Supporting Information

Microarray based Screening of Peptide Nano Probes for HER2 positive Tumor

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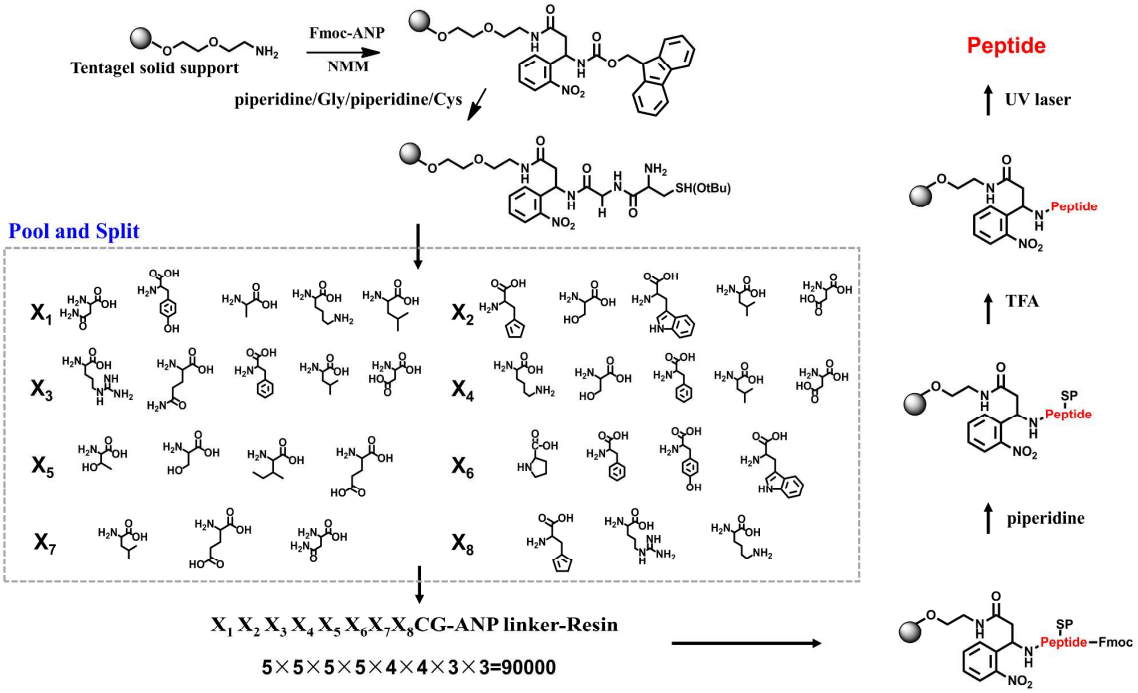
1. Synthesis process of the photo labile OBOC peptide library towards HER2

Fmoc strategy SPPS (solid phase peptide synthesis) was employed for synthesis of the OBOC library. Tentagel Resin (loading 0.53 mmol/g) was used as the solid phase support. Scheme S1 shows the synthesis process. A photo cleavable linker, ANP (3-Amino-3-(2-nitrophenyl) propionic acid) was attached on the beads in the first position of the peptide chain at C terminal. During the OBOC library synthesis, solid support beads were split equally in each cycle and different amino acids were added. After amino acid coupling using the HBTU coupling strategy, the beads were pooled together. It means that amino acid coupling process was carried out in the "split" step while the deprotection process was carried out in "pool" step. In the scheme, NMM is the abbreviation for N-Methyl morpholine, DMF is the abbreviation for N, N-dimethylformamide, TFA is the abbreviation for Trifluoroacetic acid and SP is stand for side chain protecting group. When the positive sequences were determined, peptides were *de novo* synthesized. H6 and H10 were synthesized through Fmoc strategy. The synthesized peptides were analyzed and purified by using a Hitachi HPLC (High Performance Liquid Chromatography) system (L-7100, Japan) on a TSK gel ODS-100V column (150 mm×4.6 mm) at a flow rate of 1.0 mL min^{-1}. Gradient: 0-25 min, 5-80% acetonitrile containing 0.1% TFA. The purified peptides were characterized by MALDI-TOF-MS (Bruker Daltonics).



