Supplementary Information (SI) for Journal of Materials Chemistry B.

Supporting Materials

Multi-Functional Near-infrared Fluorescent Polymer Dot-siRNA for Gene Expression Regulation

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1. Supplemental materials and methods

Investigating the duration of Pdots fluorescent signal in live cells

After investigating the Pdots cellular uptake and cytotoxicity under different incubation times and different Pdots concentrations, the duration of the Pdots fluorescent signal was determined. After analyzing the above results, we realized that 10 µg/mL of Pdots under 4 h incubation with the cells had a minimally toxic effect with a good fluorescence intensity. Based on this information, we wanted to determine how long the Pdots fluorescent signal persisted in live cells.

The BMVFb cells were cultured in 8 replicates of 8-well Lab Tek Chamber slides at a target plating density of 2,500 cells/well. After the cells adhered, 10 µg/mL of Pdots were added to the medium for 4 h to allow cellular uptake. The replicates of the 8-chamber slides were used for long term monitoring of the cells from 0 day until 14 days with one chamber collected at each designated time point. The time points were collected every two days at which point the media was removed, the cells were gently washed with 1X PBS (with Ca^{2+} and Mg^{2+}) and the cells fixed by adding 400 µL of 4% paraformaldehyde in 1X PBS for 15 min at RT. For the remaining cultures, the medium was replaced every four days. Once all time points were collected, the cells were labelled with DAPI (nuclear label) and Alexa Fluor 488 phalloidin (actin cytoskeleton label). The cells were visualized using an Olympus FV3000 laser scanning confocal microscope under 20X objective and images were collected for each slide.

siRNA encapsulation efficiency by RiboGreen™ RNA Quantitation Assay

To evaluate the siRNA encapsulation capacity of the Pdots with electrostatically bound siRNA, the RediPlate™ 96 RiboGreen™ RNA quantitation assay was carried out. A range of ratios of Pdot to siRNA in 20 µL were combined with 180 µL of RediPlate TE buffer and free siRNA was assayed in parallel as a positive control. The combined solutions were gently mixed in the plate

and incubated in the dark for 10 min at RT. The fluorescence intensity of the Pdot-siRNA was quantified with a fluorescence-based microplate reader (Ex/Em: 500/525 nm) and the free siRNA was set as 100% fluorescence for reference.

