

## Supporting Information

Controlled Organization of Inorganic Materials Using Biological Molecules for Activating Therapeutic Functionalities

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## Sequences

DNA strands were designed to form RNA/DNA hybrids with sense and antisense strands of Dicer Substrate RNAs (DS RNAs) selected against Green Fluorescent Protein. Once formed, those hybrids have single-stranded DNA toeholds (underlined) which are designed to interact with each other to initiate branch migration.

DNA for Sense\_12\_Biotin

5'-/5Biosg/GGAGACCGTGACCGGTGGTGCAGATGAACTTCAGGGTCA

DNA for Antisense\_12\_Biotin

5'-/5Biosg/TGACCCTGAAGTTCATCTGCACCACCGGTCACGGTCTCC

RNA Sense

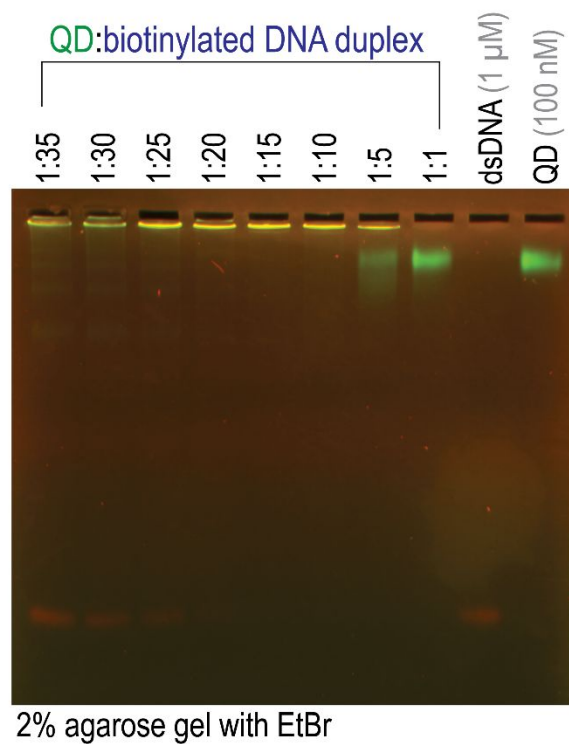
5'-/5Phos/ACCCUGAAGUUCAUCUGCACCACCG

RNA Antisense

5'-CGGUGGUGCAGAUGAACUUCAGGGUCA

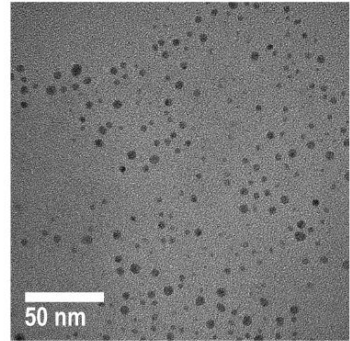
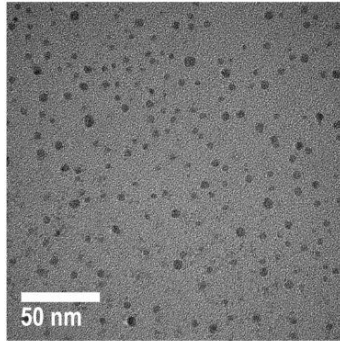
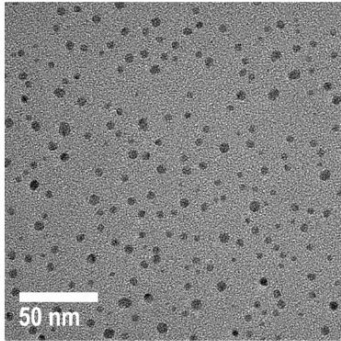
Beamline	11-BM CMS
Photon Energy (keV)	13.5
Horizontal × Vertical Beam size ( $\mu\text{m} \times \mu\text{m}$ )	200 x 200
Approximate Flux (photons/sec)	$10^{11}$
Sample-to-Detector Distance (m)	5.05
Detector Manufacturer	Dectris
Detector Model	Pilatus 1M
Detector Pixel Size ( $\mu\text{m} \times \mu\text{m}$ )	172 x 172

