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ARTICLE

Degradable Redox-Responsive Disulfide-Based Nanogel Drug Carriers via Dithiol Oxidation Polymerization

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Electronic supplementary information (ESI)

Figure S1. (**A**) 2,2'-(ethylenedioxy)diethanethiol (EDDET) δH(400 MHz; DMSO-d_6) 2.3 (2 H, m, CH_2), 2.6 (2 H, m, CH_2), 3.3 (2H, s, SH), and 3.5 (4 H, m, CH_2). (**B**) pentaerythritol tetramercaptoacetate (PETMA) δH(400 MHz; DMSO-d_6) 3.3 (1 H, s, SH), 3.4 (2 H, s, CH_2), and 4.2 (2 H, 2, CH_2). (**C**) dithiothreitol (DTT) δH(400 MHz; DMSO-d_6) 2.45 (2 H, m, CH), 2.55 (2 H, m, CH), 3.5 (2 H, s, SH), and 43.5 (2 H, 2, CH). (**D**) 1:50 monomer:cross-linker (mol/mol) bulk gel incubated with DTT reducing agent (2X free thiol molar equivalence) for 24 hr δH(400 MHz; DMSO-d_6.

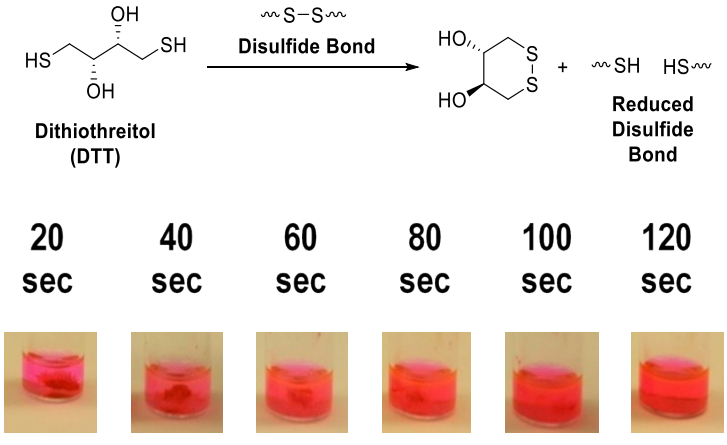

Figure S2. PETMA-based bulk hydrogels degraded in response to DTT to release Rhodamine B. All dye molecules were released within two minutes.

Total Flow Rate (mL/min)	H_2O_2 wt. %	DLS (nm)	Appearance	Ratio	Yield
12	3%	73	opaque blue sheen	1:10	22.41%
12	6%	69	opaque blue sheen	1:10	27.03%
12	12%	74	opaque blue sheen	1:10	40.39%
12	24%	70	opaque blue sheen	1:10	27.74%
12	30%	----	opaque blue sheen	1:10	N/A
nanoprecip.	3%	99	opaque blue sheen	1:10	28.63%

Before Dialysis
PETMA Nanogel = 63 nm

After Dialysis
PETMA Nanogel = 68 nm

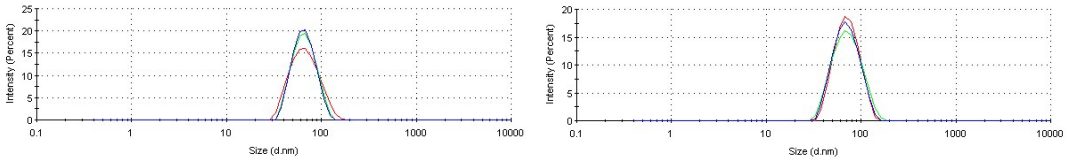

Figure S3. Table listing different formulation conditions. Formulated nanogels were prepared using a microfluidic mixing instrument. An organic phase containing monomer, cross-linker, and base dissolved in water:acetone 1:1 (volume) solution was combined with an aqueous phase containing different weight percentages of H_2O_2. The ratio of organic:aqueous phase was 1:10 (volume) and the flow rate was 12 mL/min.

