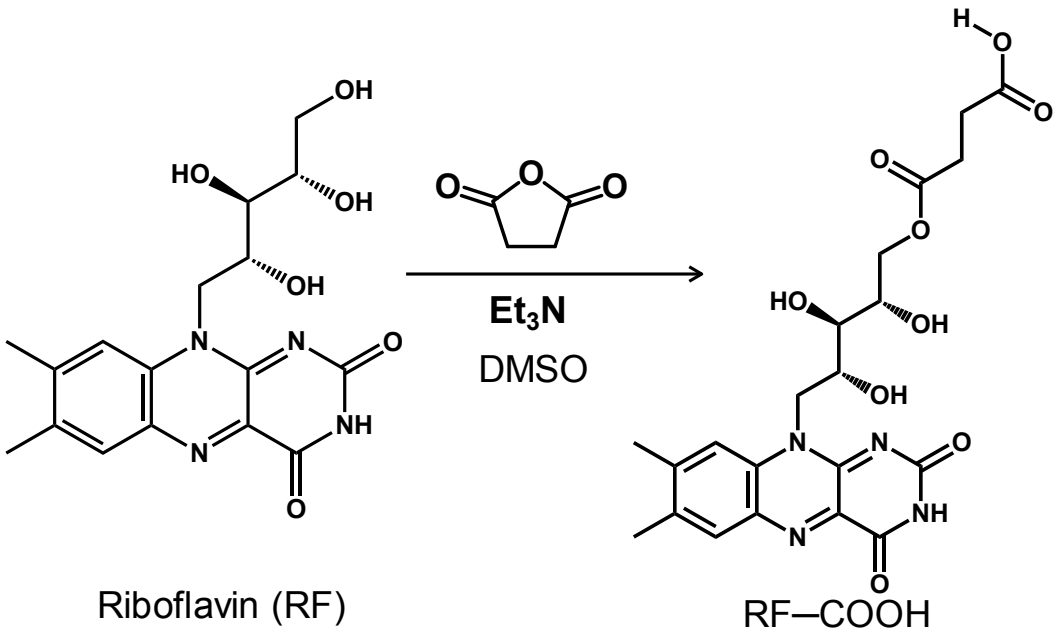
Table S1: Result of alternative **s**ynthetic condition in making riboflavin functionalized QD by changing riboflavin concentration.

Amount of riboflavin (mg)	Number of riboflavin / QD
0.5	20-40
1	40-70
2	70-80

Scheme S1. Synthesis scheme of carboxylated riboflavin (RF-COOH) from riboflavin (RF).