Scheme S1. Synthesis process of the photo labile OBOC peptide library towards HER2

2. *In situ* MALDI-TOF sequencing and identification of the peptide

MALDI-TOF MS analysis was performed on a Bruker ULTRAFLEXTREME mass spectrometer (Bruker Daltonics, Germany) equipped with a nitrogen laser with reflectron and positive-ion modes. The laser power energy was adjusted between 0% and 100% to provide laser pulse energy between 0 and 100 μJ per pulse. Each mass spectrum was acquired as an

average of 500 laser shots. For *in situ* "one well one bead" analysis, TOF-TOF MS spectra were characterized by searching the MASCOT server after uploading the sequences list into the Swissport database. Representative MS/MS spectra for the single positive peptide bead were shown in Figure S1.

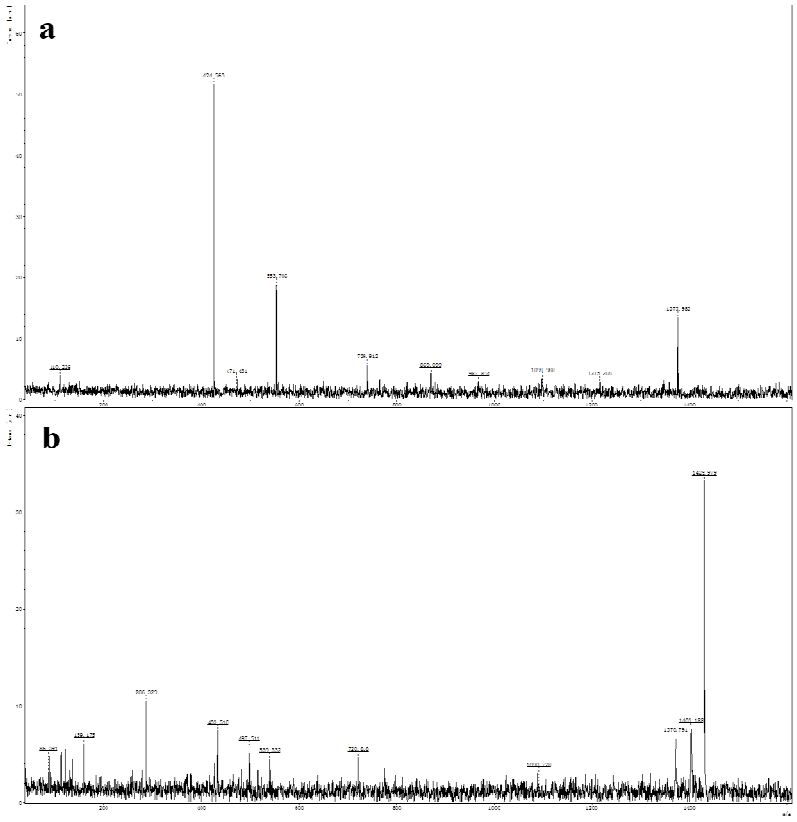


Figure S1. Representative MS/MS spectra for the positive peptide beads cleaved off from individual beads. (a) TOF-TOF MS spectrum of the H6 (b) TOF-TOF MS spectrum of the H10.

3. Flow cytometry of the FITC-labeled peptides towards HER2 positive cells

Peptide binding assays were performed by incubating SKBR-3 cells and control cells (293A cells) with FITC-labeled peptide H10 or H6. After incubation for 30 min at 37°C, the cells were washed with ice cold PBS. Flow cytometry analysis was performed, all the experiments for the binding assay were repeated 3 times. The binding rates of FITC-H6 and FITC-H10 to SKBR-3 were 69.59% and 53.32%, respectively. (Figure S2).

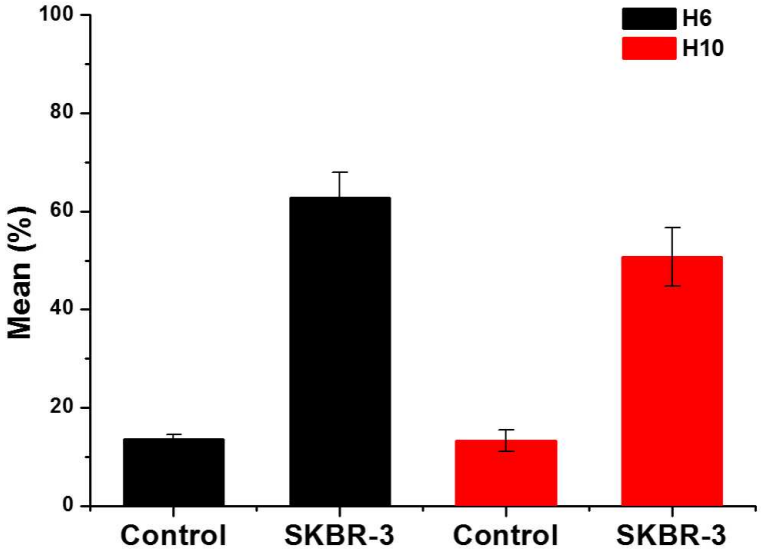


Figure S2. Binding specificities of peptides towards SKBR-3 cells were measured by flow cytometry. (Standard error of the mean were obtained from three independent experiments, n=3).