2. Supporting figures

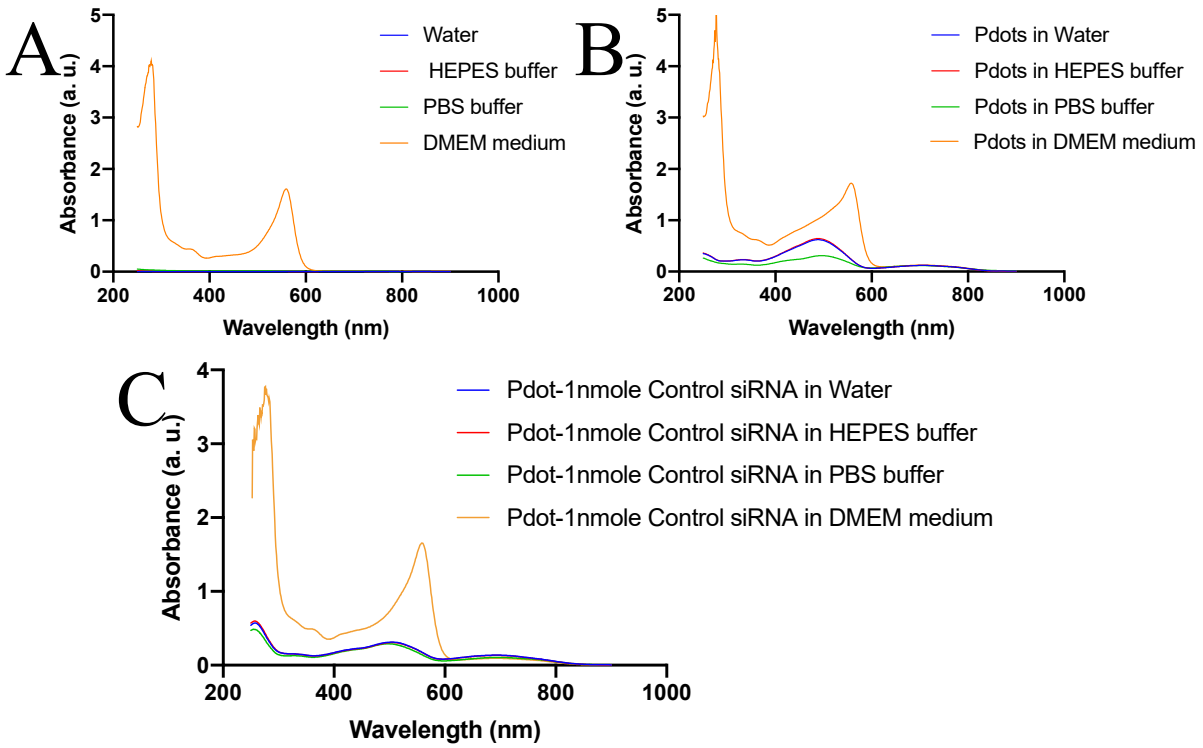

Figure S1. Absorbance characterization of Pdots and Pdot-Control siRNA in water, biocompatible buffers (HEPES & PBS), and cell culture medium (DMEM). (A) The absorbance investigation of water, HEPES buffer, PBS buffer and DMEM medium. (B) The absorbance properties of Pdots in water, HEPES buffer, PBS buffer and DMEM medium. (C) The absorbance properties of Pdot-1nmole Control siRNA in water, HEPES buffer, PBS buffer and DMEM medium.

Table S1. Summary of Pdots and Pdot-Control siRNA in terms of size, zeta potential, PDI and conductivity in water, HEPES buffer, PBS buffer and DMEM medium. The test revealed that the nanomaterial average diameter differed in buffer versus cell culture media.

Water	Pdots	Pdot-0.5nmole C-siRNA	Pdot-1nmole C-siRNA
Size (nm)	64.76 ± 2.09	78.63 ± 2.79	78.87 ± 2.51
Zeta (mV)	28.40 ± 5.27	-31.90 ± 0.64	-18.40 ± 0.41
PDI	0.11 ± 0.01	0.26 ± 0.02	0.20 ± 0.02
Cond (mS/cm)	1.05 ± 0.06	0.015 ± 0.004	0.281 ± 0.004
HEPES	Pdots	Pdot-0.5nmole C-siRNA	Pdot-1nmole C-siRNA
Size (nm)	64.25 ± 0.60	75.05 ± 3.46	77.66 ± 1.05
Zeta (mV)	37.40 ± 8.28	-37.00 ± 10.07	-18.37 ± 0.25
PDI	0.13 ± 0.02	0.24 ± 0.02	0.20 ± 0.01
Cond (mS/cm)	0.43 ± 0.01	0.45 ± 0.01	0.52 ± 0.01
PBS	Pdots	Pdot-0.5nmole C-siRNA	Pdot-1nmole C-siRNA
Size (nm)	62.15 ± 4.61	587.70 ± 57.61	479.20 ± 73.88
Zeta (mV)	-6.71 ± 0.83	-5.77 ± 1.51	-9.23 ± 3.91
PDI	0.19 ± 0.02	0.24 ± 0.01	0.25 ± 0.022
Cond (mS/cm)	19.20 ± 0.43	18.50 ± 1.27	18.30 ± 1.29
DMEM	Pdots	Pdot-0.5nmole C-siRNA	Pdot-1nmole C-siRNA
Size (nm)	5.78 ± 0.87	5.98 ± 1.55	6.03 ± 1.43
Zeta (mV)	-8.34 ± 0.50	-5.96 ± 1.75	-4.77 ± 2.07
PDI	0.59 ± 0.01	0.62 ± 0.03	0.59 ± 0.01
Cond (mS/cm)	17.50 ± 1.08	17.00 ± 0.88	17.20 ± 1.23