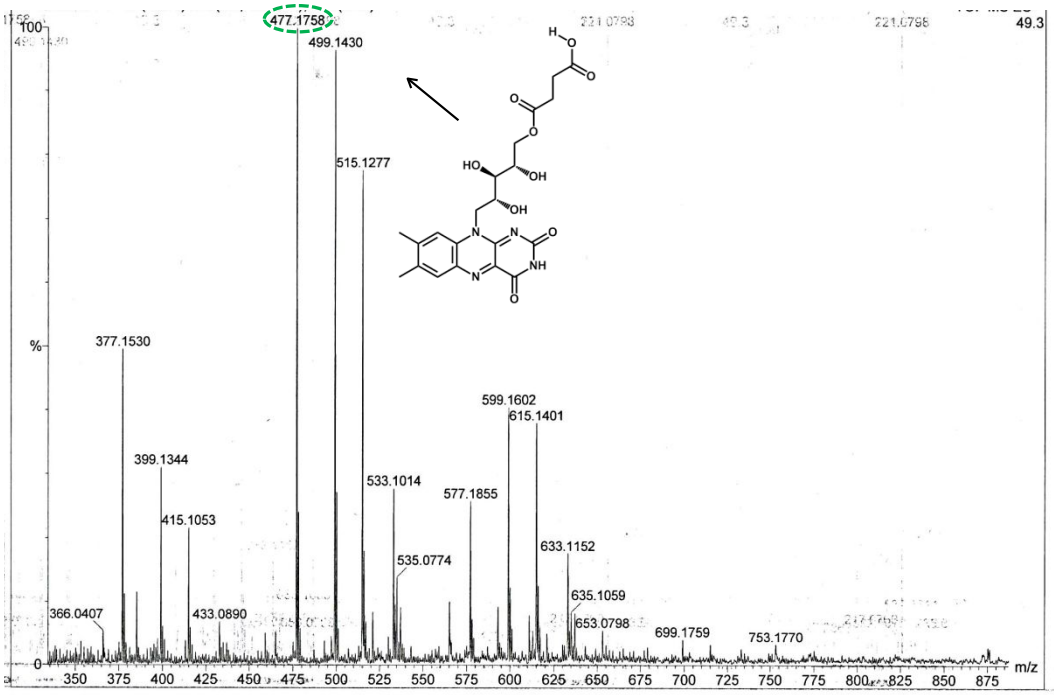


Figure S1. HRMS of RF-COOH. The peak at 477.17 suggest the formation of RF-COOH. [calculated for C_{21} $H_{24}N_4O_9$ (MH+) : 477.15, found : 477.17].

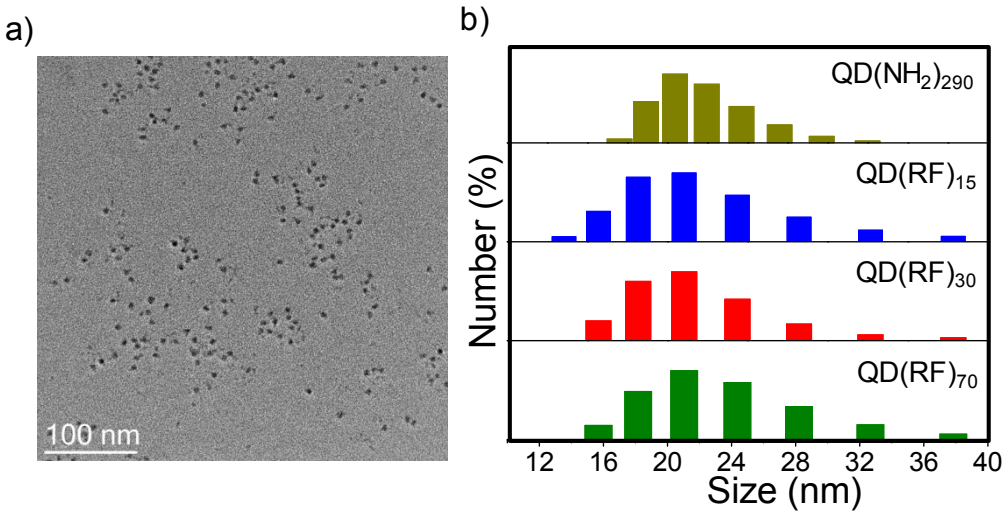


Figure S2. a) TEM image of $QD(NH_2)_{290}$, showing the inorganic QD core having 4-5 nm size. b) Hydrodynamic size of nanoparticles in phosphate buffer of pH 7.4, as observed by dynamic light scattering.

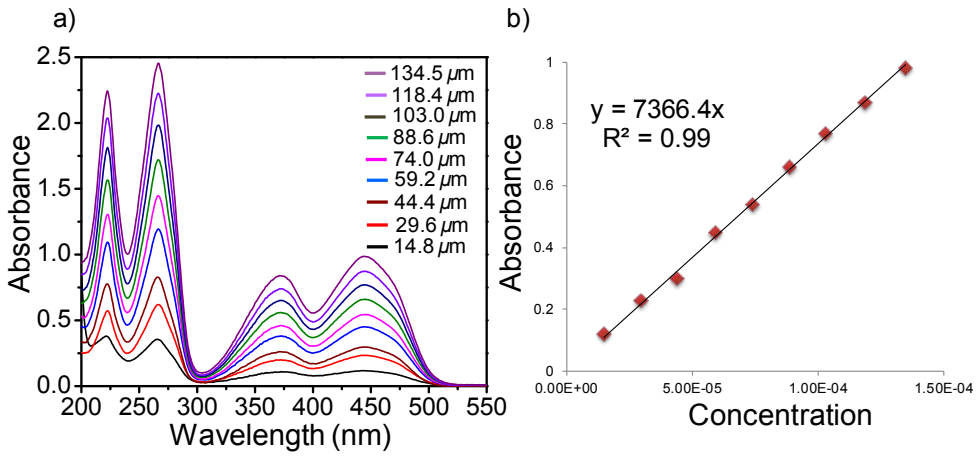


Figure S3. a) UV-visible absorbance spectra of different concentration of riboflavin. b) A linear calibration curve was obtained by plotting absorbance value at 445 nm against concentration of riboflavin as follows: Y = 7366.4 X with R^2 value 0.99 (Y= absorbance and X= concentration of riboflavin).

