Supplementary Information

Biophysical restriction of growth area using a monodispersed gold sphere nanobarrier prolongs mitotic phase in HeLa cells

Dae-Woong Jung[a,d,#], Hyun-Joo Ro[a,b,c,#], Junmin Kim[a,d], Seung Il Kim[a,b,c], Gi-Ra Yi[d], Gaehang Lee[a,*], and Sangmi Jun[a,b,c,*]

[a] Korea Basic Science Institute, Daejeon, 34133, Republic of Korea
[b] Convergent Research Center for Emerging Virus Infection, Korea Research Institute of Chemical Technology, Daejeon, 34114, Republic of Korea
[c] Bio-Analytical Science, University of Science & Technology, Daejeon, 34113, Republic of Korea
[d] Department of Chemical Engineering, Sungkyunkwan University, Suwon, 16419, Republic of Korea

[#] Equal contribution.
[*] Corresponding authors. Korea Basic Science Institute, Daejeon, 34133, Republic of Korea.
E-mail: ghlee@kbsi.re.kr (G. Lee), smjun@kbsi.re.kr (S. Jun)

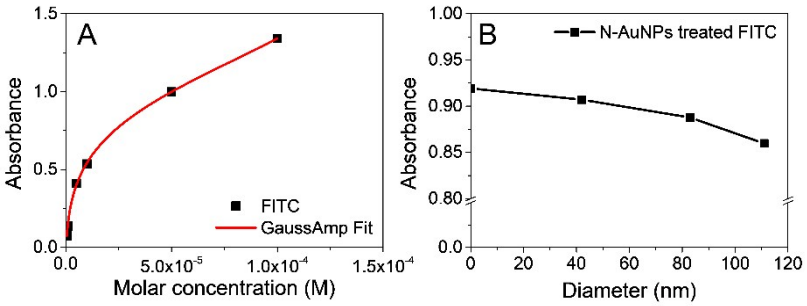

Fig. S1 (A) Standard calibration curve of the absorbance of FITC solution and (B) the absorbance of 42-, 83-, and 111-nm N-AuNSs-treated FITC solutions at 456 nm.

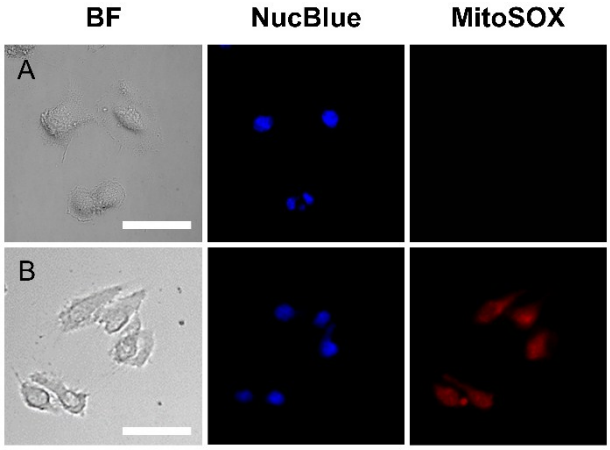

Fig. S2 Fluorescence micrographs of (A) HeLa cells (untreated control) and (B) mitochondrial ROS-positive HeLa cells stained with TBHP (positive control). Scale bar, 50 μm.