**Table S1.** CMS Beamline Experimental Setup

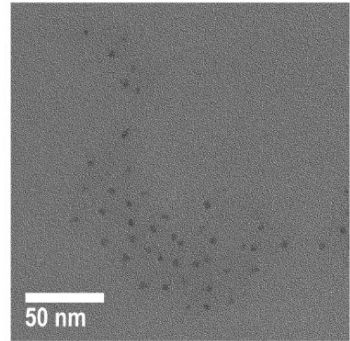
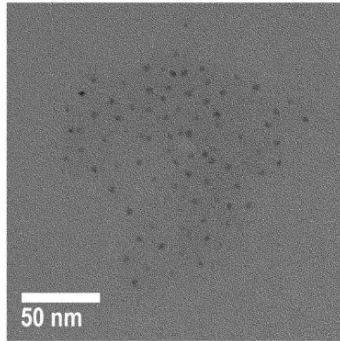
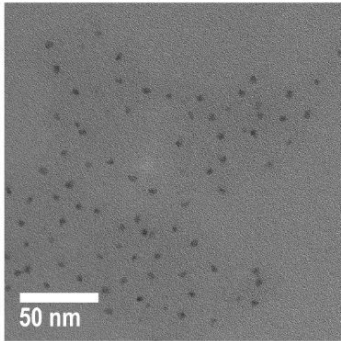


**Figure S1.** Binding assay of QD545 with increasing concentrations of biotinylated DNA duplexes used to confirm the number of biotin binding sites per QD required for assembly. QD is 100 nM in all conditions. The band corresponding to excess dsDNA becomes faintly visible beginning at the 1:15 ratio, so a 1:10 ratio was used for all QD:nucleic acid assemblies.

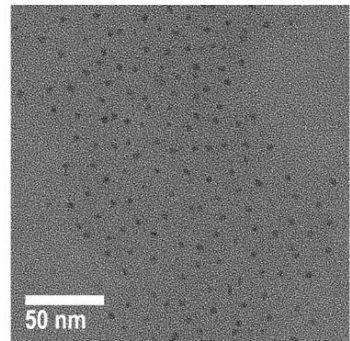
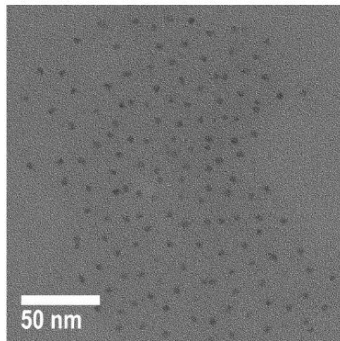
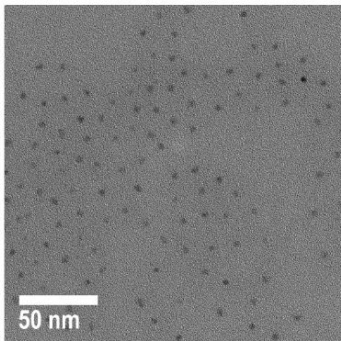
**A** QD + dsDNA



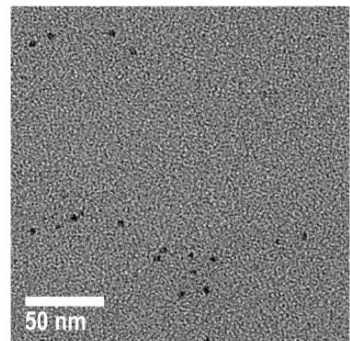
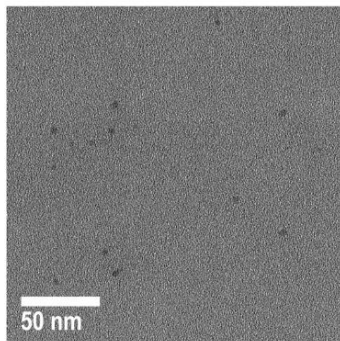
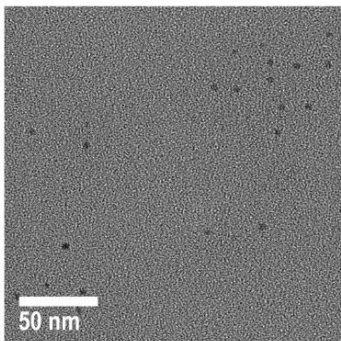
**B** (QD-ssDNA1)+(QD-ssDNA2)