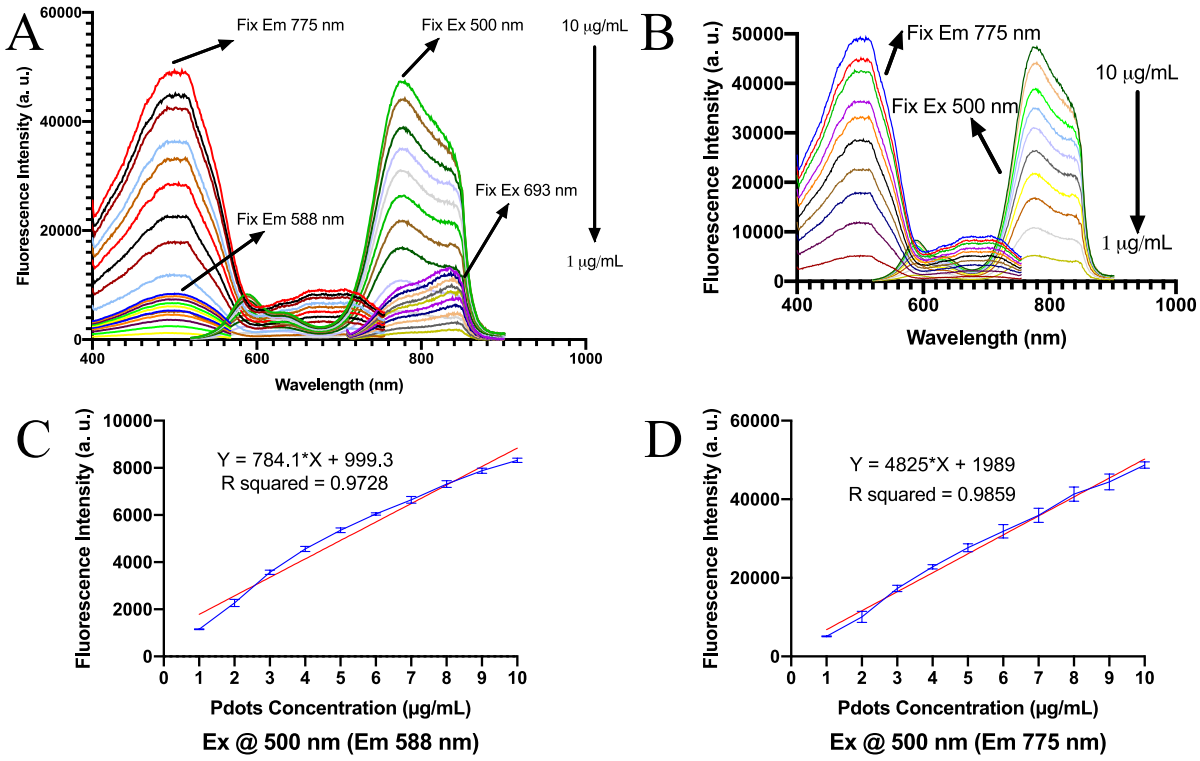

Figure S2. Fluorescence properties of Pdots in a dose series from 1 μg/mL to 10 μg/mL. (A) Overall fluorescent spectra of Pdots through fixed excitation at 500 nm and 693 nm and fixed emission at 588 nm and 775 nm. (B) Fluorescent spectra by fixed excitation at 500 nm and emission at 775 nm. (C) Linear regression analysis of different concentrations of Pdots versus excitation fluorescence intensity at 500 nm with emission fixed at 588 nm. (D) Linear regression analysis of different concentrations of Pdots versus excitation fluorescence intensity at 500 nm emission fixed at 775 nm.