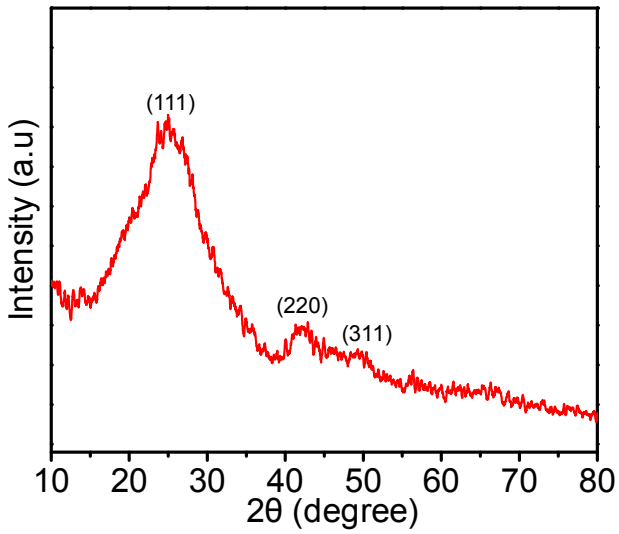


Figure S4. The XRD pattern of riboflavin functionalized QD showing the crystal phases of CdSe/ZnS (ref. JCPDS file No. 19–0191).

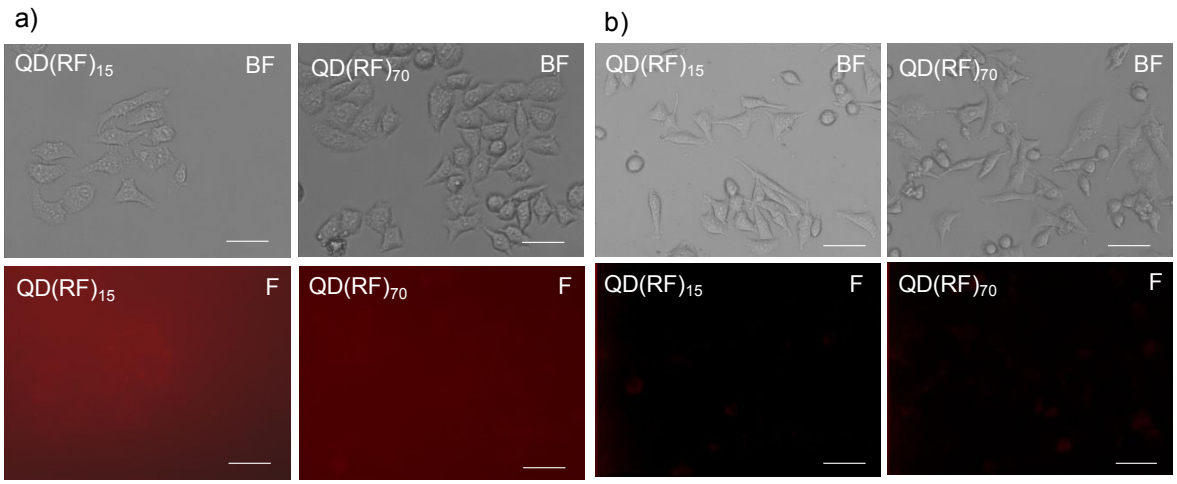


Figure S5. a) Bright field (BF) and fluorescence (F) image of $QD(RF)_{15}$/$QD(RF)_{70}$ labeled KB cells at 4 °C. Cells are incubated with QD sample for 2-3 h at 4 °C. b) Fluorescence image of KB cells pretreated with mixture of sodium azide and deoxy glucose followed by $QD(RF)_{15}$/$QD(RF)_{70}$. Insignificant QD labeling suggests that the labeling of $QD(RF)_{15}$/$QD(RF)_{70}$ occurs via energy dependent pathway. Scale bar represents 50 μm.