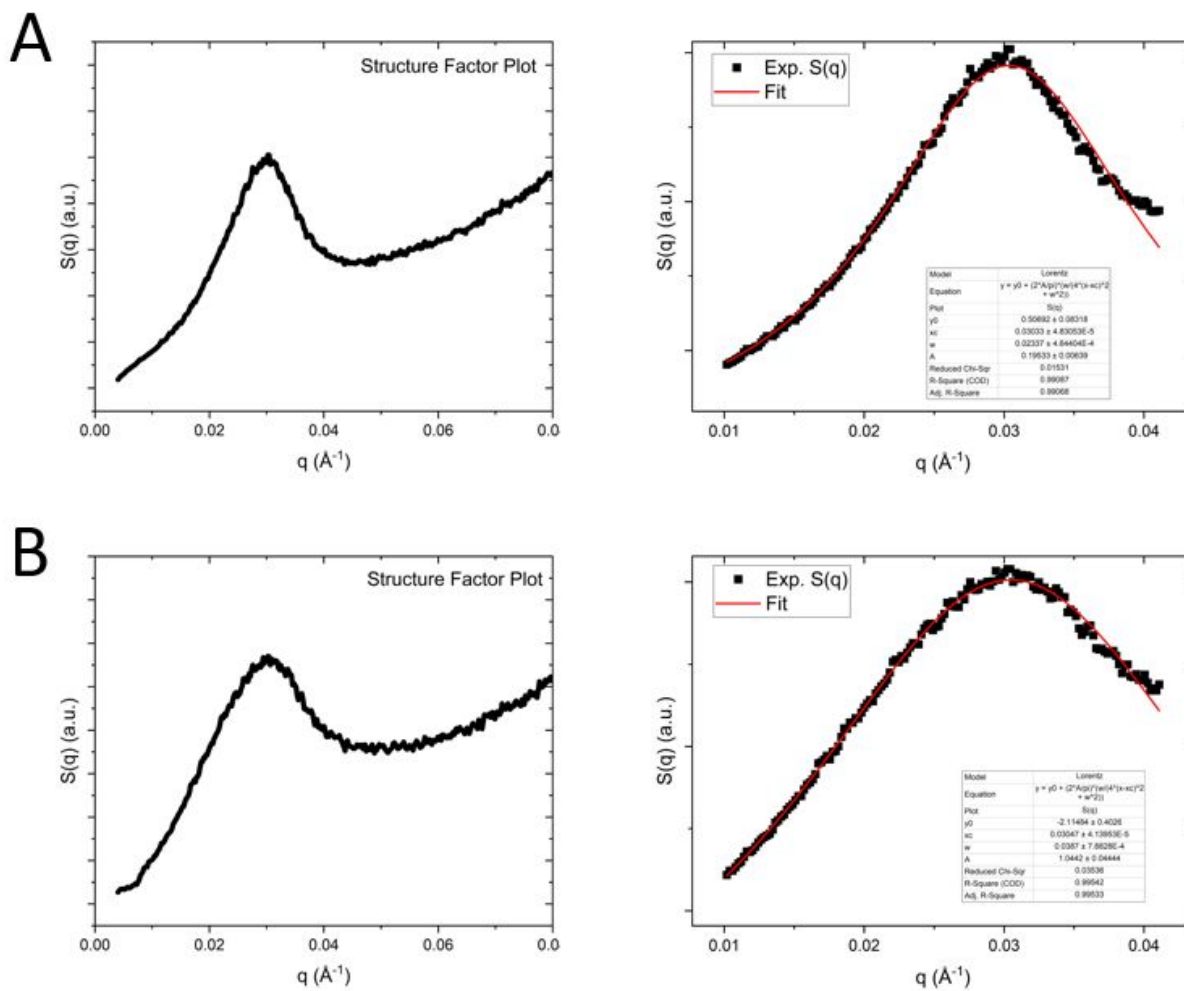
**C** (QD-Hybrid1)+(QD-Hybrid2)