A

Dynamic Light Scattering (DLS) of Different Mesh Size Nanogels (nm)						
CL:M	Intensity	STDEV	Volume	STDEV	Number	STDEV
1 to 5	55.56	18.44	42.18	14.08	34.19	9.124
1 to 25	111.5	33.92	93.59	32.32	74.30	20.64
1 to 50	111.2	31.84	95.28	30.82	77.73	20.48
1 to 75	92.30	23.70	89.70	22.48	77.83	16.53
1 to 100	99.37	24.58	87.37	23.97	75.15	17.75

B

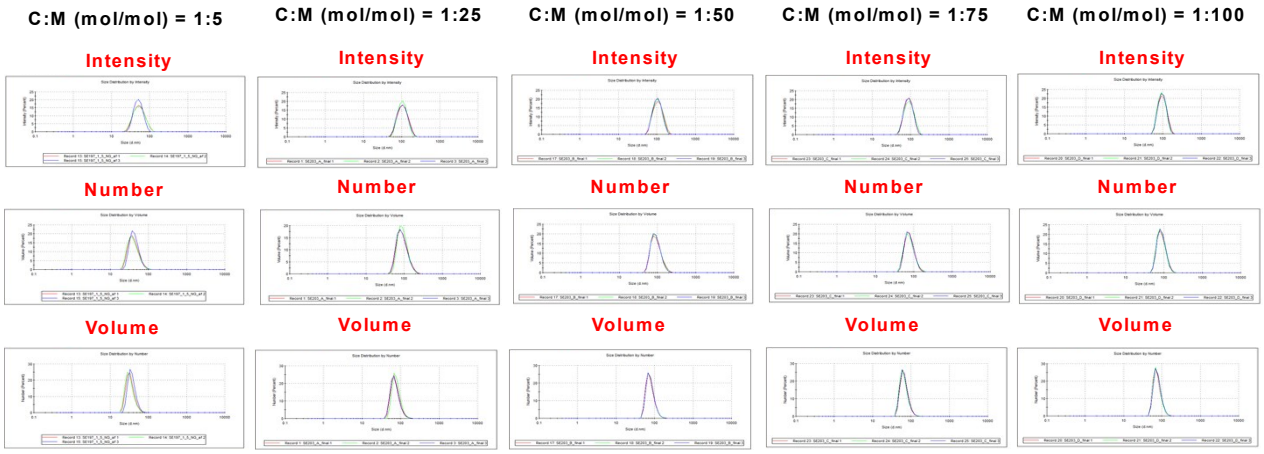

Figure S4. (**A**) Table of particle sizes of different PETMA nanogels of different cross-linking densities after dialysis purification. (**B**) Intensity-, number-, and volume-weighted DLS plots of corresponding nanogels. Formulated nanogels were prepared using a microfluidic mixing instrument. An organic phase containing monomer, cross-linker, and base dissolved in water:acetone 1:1 (volume) solution was combined with an aqueous phase containing 12 wt. % H_2O_2. The ratio of organic:aqueous phase was 1:10 (volume) and the flow rate was 12 mL/min. Particle sizes before after dialysis purification were measured by DLS.