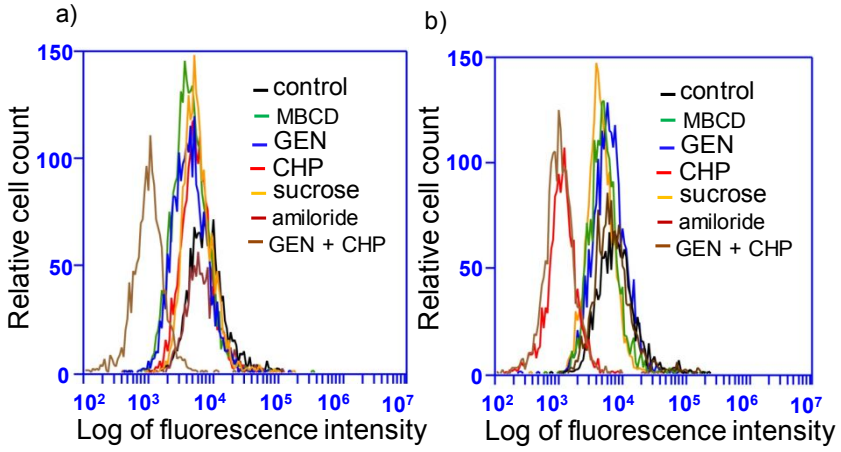


Figure S6. Flow cytometry based quantitative estimation of uptake of $QD(RF)_{15}$ (a) and $QD(RF)_{70}$ (b) in KB cells in presence of different endocytosis inhibitors. This result has been used to prepare Figure 4a and 4b.

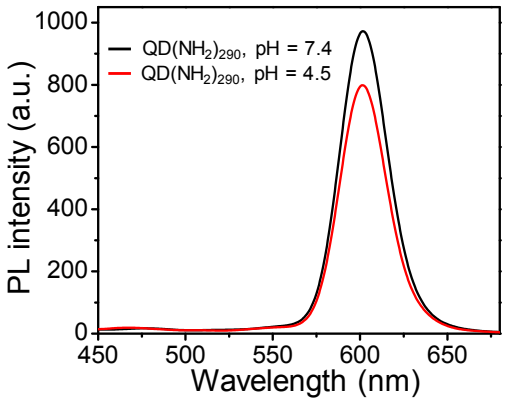


Figure S7. Fluorescence of riboflavin functionalized $QD(RF)_{15}$ and $QD(RF)_{70}$ at pH 7.4 and 4.5 taken after 15 h of preparing solution, suggesting that fluorescence of riboflavin functionalized QD is stable in both cytosol and lysosomal pH.

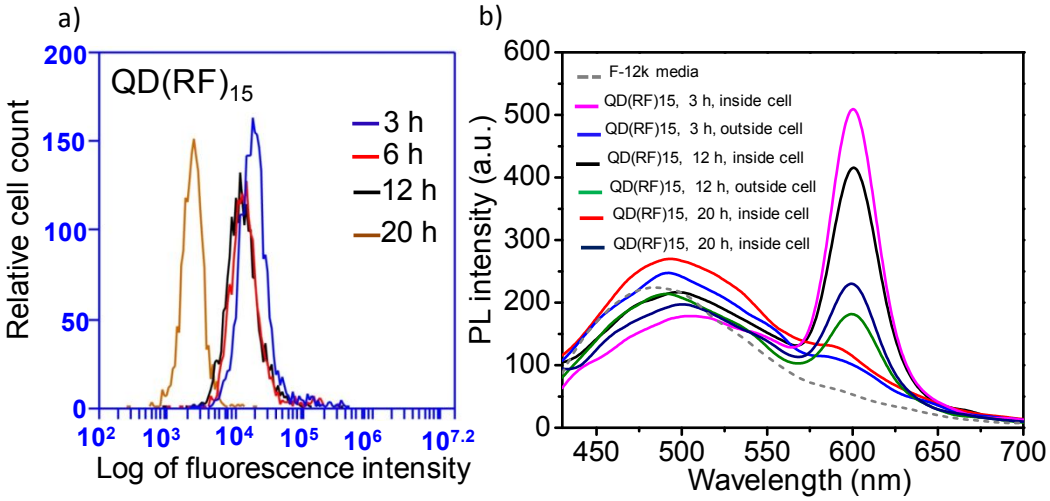


Figure S8. a) Flow cytometry based quantitative estimation of QD in KB cells at different time interval. Results show that fluorescence emission of QD from $QD(RF)_{15}$ labeled cells remain intact upto 6 h and then decreases gradually within 20 h. b) Fluorescence based quantitative estimation of QD of $QD(RF)_{15}$ inside and outside of the cell. This result has been used to prepare Figure 5c. Results show that QD fluorescence intensity decreases with time inside the cell but increases outside of the cell.