4. Quantitative RT-PCR analysis of HER2 mRNA levels in pRNAi-HER2 transfected SKBR3 cells.

Two short interfering RNA (siRNA) sequences were selected based on HER2 using siRNA design software from Invitrogen Life Technologies. PCR amplification HER2a and HER2b cDNA was inserted into vector pRNAi underwent restriction digest. The recombinant plasmids were named pRNAi-HER 2a and pRNAi-HER 2b. Then recombinant plasmids were transfected by Lipofectamine 2000 reagent (Invitrogen) into SKBR-3 cells. HER2 knocking-down levels were examined after 48 hours. Total RNA was extracted using RNAeasy kits (Qiagen). Real-time PCR (Polymerase Chain Reaction) were carried out using SYBR Green PCR Master Mix (Life Technologies). Compared with control and pRNAi, transfection with pRNAi-HER2a and pRNAi-HER2b can significantly downregulate HER2 expression of SKBR-3. The downregulate efficiencies are 31% and 43%, respectively (Figure S3). pRNAi-HER 2b showed more inhibition of HER2 expression and was selected in further cell experiments.

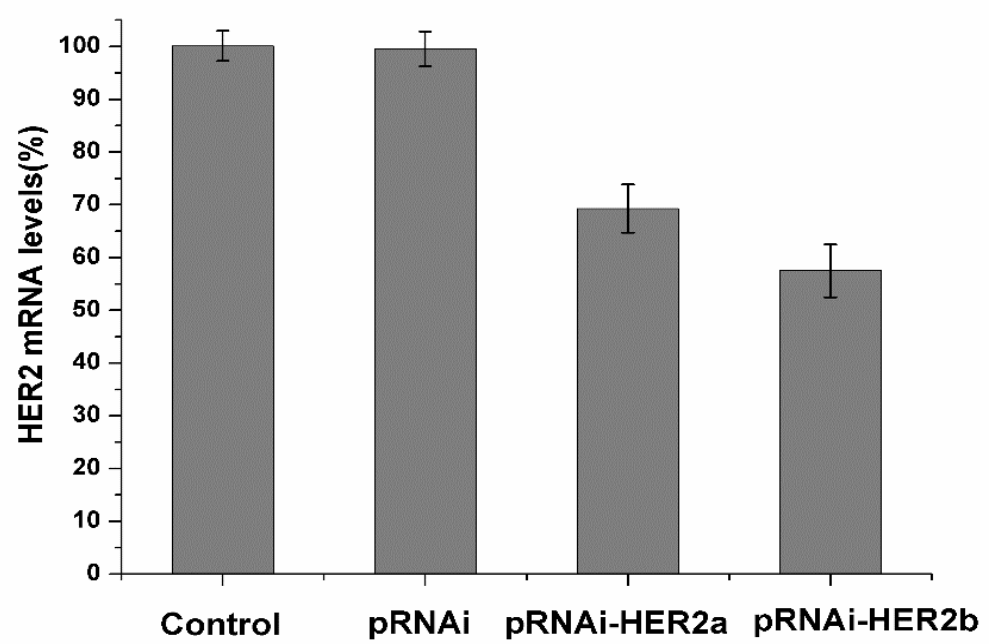


Figure S3. Quantitative RT-PCR analysis of HER2 mRNA levels in pRNAi-HER2 transfected SKBR3 cells. Control (with no plasmid), and the pRNAi vectors as mock, pRNAi-HER2a and pRNAi-HER2b (Standard error of the mean were obtained from three independent experiments, n=3)

5. SPRi detection between the peptides and the control proteins

Dissociation constants between the peptide ligands and some control proteins were also determined by SPRi under the same experiment condition. The dissociation constants were calculated from kinetic constants obtained by curve-fitting association and dissociation rates to real-time binding and washing data. Two proteins from human epidermal growth factor receptor (HER) family, EGFR (epidermal growth factor receptor, HER1) and HER3 were chosen to verify the specificity of the peptide towards HER2. Human Serum Albumin was chosen to check the non-specific absorption. As shown in Figure S4, the SPRi curves of the peptide towards the three control proteins were nearly to the 'square wave' of which the signals only increases during analyte concentration increasing, and goes back to baseline after washing. These typical curves indicated that there is no specific binding of the peptides towards the three control proteins.