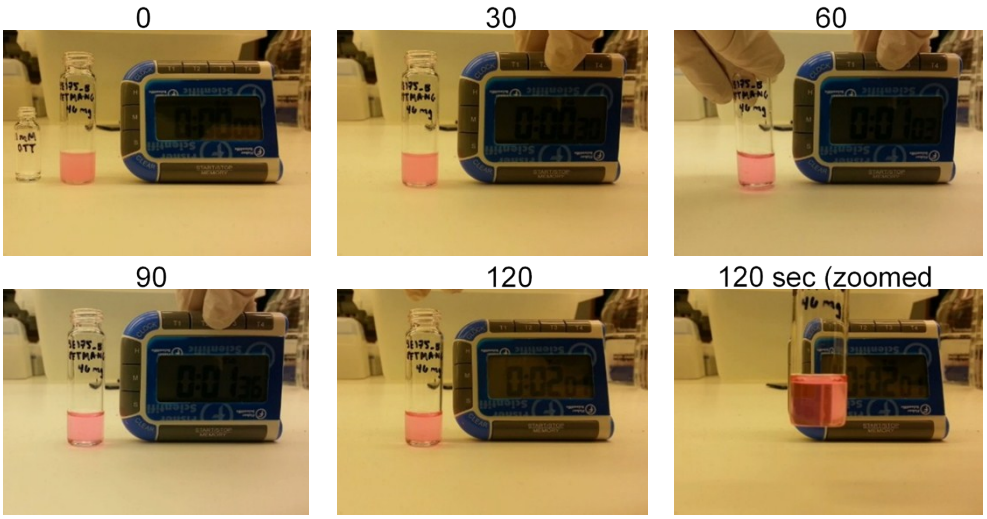

Figure S5. Degradation of PETMA, Rhodamine B loaded nanogels after the addition of dithiothreitol (DTT) disulfide bond reducing agent. Degradation occurred in less than two minutes for this sample size (10 mg).