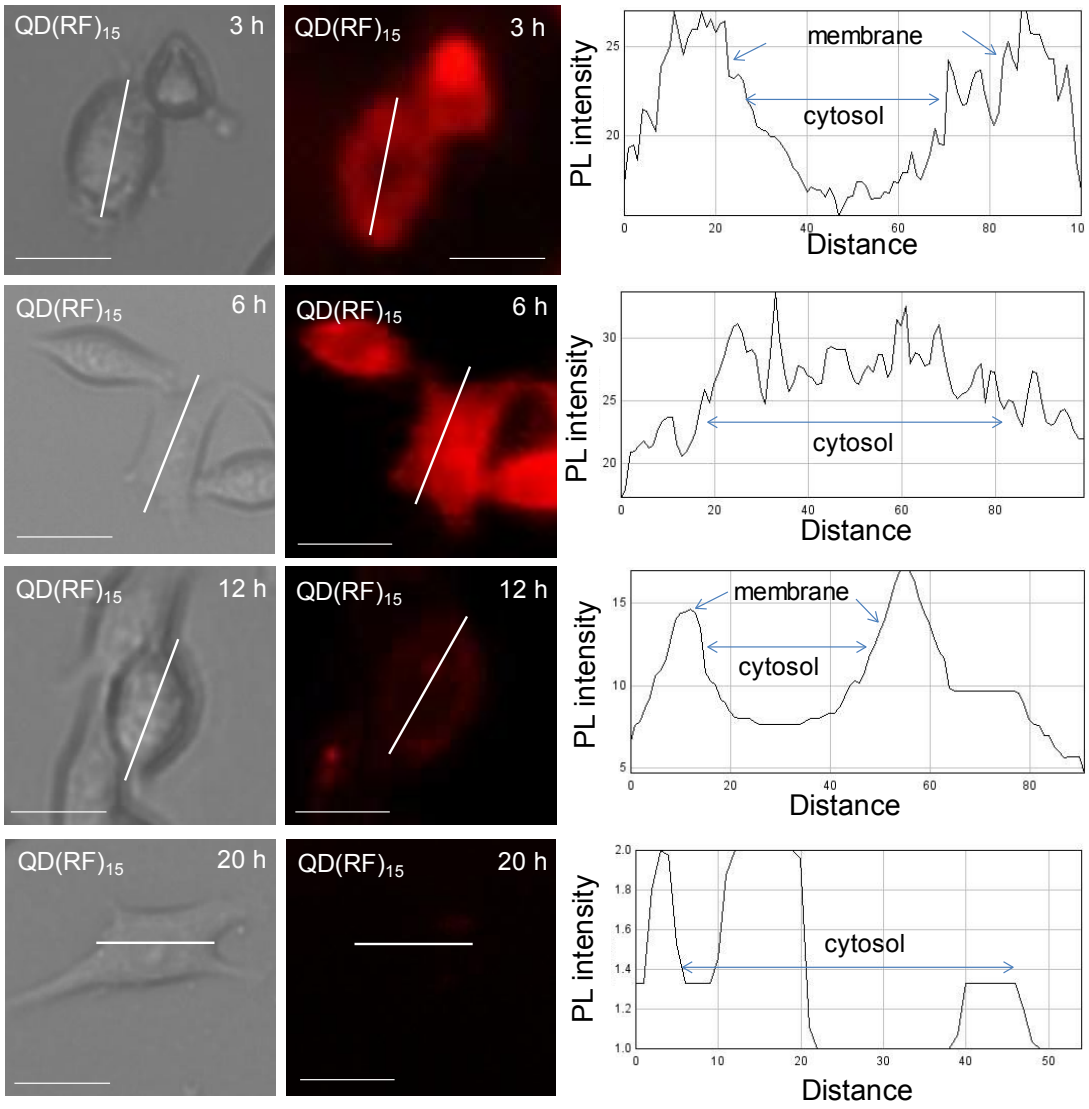


Figure S9. High magnification fluorescence images and fluorescence intensity profile diagram (using Image J software) of $QD(RF)_{15}$ labeled KB cells at different time interval. In case of $QD(RF)_{15}$, maximum fluorescence intensity comes from membrane at 3 h and at 6 h maximum fluorescence intensity comes from cytosol. After 12 h, fluorescence intensity decreases from cytosol and intensity comes from cell membrane. Fluorescence intensity becomes insignificant throughout the cell at 20 h. Results conclude that $QD(RF)_{15}$ enter into cells and stay in cytosol upto 8 h followed by exocytosized within 20 h. Scale bar represents 25 μm.