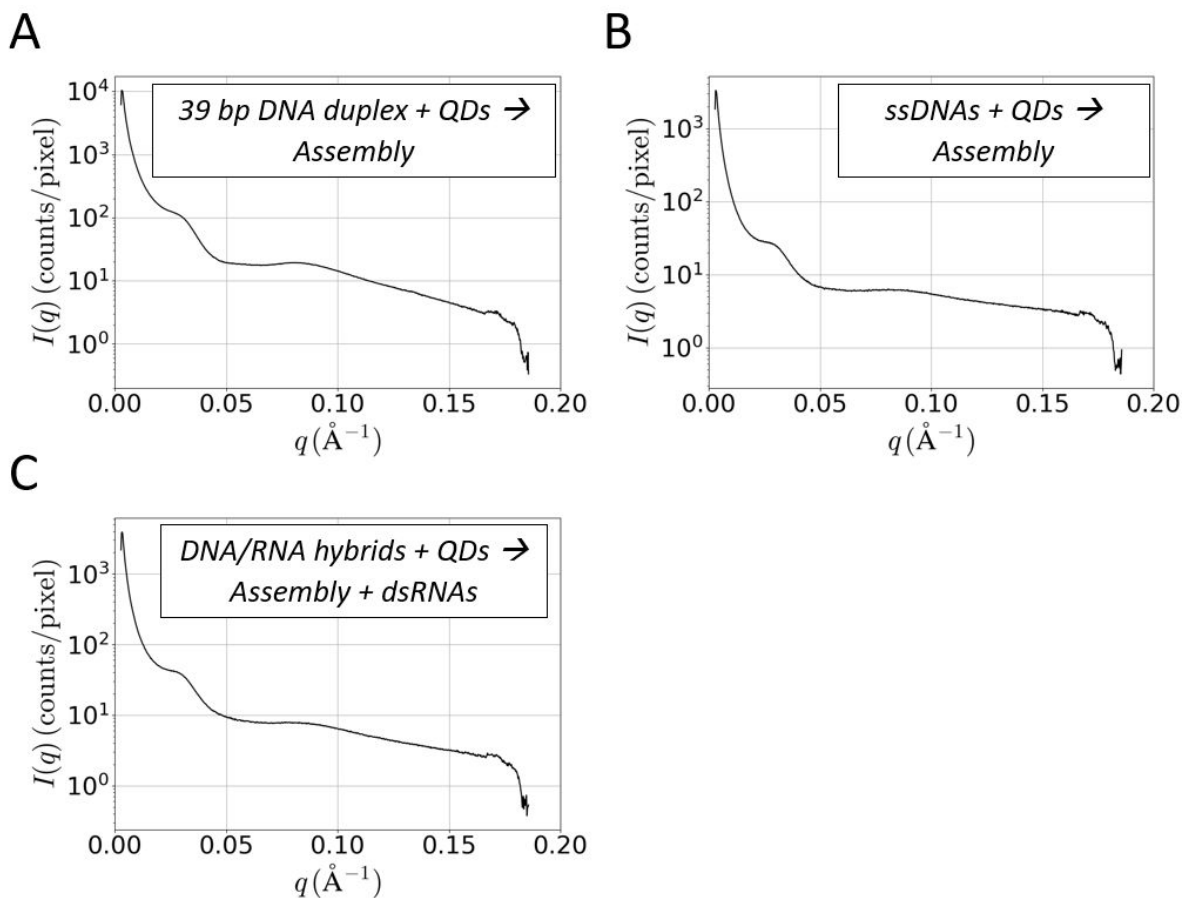
**D** Free QDs



**Figure S2.** Three representative TEM images were used for calculations of center-to-center distances in ImageJ. Assemblies were formed via (A) QD+dsDNA, (B) (QD-ssDNA1)+(QD-ssDNA2), and (C) (QD-Hybrid1)+(QD-Hybrid2). (D) Free QDs were used as a control.