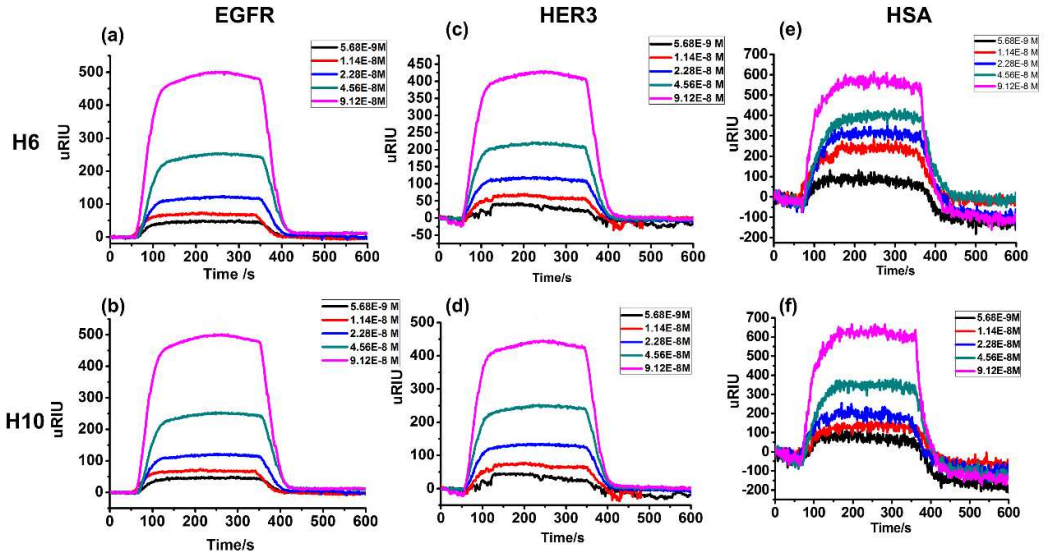


Figure S4. SPRi detection of the biding affinity of H6 and H10 towards some control proteins

6. MTT assay of the two peptides towards cancer cells

Cell growth or inhibition was measured by MTT assay, 5×10^3 SKBR-3 cells were seeded overnight in each well of a 96-well plate in RPMI-1640 medium. Various concentrations of H6 and H10 were added in each well for 48 hours followed by the adding of MTT solution for 4 hours at 37 °C. The solution was removed and 200 μL of dimethyl sulfoxide (DMSO) was added to each well. After 10 minutes of vibration mixing, the optical density (OD) at 570 nm was measured using an ELISA reader. The survival rates of SKBR-3 cells exposed to H6 and H10 at concentrations ranging from 0.1 μM to 100 μM were close to 100% (Figure S5). It is indicated that peptides show the good biocompatibilities.