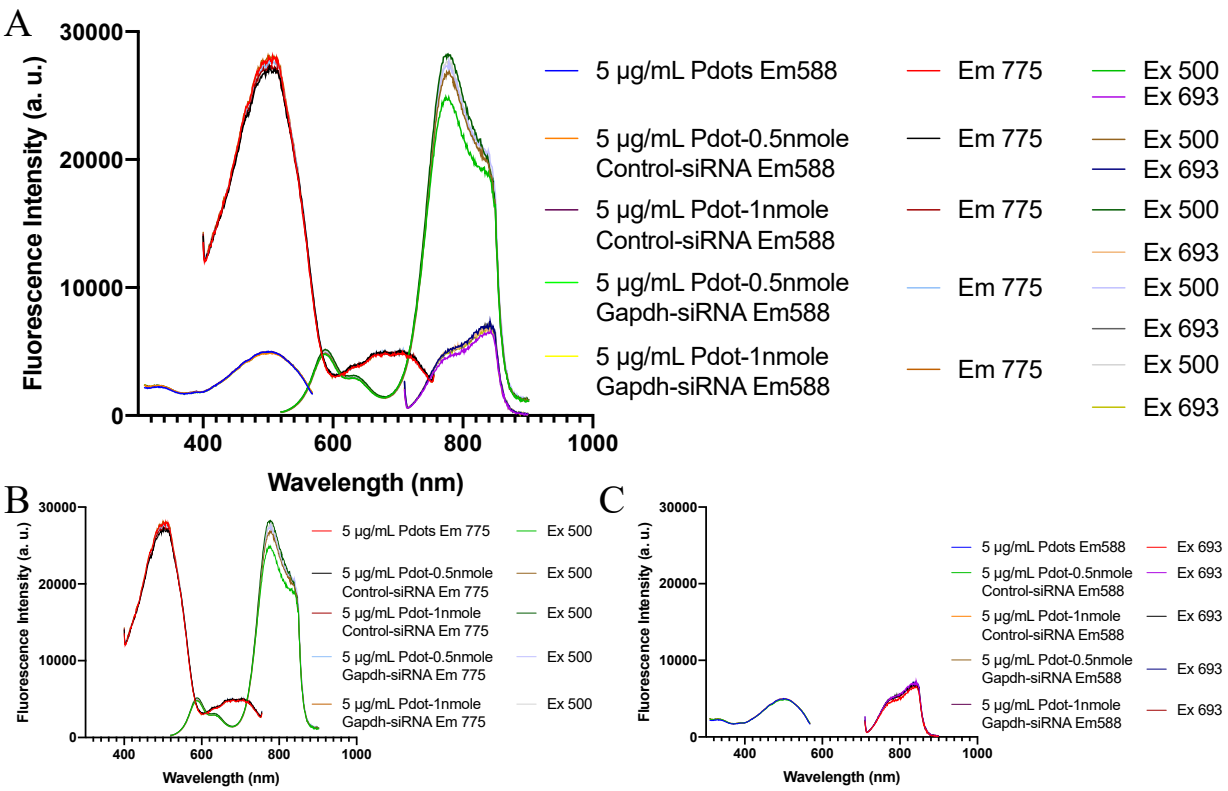

Figure S3. Fluorescence properties of Pdots, Pdot-control siRNA and Pdot-Gapdh siRNA. (A) Fluorescence properties of Pdot-0.5 nmole control siRNA, Pdot-1.0 nmole control siRNA, Pdot-0.5 nmole Gapdh siRNA, and Pdot-1.0 nmole Gapdh siRNA were compared with Pdots alone. (B) Fluorescence spectra of Pdot-control siRNA and Pdot-Gapdh siRNA with excitation fixed at 500 nm and emission at 775 nm. Fluorescence at 500 nm and 775 nm had a similar intensity. (C) Fluorescence spectra of Pdot-control siRNA and Pdot-Gapdh siRNA is shown with the excitation wavelength fixed at 693 nm and emission fixed at 588 nm.