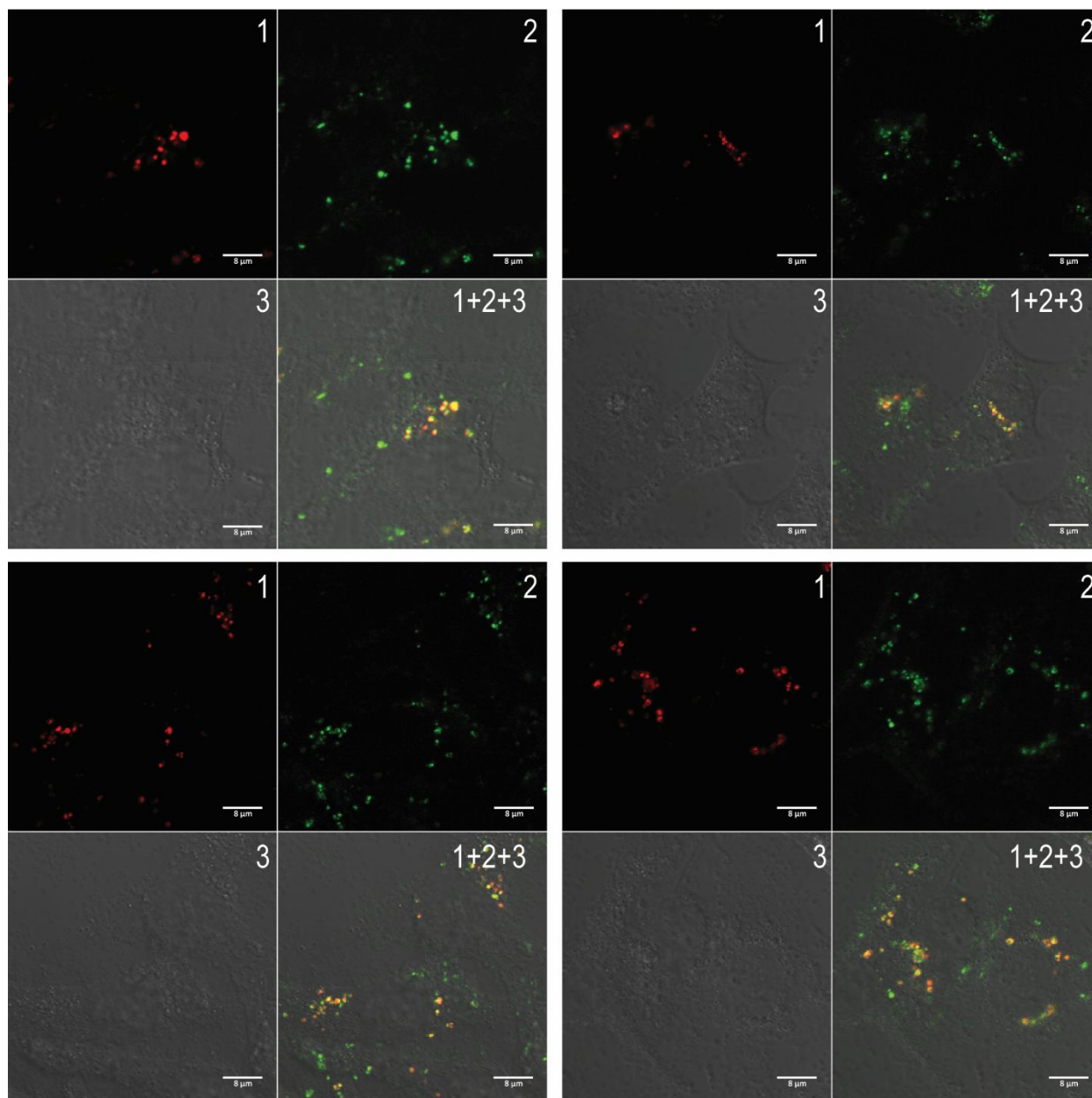
**Figure S3.** Additional SAXS analysis showing the Structure Factor Plot and Lorentzian Fit of two QD assemblies: (A) QD+dsDNA and (B) (QD-Hybrid1)+(QD-Hybrid2).



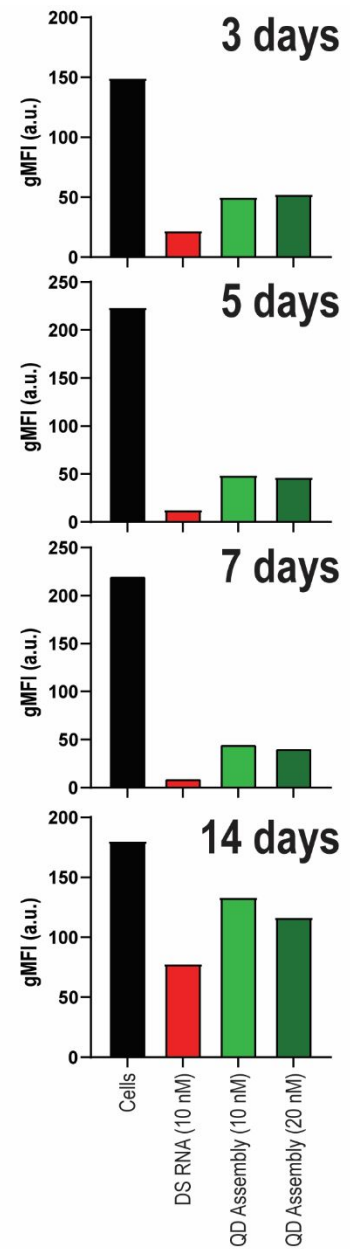
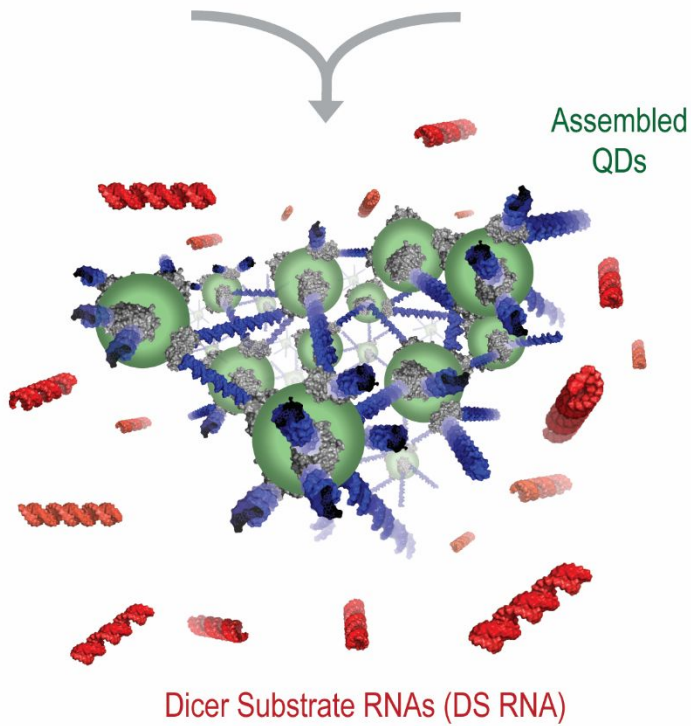
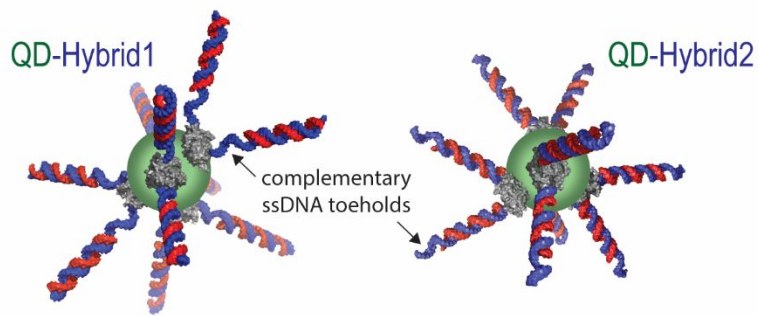
**Figure S4.** Intensity profiles,  $I(q)$ , for each of the system designs described in Figures 1 and 2: **(A)** QDs mixed with double-biotinylated DNA duplexes, **(B)** QDs decorated with complementary ssDNA, and **(C)** QDs decorated with DNA/RNA hybrids that re-associate via the complementary ssDNA toehold interaction and release DS RNAs.