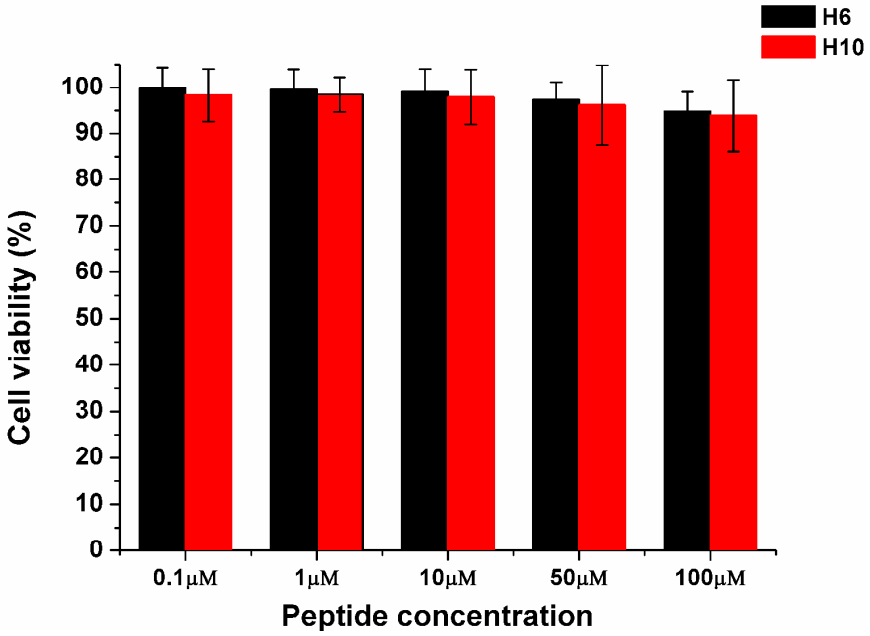


Figure S5. Activities of H6 and H10 peptides on SKBR-3 cell growth were measured by MTT assay (standard error of the mean were obtained from three independent experiments, n=3)

7. Half-lives determination of the two peptides

Half-lives determination were carried out following the literature.[1] Japanese rabbits were used as the animal model. Peptide probes were injected into the rabbits through the left ear vein at the dose of 5 mg/kg. Blood was collected from the right ear at the time of 15, 30, 60, 120 and 240 min. The blood samples were processed and the serums were separated. The serum samples at different periods were measured by high performance liquid chromatography (HPLC). Peptide concentrations in the serum were calculated by the integration of the peak areas of the chromatogram.

One compartment open model[2] was chosen and half-lives of the two peptides were calculated from the formula $\ln C = -kt+\ln C_0$. The plasma half-lives of the peptides were determined as 17 min of H6 and 13 min of H10 (Figure S6). Since shorter half-life, peptide H10 cleared faster than H6 in blood flow.

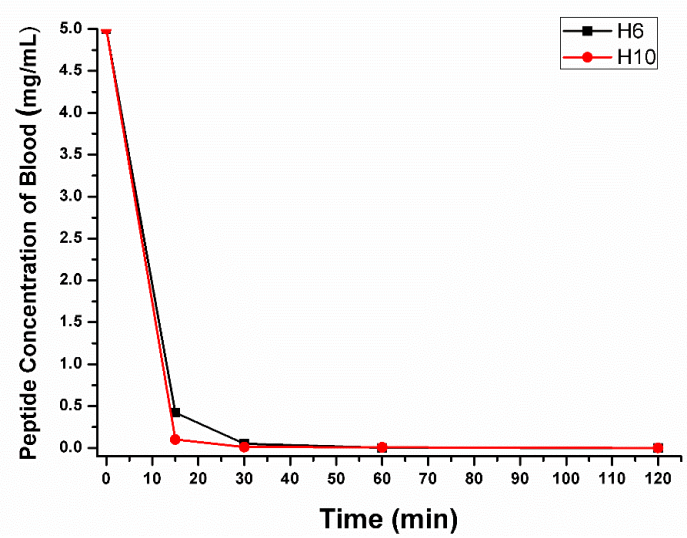


Figure S6. Distribution of peptide concentration of blood in rabbits at different time

8. MTT assay of the peptide-QDs compared with QDs only towards cancer cells

To examine the biocompatibility and toxicity of peptide-QDs, MTT assay was used. 5×10^3 SKBR-3 cells were seeded overnight in each well of a 96-well plate (RPMI-1640 medium contained). QDs or peptide-QDs were added with a series of concentrations for 48 hours. The MTT solution was added to each well and the plate was incubated for 4 hours at 37°C. The solution was removed and 150 μL of dimethyl sulfoxide (DMSO) was added to each well. After 10 minutes of vibration mixing, the optical density (OD) at 570 nm was measured using an ELISA reader. Compared with QDs, the survival rates of SKBR-3 cells add peptide-QDs have obviously increased. Especially, under the condition of 1 μM peptide-QDs for 48 hours, the cell survival rate is above 50% (Figure S7). It is suggested that peptide modification could further reduce the toxicity of QDs and enhance the biocompatibility of the QDs.

