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Supporting Information

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Targeted Delivery of siRNA-Generating DNA Nanocassettes Using Multifunctional Nanoparticles

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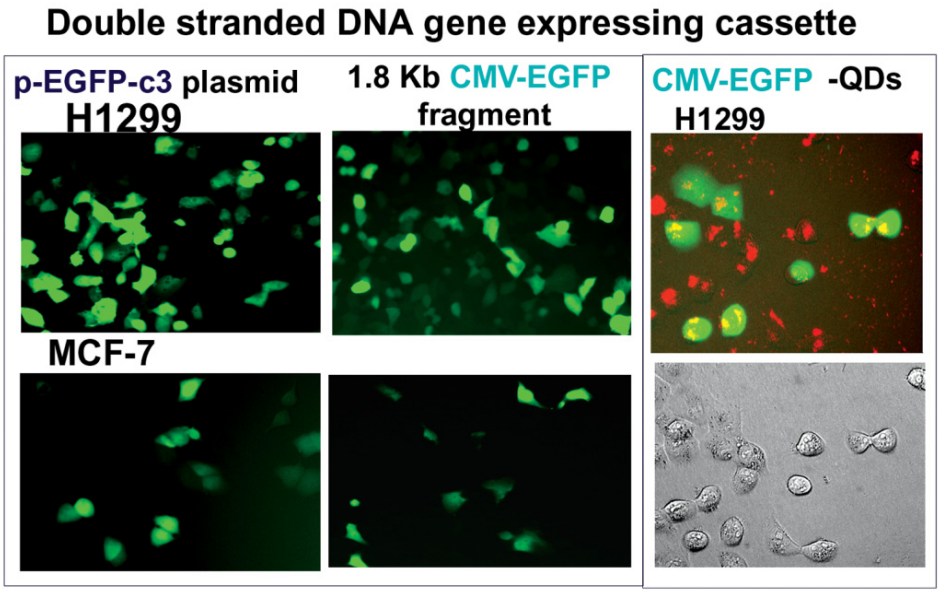
Supplemental Information

DNA-nanocassettes can efficiently express the EGFP gene following transfection into cells

To determine the feasibility and efficiency of gene expression from the DNA-nanocassettes after being delivered into cells, 1.8 Kb PCR fragments containing a cytomegalovirus (CMV)-promoter and an EGFP marker gene were amplified from pEGFP-c3 plasmid (Clontech Laboratories, Inc, Mountain View, CA) using forward primer 5'-CACATGTTCTTTCCTGCGTTA-3' and reverse primer 5'-CGATTTCGGCCTATTGGTTAA-3' modified with 5'-end amine and phosphorothioate. The EGFP gene expressing nanocassettes were further conjugated to QDs using EDAC mediated reaction. pEGFP-c3 plasmid and EGFP DNA nanocassettes, with or without conjugated to QDs, were transfected into a human lung cancer H1299 and breast cancer MCF-7 cell lines using Lipofectamine 2000 (Invitrogen). Cells were then examined under an inverted fluorescence microscope (Olympus America Inc). A strong fluorescence signal was detected in tumor cells 48 h following transfection. The intensity of GFP positive signal in CMV-EGFP DNA nanocassette-transfected group is slightly lower than that of p-EGFP-plasmid transfected group (**Supplemental Figure 1**). We also found that the conjugation of DNA nanocassettes to QDs retained the ability of the nanocassettes to express the GFP gene in tumor cells (**Supplemental Figure 1**).



Supplemental Figure 1. Determination of the level of gene expression using the DNA gene expressing nanocassettes.