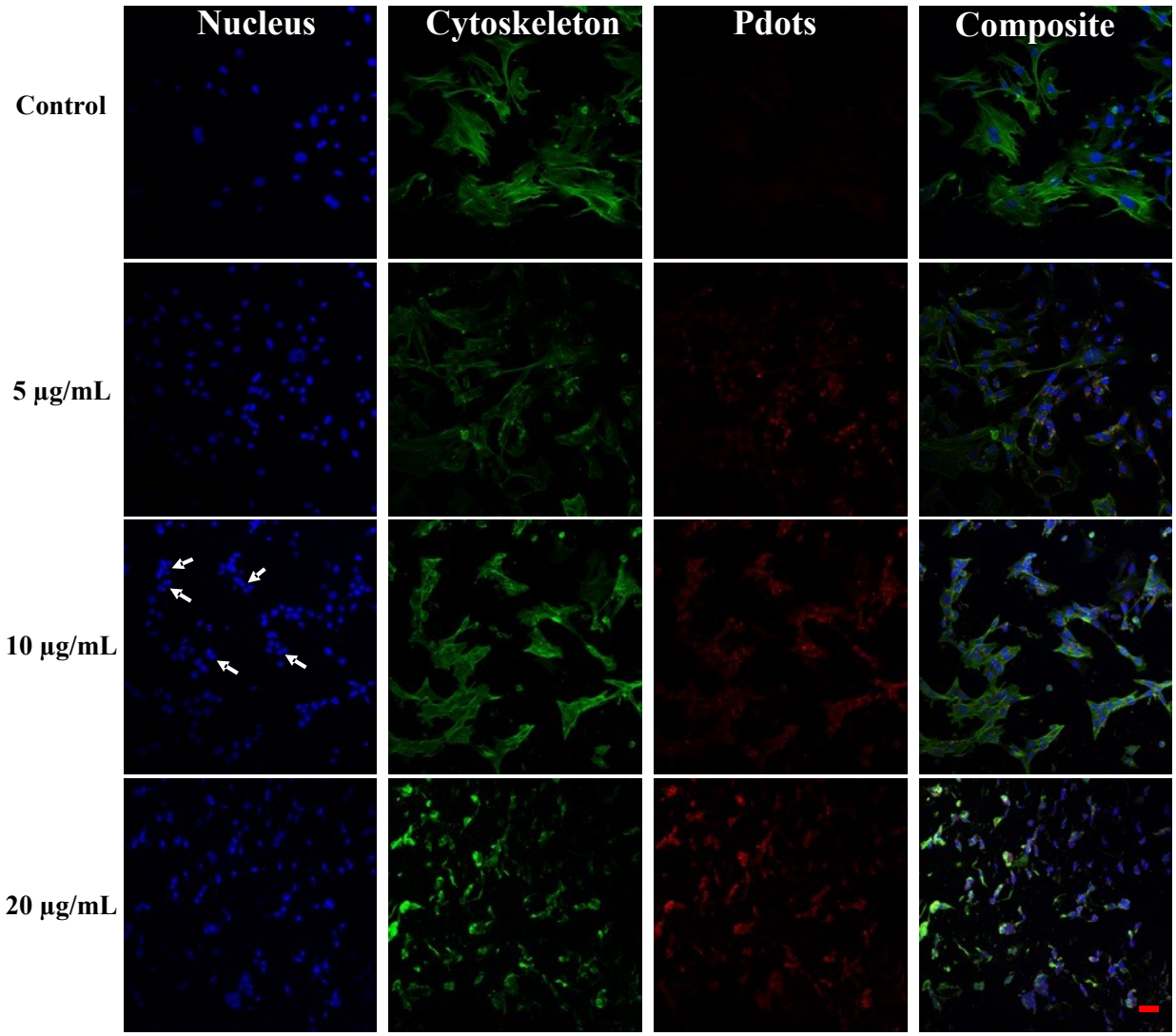

Figure S4. Imaging of BMVFb cells incubated with Pdots for 8 h. BMVFb were cultured in 8-well Lab Tek Chamber slides at a target plating density of 2,500 cells/well, treated with different concentrations of Pdots (red, 588 nm emission) for 8 h and then post-fixation labeled with DAPI (blue, nuclei) and Alexa Fluor 488 phalloidin (green, actin). The white arrows highlight intermediate cell cells that are likely undergoing apoptosis and have small, pyknotic nuclei. The scale bar is 50 µm for all panels and the three-channel overlay is shown for comparison with the individual channels.