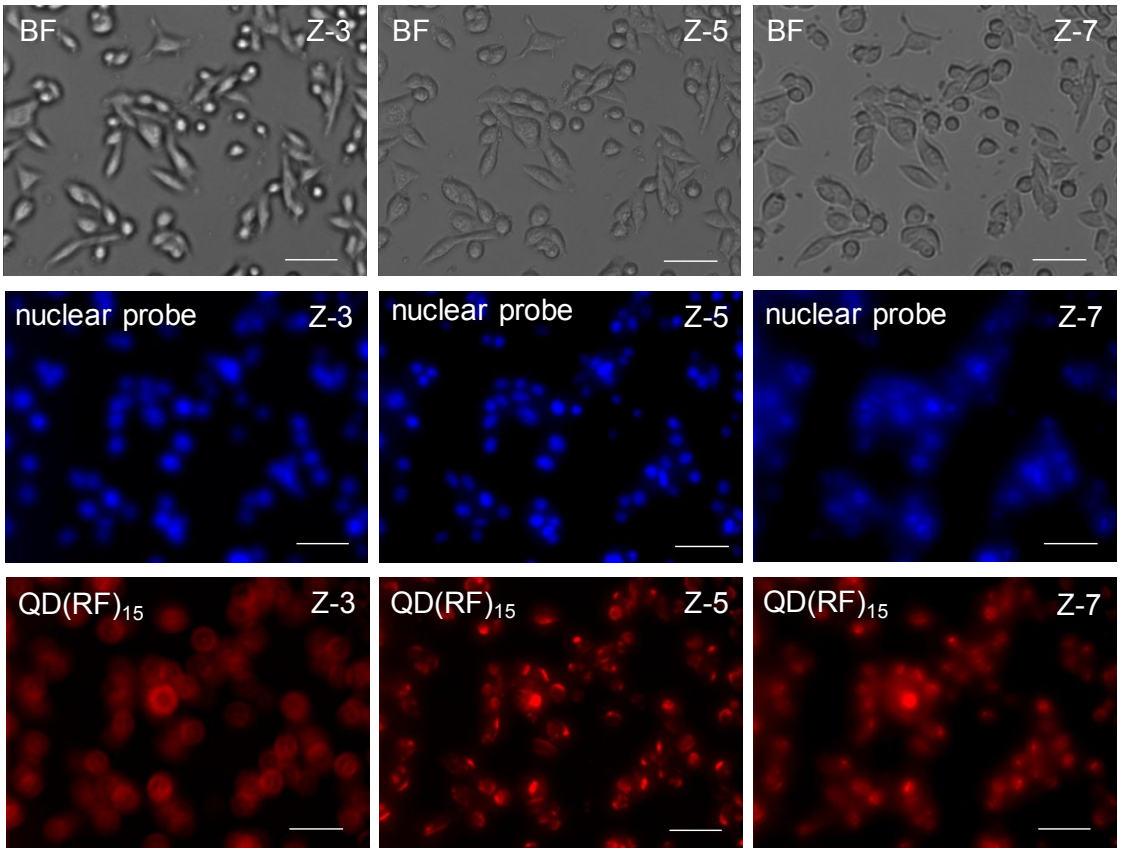


Figure S10. Fluorescence image of KB cells labeled with $QD(RF)_{15}$ and nuclear probe. Cells are imaged at different Z-planes (from top to bottom with consecutive Z-axis slices of 0.75 μm starting from Z1 to Z10). Here cells are incubated with QD sample for 3 h and washed cells are treated with fresh media and kept for next 24 h and images were taken after 8 h. From the images it is found that the emission from QD and nuclear probe comes from same Z-5 plane, suggesting that QDs and the nucleus are in the same plane. Scale bar represents 50 μm.