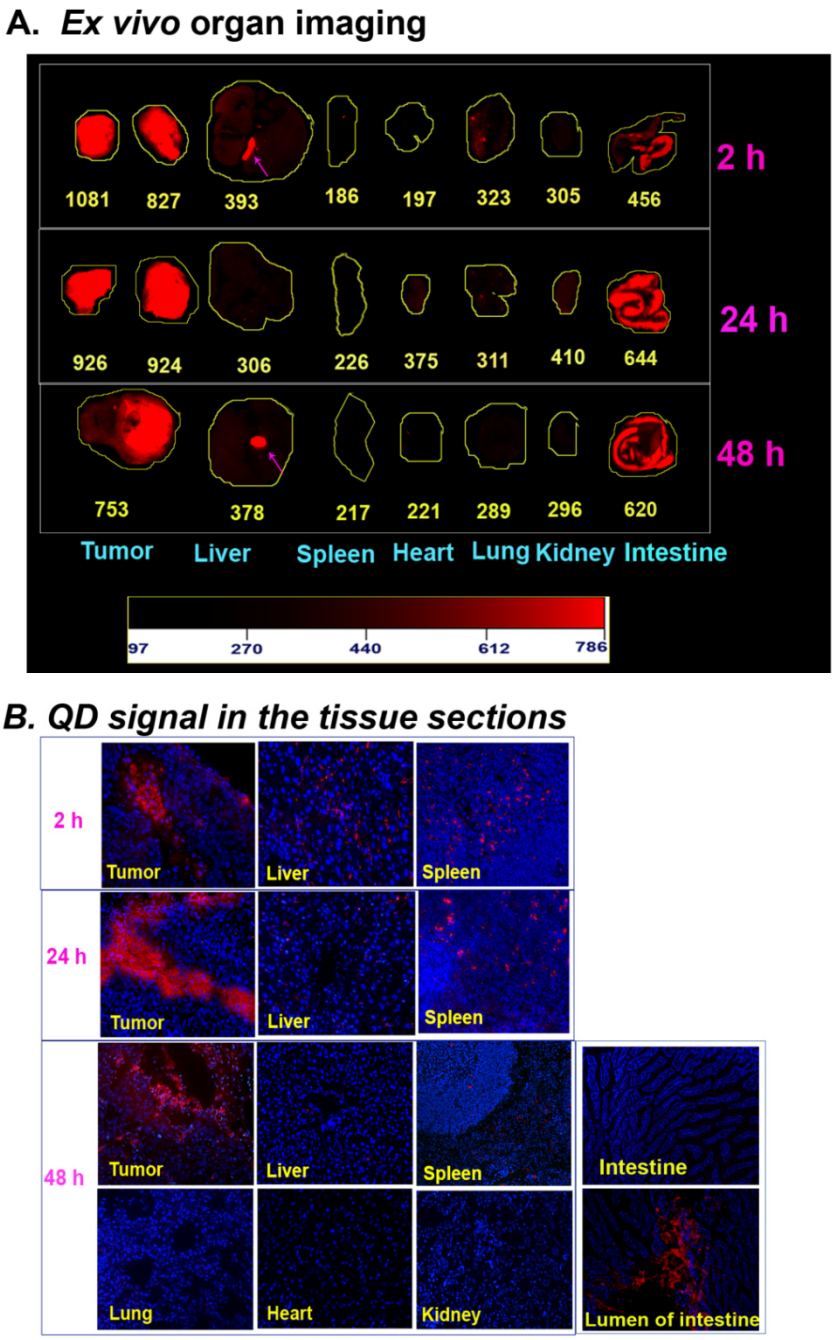
Human lung cancer H1299 and breast cancer MCF-7 cell lines were cultured on 24-well plates for 24 h and then transfected with pEGFP-c3 plasmids, CMV-EGFP DNA fragments or CMV-EGFP-QDs (1 μg of DNA). At 48 h after transfection, cells were examined under an inverted fluorescence microscope. GFP (green); QDs (red). The bright field image was the same area as the dual fluorescent image of GFP and QDs.

Determination of targeted delivery and biodistribution of the uPAR-targeted nanoparticles carrying Luc siRNA nanocassettes in a human breast cancer xenograft model.

Biodistributions of the uPAR-targeted nanoparticle siRNA carriers were examined in an orthotopic primary human breast cancer xenograft model in nude mice. At 2, 24, and 48 h following the tail vein delivery of 200 pmol of hATF-QDs-Luc siRNA, the mice were sacrificed and tumors and normal organs were collected for optical imaging (**Supplemental Figure 2**). Strong QD signals were detected in the tumors as early as 2 h after the nanoparticle injection and lasted over 48 h. However, very low levels of the QD signal were detected in the liver, spleen, kidney, lung and heart (**Figure 2 A**). Histological examination of the tumor and normal tissue

sections further confirmed the results of *ex vivo* imaging. QDs were detected in the tumor cell and tumor stromal areas 2 h following the nanoparticle delivery. At 24 h, larger clusters of QD positive tumor and stromal cells were detected in the tumors (**Figure 2 B**). By 48 h, QDs diffused into larger tumor areas and the signal intensity was reduced. In normal tissue sections, intermediate levels of QDs were only detected in the liver and spleen 2 hr following the nanoparticle administration. The QD signals were markedly reduced 48 h after the nanoparticle injection (**Figure 2 B**). However, QD signal was not detected in the lung, kidney and heart in the mice that received the targeted nanoparticles (**Figure 2B**).

In the *ex vivo* imaging, we also detected a high level of QDs in the gallbladder (pink arrows), suggesting that QDs are eliminated from the liver and bile (**Figure 2 A**). Supporting this notion, we found strong QD signals in the intestines 2 to 48 h following the nanoparticle delivery (**Figure 2 A**). In the tissue sections, QDs were only detected in the lumen of the intestines but not in the intestinal mucosa (**Figure 2B**).



Supplemental Figure 2 Selective tumor targeting and biodistribution of the uPAR-targeted QDs carrying Luc siRNA in a human breast cancer xenograft model

A. *Ex vivo* optical imaging of tumor and normal organs 2, 24, and 48 h following the tail vein injection of 200 pmol of hATF-QDs-Luc siRNA nanocassettes. Kodak FX *in vivo* imaging system equipped with a filter set of Ex 465 and Em 620 nm was used for optical imaging. Pink arrows: QD signal in the gallbladder. Numbers shown are the mean fluorescence signal intensity of the organ.

B. Fluorescence microscopic examination of biodistribution of QDs in frozen tissue sections. Red: QDs. Blue: DAPI counterstaining.