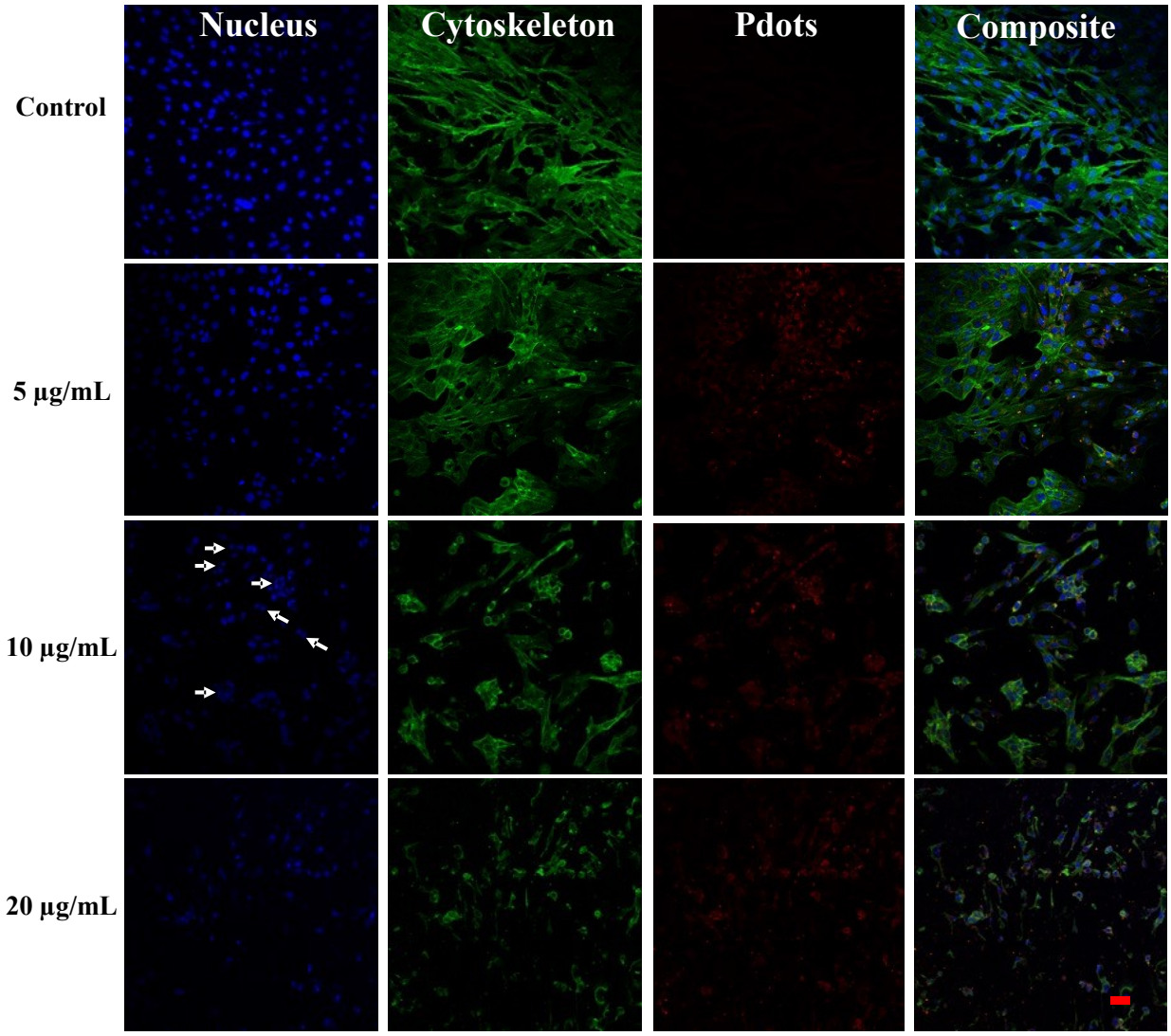

Figure S5. Imaging of BMVFb cells incubated with Pdots for 12 h. BMVFb were cultured as described for Figure S3 but were incubated with varying Pdots concentrations (red, 588 nm emission) for 12 h and then post-fixation labeled with DAPI (blue, nuclei) and Alexa Fluor 488 phalloidin (green, actin). The white arrows in the figure (10 µg/mL of Pdots) identify cells with small, round nuclei. The scale bar is 50 µm for all panels.