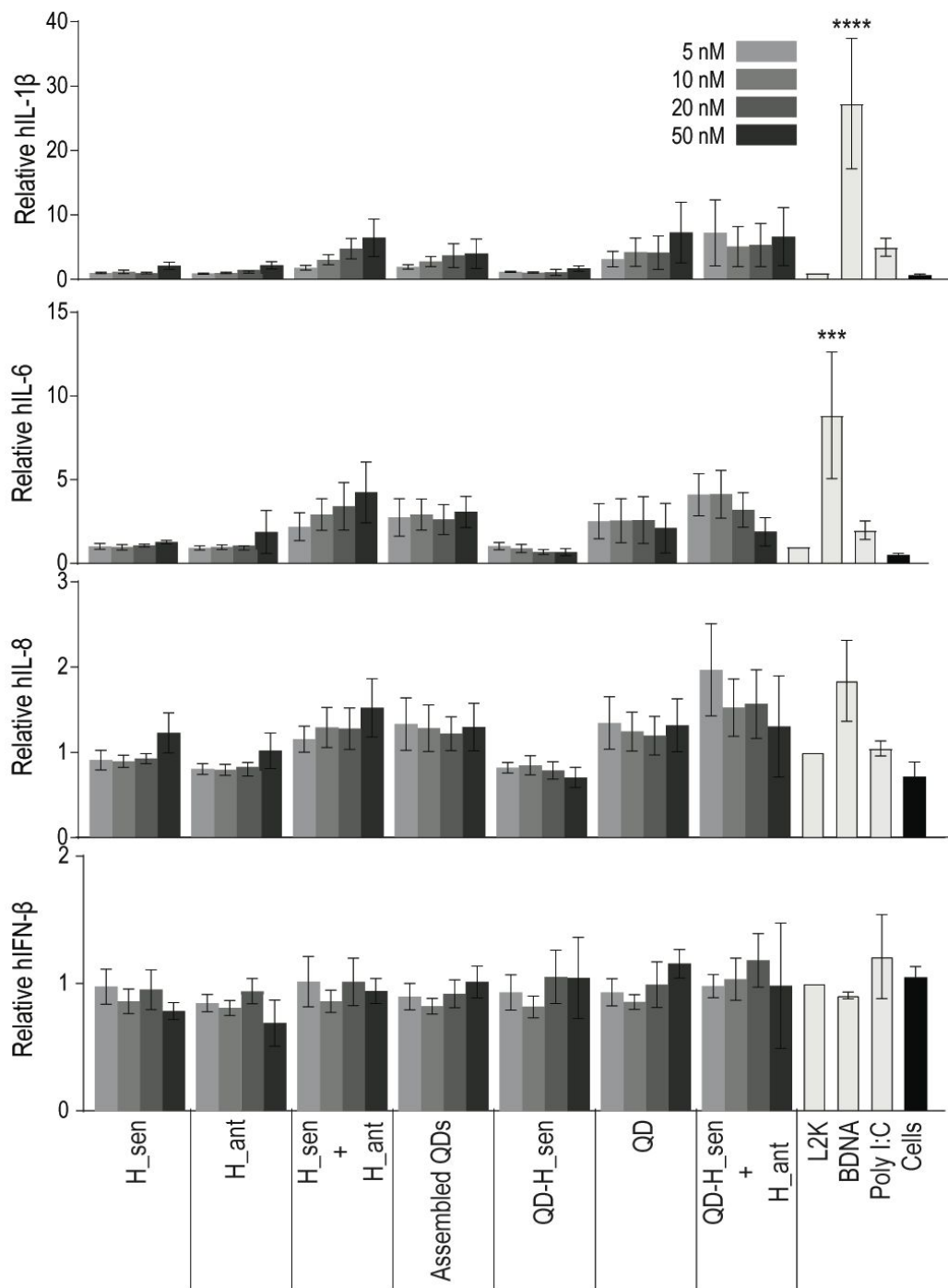




**Figure S5.** Co-localization of QD545 (green) and QD605 (red) entering the composition of intracellularly assembled QDs analyzed by confocal microscopy. Image numbers correspond to: (1) QD605 emission, (2) QD545 emission, and (3) differential interference contrast (DIC) images. Images (1+2+3) are the superposition of three different images. Scale = 8  $\mu\text{m}$ .

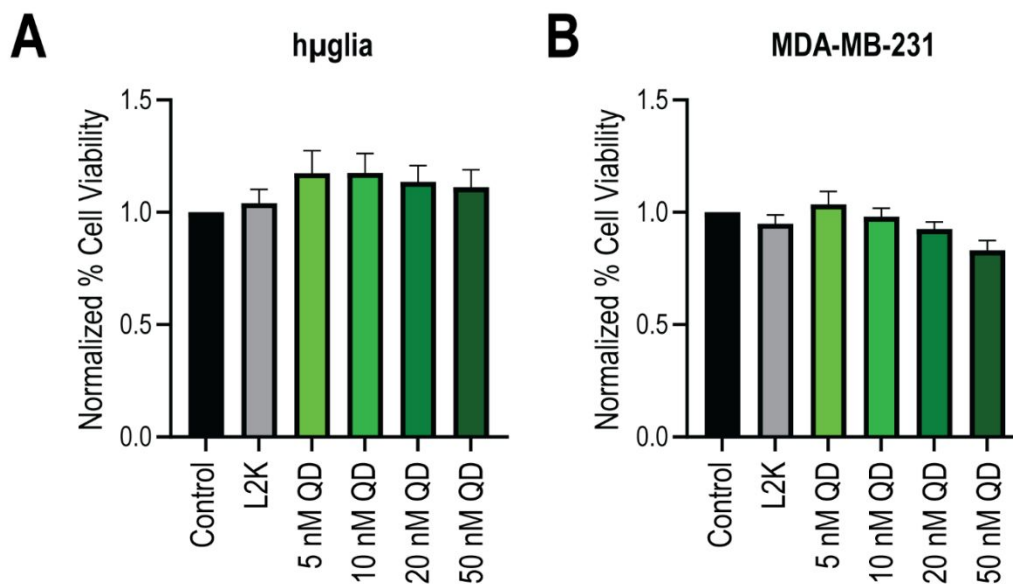


**Figure S6.** Activation of RNAi through QD assembly and release of DS RNAs using GFP knockdown assays for human breast cancer cells expressing GFP. Three, five, seven, and fourteen days after the co-transfection of cells with hybrid-functionalized QDs, GFP expression was analyzed with flow cytometry. As a control, transfections with the pre-formed DS RNA duplexes against GFP were used. gMFI corresponds to the geometric mean fluorescence intensity. Bars denote mean  $\pm$  SEM of n=20,000 individual events.