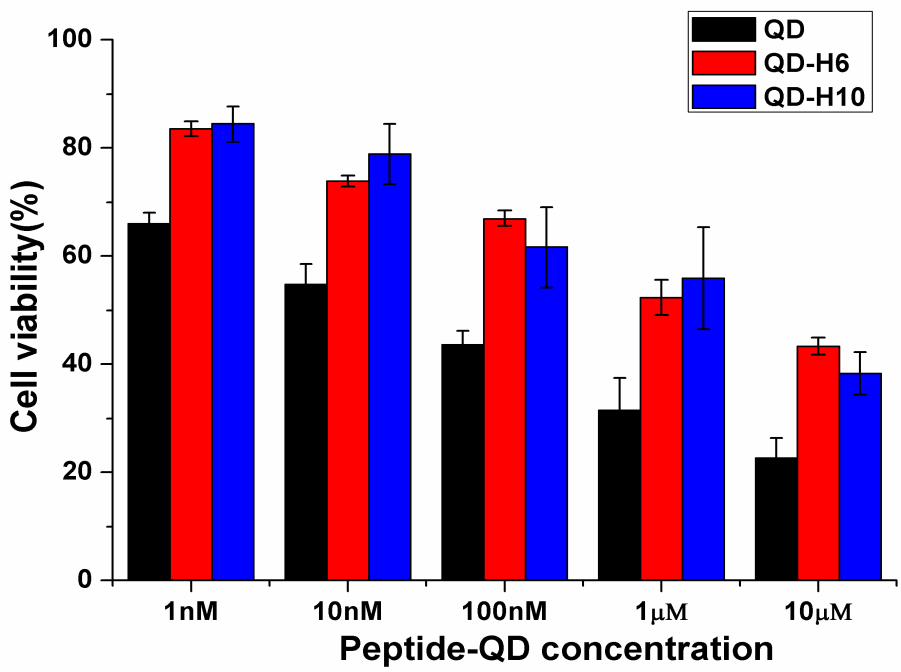


Figure S7. Activity of H6-QDs and H10-QDs on SKBR-3 compared with QDs only on cell growth measured by MTT assay (standard error of the mean were obtained from three independent experiments, n=3)

9. MTT assay of the two peptides towards normal cells

The experiment aims to test whether the peptide-QDs would affect other kinds of cells. Three types of non-cancer cells were chosen: human embryonic kidney (HEK) 293 cells, human umbilical vein endothelial cell (HUVEC) and standard fibroblast cell 3T3 cells. MTT assays were used to examine the cell toxicity of the peptide-QDs, 5×10^3 cells were seeded overnight in each well of a 96-well plate in medium. Then various concentrations of peptide-QDs were added for 48 hours. The MTT solution was added to each well and the plate was incubated for 4 hours at 37°C. The solution was removed and 150 μL of dimethyl sulfoxide (DMSO) was added to each well. After 10 minutes of vibration mixing, OD at 570 nm was measured using an ELISA reader. After 48 hours, the cell survival rate is approximately 80%. It is indicated that the peptide-QDs have little effect on normal cells. The toxicity of the peptide-QDs was lowered and the biocompatibility was increased.[3-5]

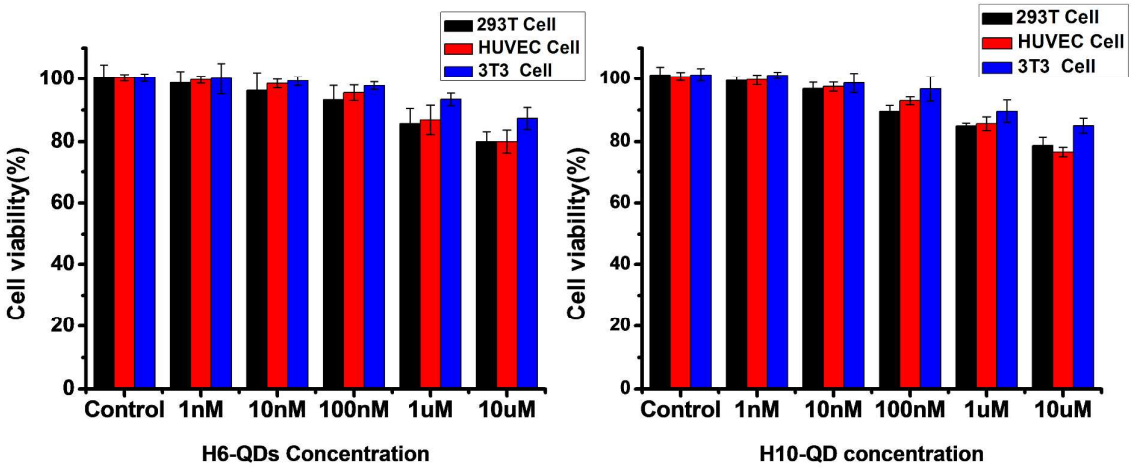


Figure S8. MTT assay of H6-QDs and H10-QDs towards normal cells (standard error of the mean were obtained from three independent experiments, n=3)

References

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