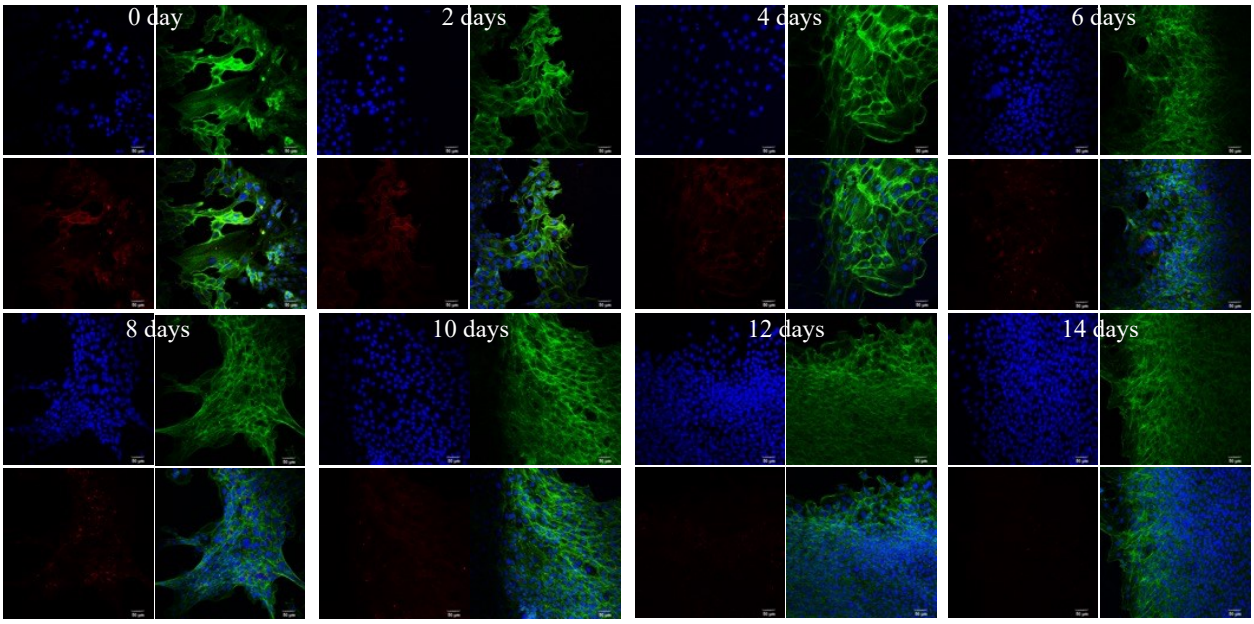

Figure S6. The Pdots signal persistence in BMVFb cells. The cells were incubated with 10 μg/mL Pdots (red, 588 nm emission) for 4 h, and the live cells were further incubated for the time points as indicated. The chamber slides were fixed and labeled with DAPI (blue, nuclei) and Alexa Fluor 488 phalloidin (green, actin). The scale bar is 50 μm.

Table S2. Primer sequence information, including full gene name, NCBI accession number, sequence (5’ to 3’), and amplicon size in base pairs (bp).