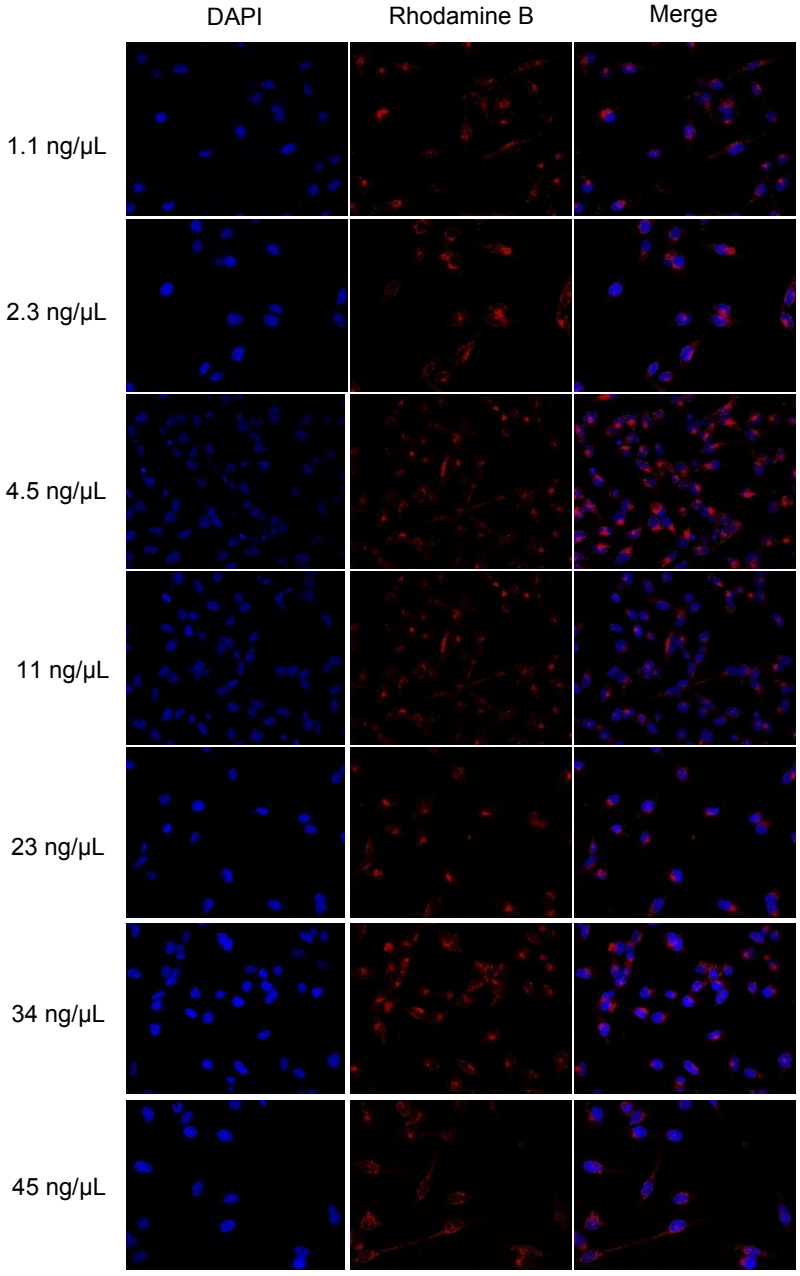

Figure S6. The optimum Rhodamine B dye concentration for analysis of cellular uptake was determined by incubating HeLa cells with increasing dye-loaded nanogel concentrations using the 1:25 nanogels for 24 hrs. HeLa cells were fixed using PFA and the nucleus stained with DAPI. Images taken on a confocal microscope at 40X magnification and images were analyzed using ImageJ.