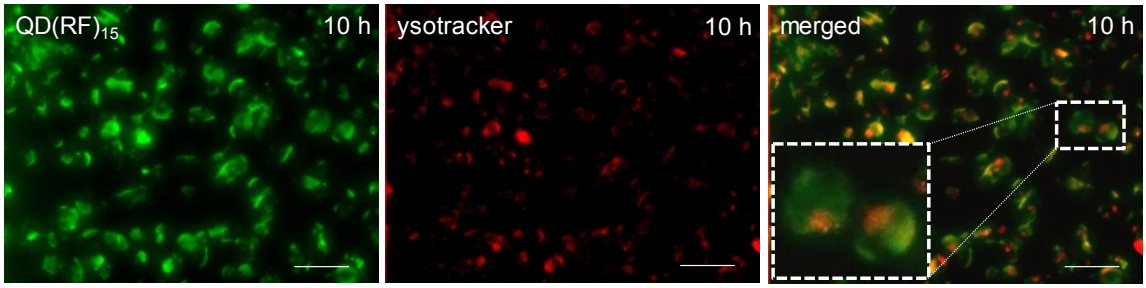


Figure S11. Colocalization study of $QD(RF)_{15}$ with lysotracker red in KB cells. Firstly cells are incubated with QD sample for 3 h and washed cells are kept for 24 h in fresh media. After treating with lysotracker red, fluorescence images are taken at 8 h under blue excitation for green fluorescent QD and green excitation for lysotracker red. Merged images shows that $QD(RF)_{15}$ is not trapped at lysosome. Red color corresponds to lysotracker and green color corresponds to QD. Scale bar represents 50 μm.

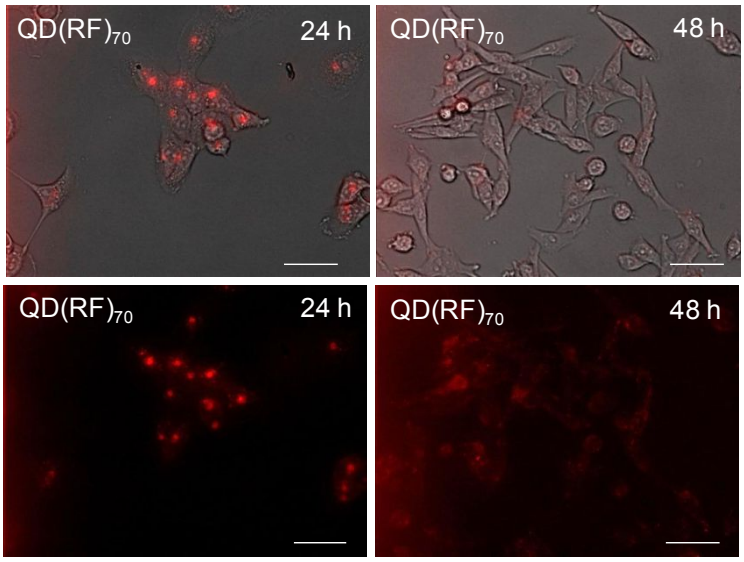


Figure S12. Fluorescence imaging study $QD(RF)_{70}$ labelled KB cells, showing the poor QD fluorescence at 48 h. This is possibly due to cell splitting. Typically KB cells are incubated with $QD(RF)_{70}$ sample for 3 h and washed cells are incubated with fresh cell culture media for 48 h and used for imaging. Scale bar represents 50 μm.

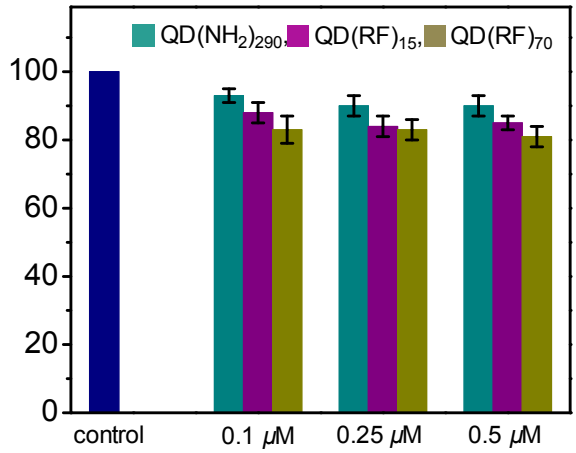


Figure S13. MTT based cytotoxicity assay of $QD(NH_2)_{290}$, $QD(RF)_{15}$ and $QD(RF)_{70}$. KB cells are incubated with QD samples for 24 h and cell viability was calculated assuming 100 % viability for cells without any nanoparticle. Results show > 80 % cell viability for all the nanoparticles in the labeling concentration range. The mean ± SD of three determinations (n = 3) are represented in bars.