Name	Accession	Sequence	Amplicon size
18S Ribosomal subunit	NR_003278.3	forward primer 5’-GACACGGACAGGATTGACAGATTGATA-3’	129bp
		reverse primer 5’-GTTAGCATGCCAGAGTCTCGTTCGTT-3’	
TATA Binding Protein (TBP)	NM_013684.3	forward primer 5' -CAAGAAATGCTGAATATAATCCCAAGC-3'	136bp
		reverse primer 5'-AGTCTGGATTGTTCTTCACTCTTG-3'	
Glyceraldehyde phosphate dehydrogenase (Gapdh)	NM_001411843.1	forward primer 5'-GTGGCAAAGTGGAGATGGTTGCC-3'	288bp
		reverse primer 5'-GATGATGACCCGTTTGGCTCC-3'	

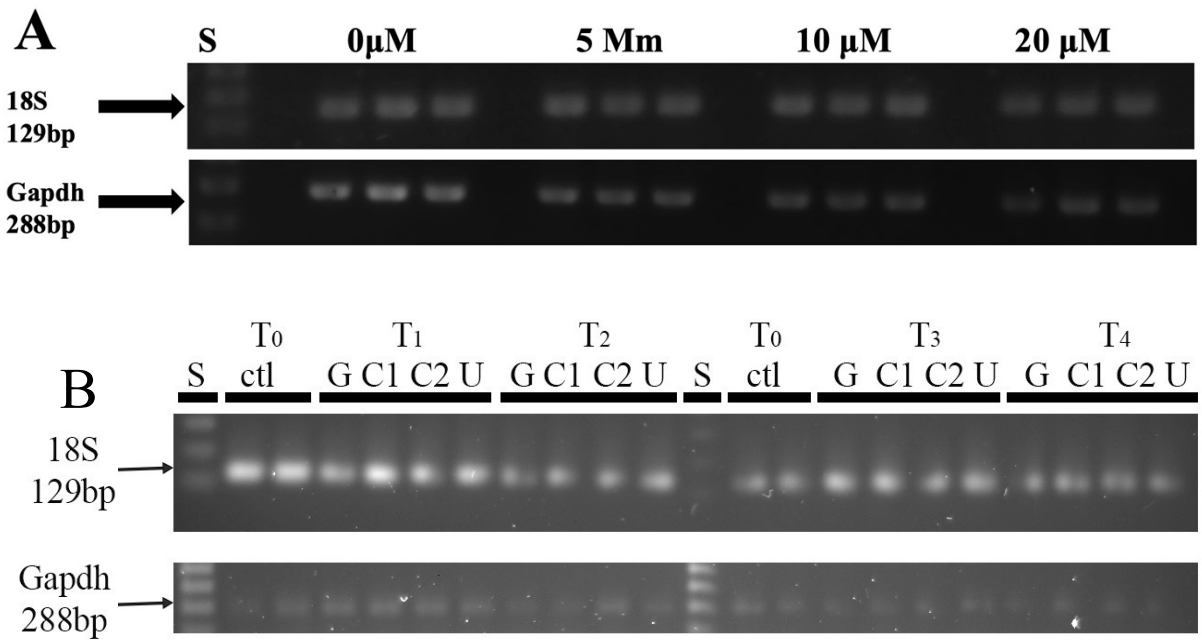

Figure S7. Optimization of dose concentration and time course for Control and Gapdh siRNA. (A) BMVEC were treated with different concentrations of Gapdh siRNA (0, 5, 10, and 20 µM) with three replicates for each treatment condition. There was a decrease in Gapdh band intensity correlating with an increase in Gapdh siRNA concentration from 5 to 20 µM. The 18S band was shown as a reference transcript for comparison. (B) Time course for Control and Gapdh siRNA treatment in BMVEC. T_0 was untreated, T_1 was collected 4 h after treatment, T_2 was 8 h after treatment, T_3 was 20 h after treatment, T_4 was 24 h after treatment. S = Standards; Ctl = Untreated, T_0; G = Gapdh siRNA; C1 = Control siRNA1, C2 = Control siRNA 2; U = Untreated, opti-MEM only. The band intensity for the 18S reference transcript was uniform across all samples. Expression of Gapdh was present in all samples; however, samples from 20 and 24 hours had progressively lower band intensity. Each optimization experiment was independently repeated five times.