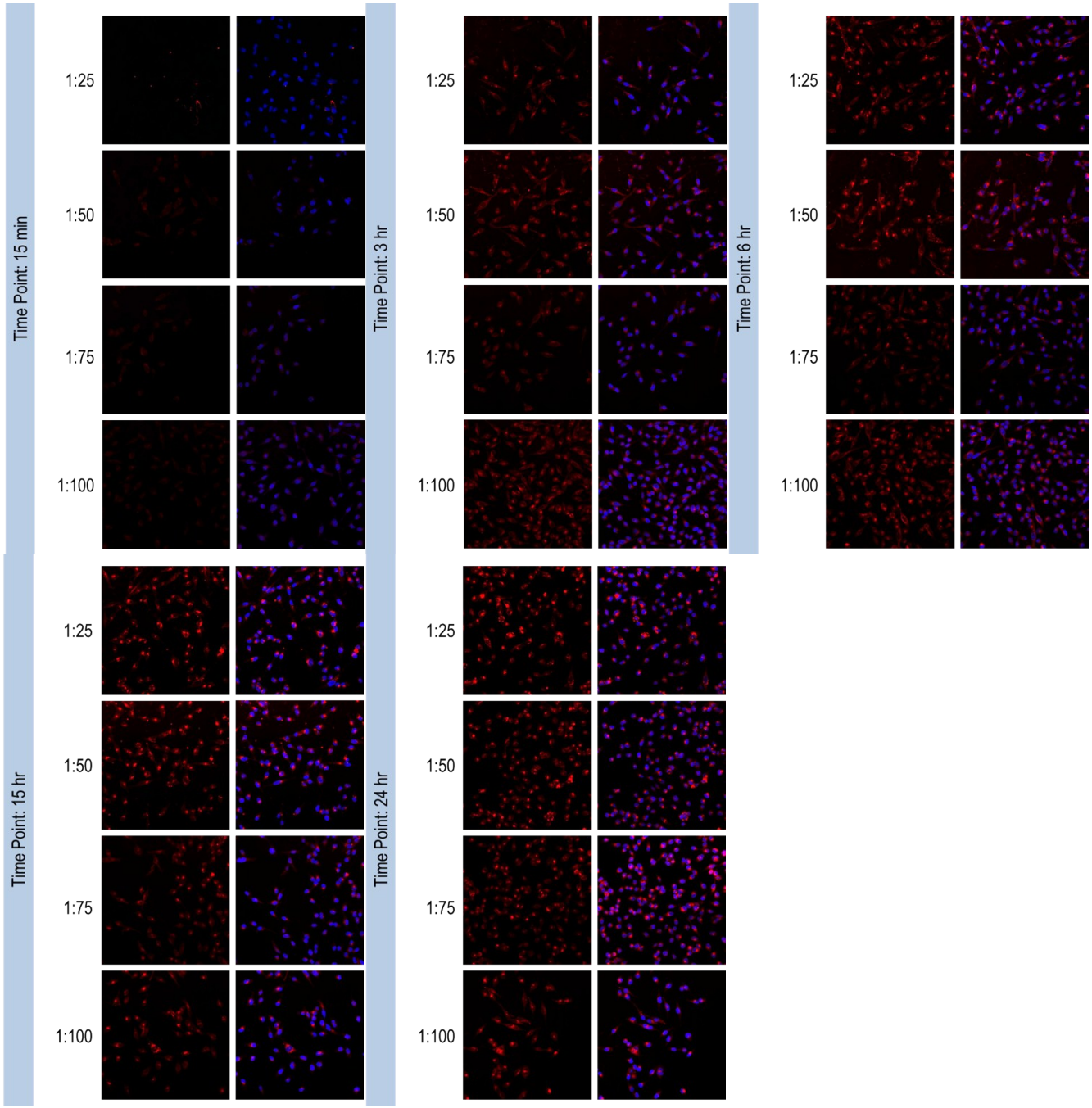

Figure S7. HeLa cells were incubated with 5 wt. % Rhodamine B loaded nanogels with a final dye concentration of 23 ng/μL and incubated at different time points (0 min, 15 min, 1 hr, 3 hr, 6 hr, 15 hr, and 24 hr). HeLa cells were fixed using PFA and the nucleus stained with DAPI. Images taken on a confocal microscope at 40X magnification and images were analyzed using ImageJ.