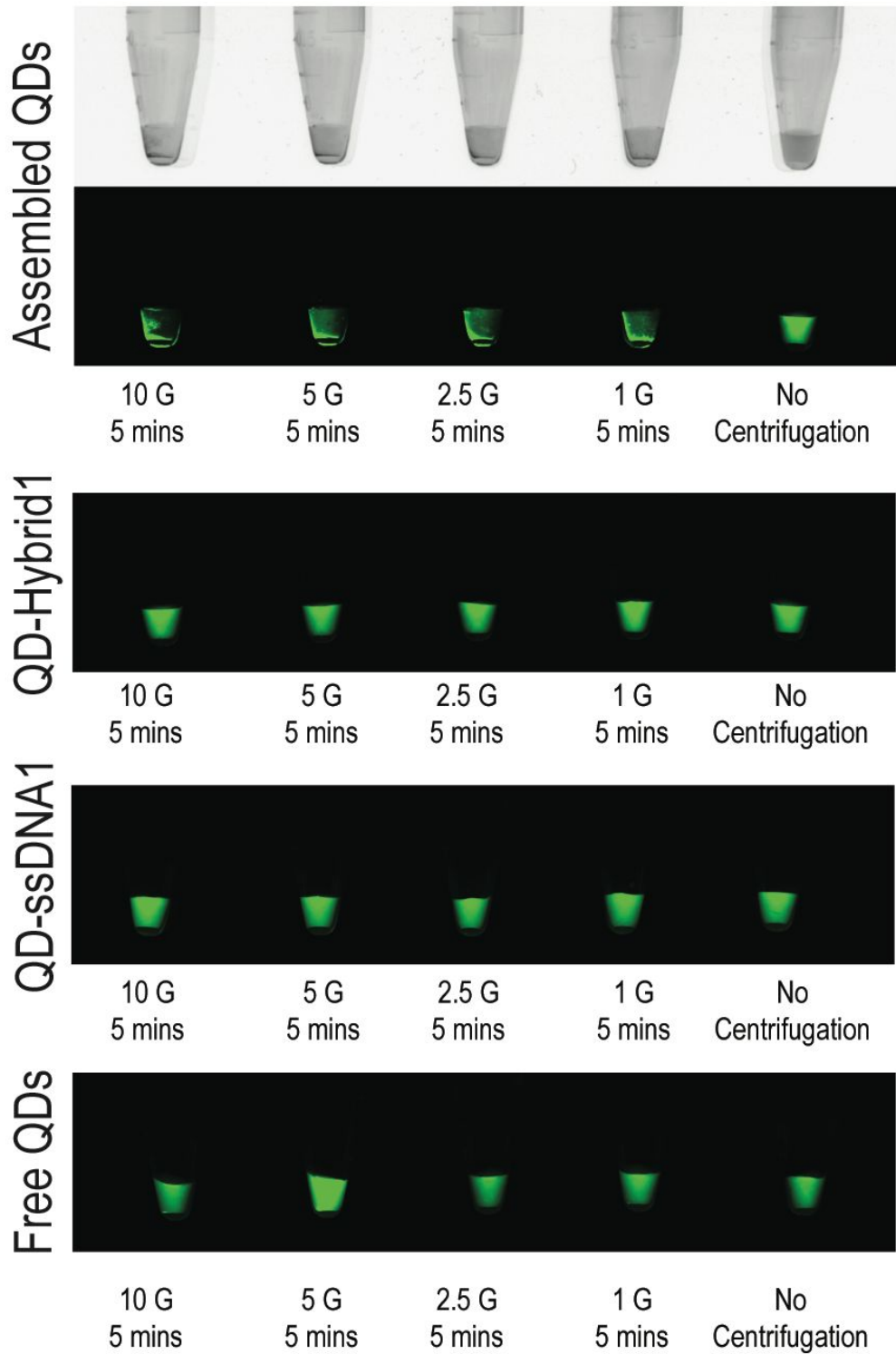


**Figure S7.** Immunostimulatory activity of QD assemblies in the human astrocyte-like cell line U87-MB. Cells were transfected and cell supernatants were collected 24 hours later. Levels of hIL-1 $\beta$ , hIL-6, hIL-8, and hIFN- $\beta$  were assessed by specific-capture ELISA. Bars denote mean  $\pm$  SEM

of n=3 independent repeats. Statistically significant results are indicated with asterisks (\*\*\*\* =  $P$ -value < 0.0001, \*\*\* =  $P$ -value < 0.001)



**Figure S8.** Cell viability assays of QDs in (A) hμglia and (B) MDA-MB-231 cell lines after 24 hours. Bars denote means  $\pm$  SEM of n=4 independent repeats for control (cells-only), L2K, and 5-20 nM QD and n=3 independent repeats for 50 nM QD.



**Figure S9.** Precipitation of QD assemblies. Assembled QDs were centrifuged for 5 minutes at different speeds and show the formation of a solid pellet in the bottom of the tube. For all other QD samples which are not assembled, no precipitation is observed.