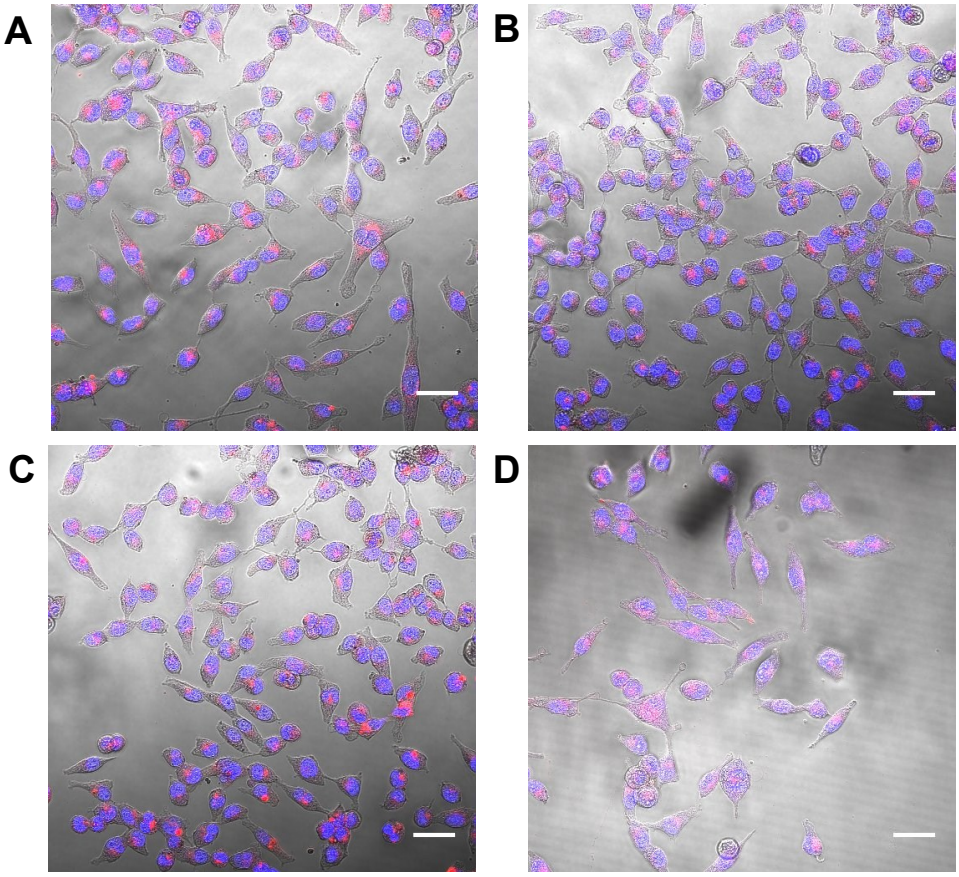

Figure S8. HeLa cells were incubated with 5 wt. % Rhodamine B loaded nanogels with a final dye concentration of 23 ng/μL. HeLa cells were fixed using PFA and the nucleus stained with DAPI. Images taken at 40X magnification and scale bars represent 50 μm scale. Confocal images of (**A**) 1:25, (**B**) 1:50, (**C**) 1:75, and (**D**) 1:100, monomer:cross-linker (mol/mol) at the 24 hr time point. An overlay of bright field with Rhodamine B (red) and cell nuclei (blue) is shown.

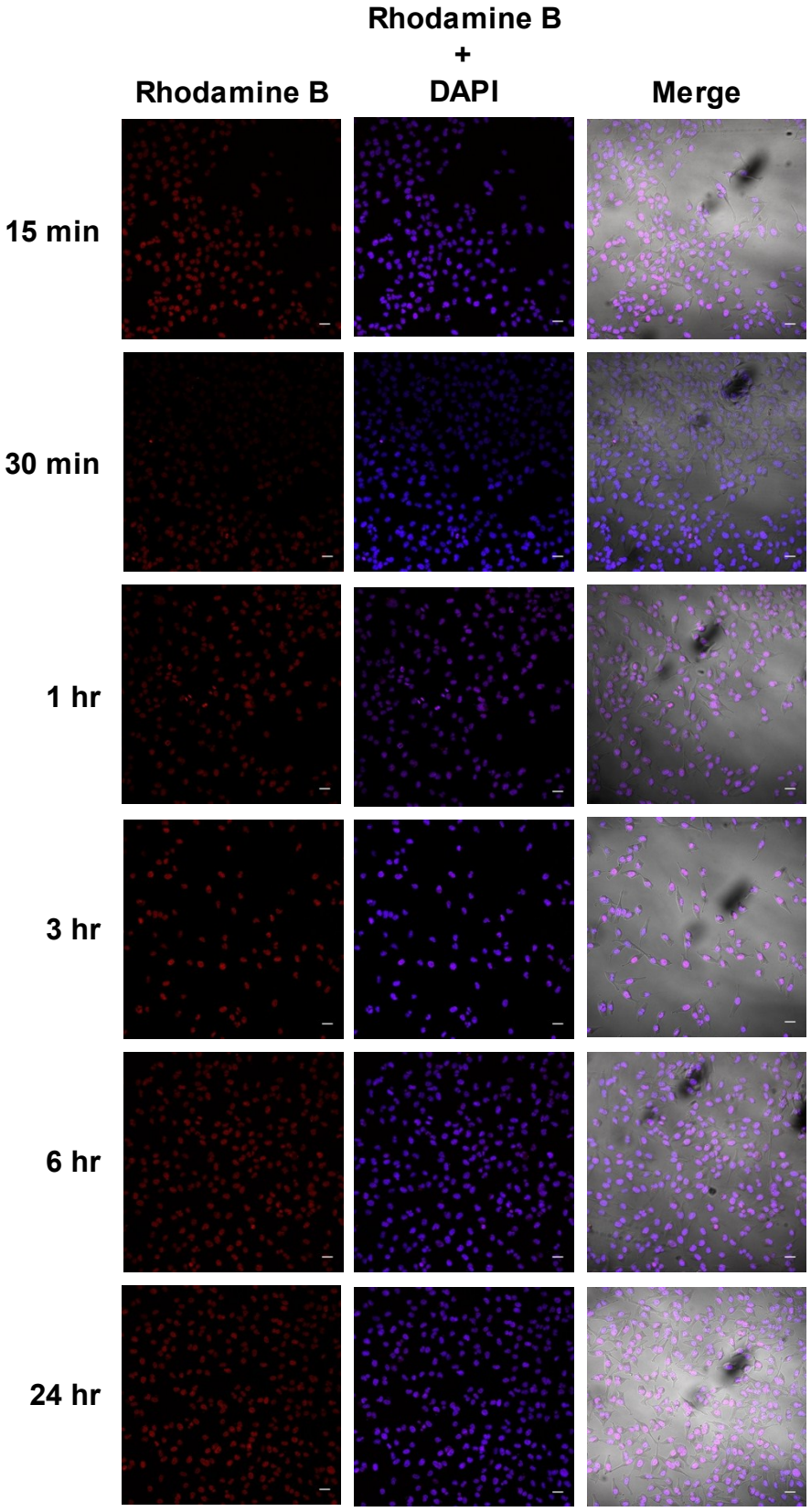

Figure S9. Confocal images of HeLa cells were incubated with a free Rhodamine B dye at different time points (0 min, 15 min, 1 hr, 3 hr, 6 hr, and 24 hr) with a final dye concentration of 23 ng/μL. HeLa cells were fixed using PFA and the nucleus stained with DAPI. Images were taken at 20X magnification and scale bars represent 50 μm scale.

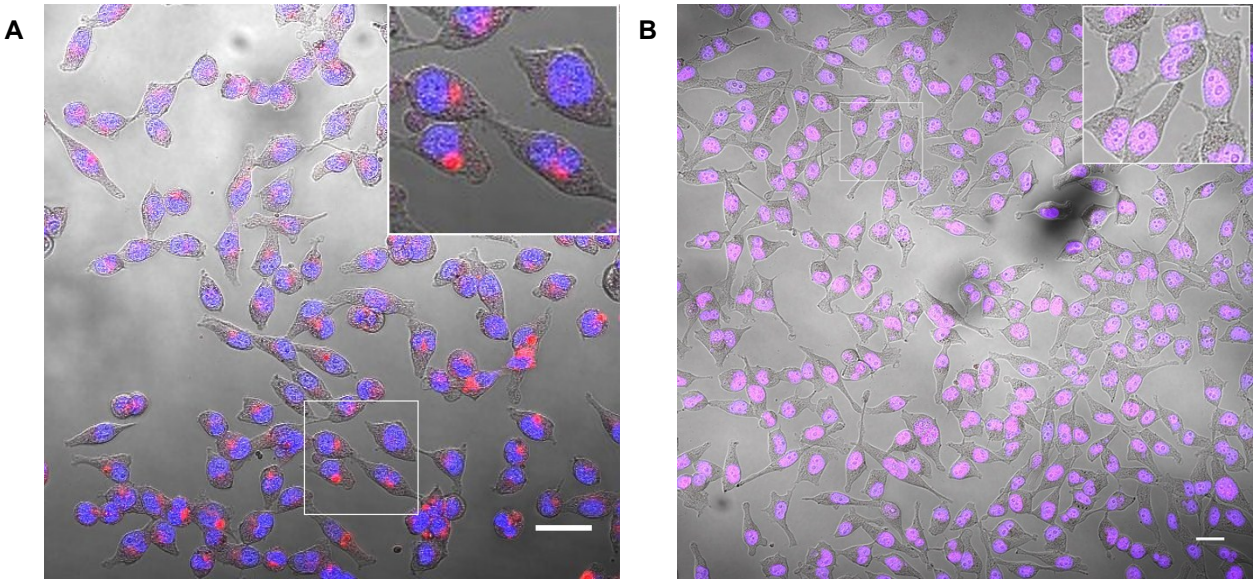

Figure S10. (**A**) HeLa cells were incubated with 5 wt. % Rhodamine B loaded 1:75 monomer:cross-linker (mol/mol) nanogels for a 24 hr time period with a final dye concentration of 23 ng/μL. Images taken at 40X magnification and scale bars represent 50 μm scale. (**B**) HeLa cells were incubated with free Rhodamine B dye for a 24 hr time period with a final dye concentration of 23 ng/μL. Images taken at 20X magnification and scale bars represent 50 μm scale. HeLa cells were fixed using PFA and the nucleus stained with DAPI.