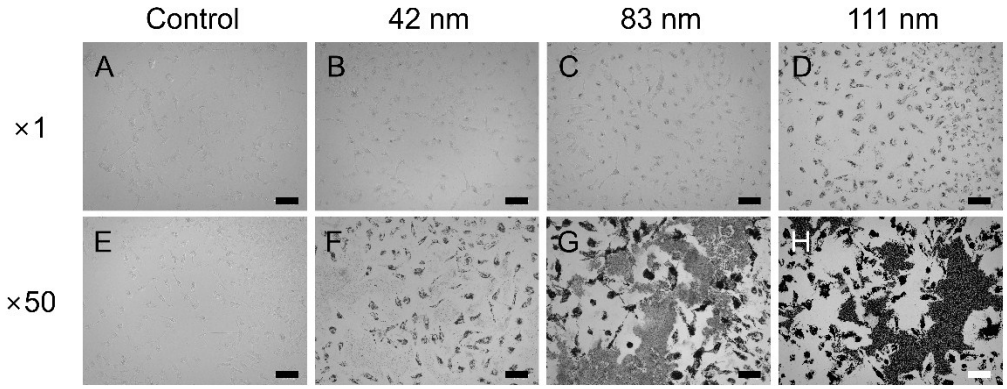

Fig. S3. Bright-field optical microscopy images of (A, E) HeLa cells (untreated control) and cells exposed to (B, F) 42-, (C, G) 83-, and (D, H) 111-nm N-AuNSs at concentrations of (A-D) ×1 and (E-H) ×50 for 24 h. Cellular uptake of N-AuNSs was confirmed by bright-field imaging. Scale bar, 100 μm.

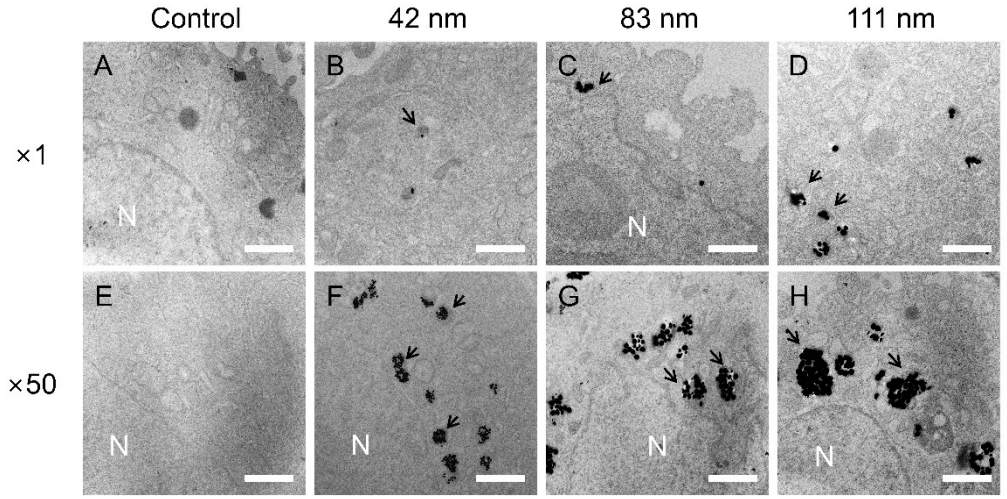

Fig. S4. TEM images showing cellular uptake and intracellular localization of (A, E) control untreated, (B, F) 42-, (C, G) 83-, and (D, H) 111-nm N-AuNSs. After 24 h of exposure, internalization was observed at concentrations of both (A-D) ×1 and (E-H) ×50. Intracellular N-AuNSs are trapped inside endosomes (arrows) and distributed in the cytoplasm. Scale bar, 1 μm. N, nuclei.

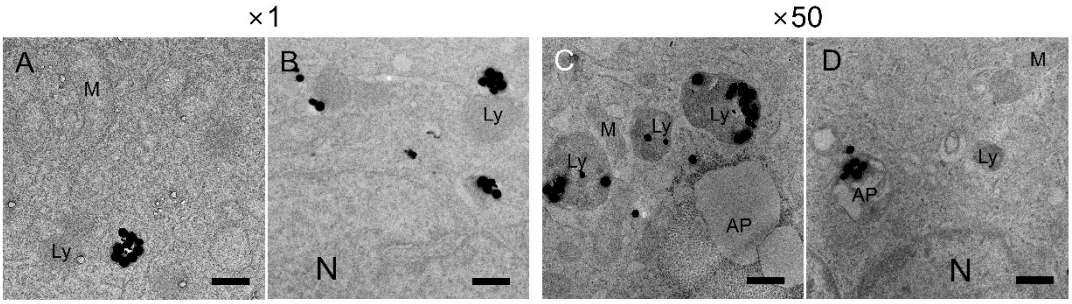

Fig. S5 TEM images of HeLa cells incubated with 111-nm N-AuNSs at concentrations of (A-B) ×1 and (C-D) ×50. Severe mitochondrial damage was not observed, but increased autophagosomes and enlarged lysosomes are observed. Scale bar, 500 nm. AP=autophagosome, Ly=lysosome, M=mitochondria, N=nucleus.

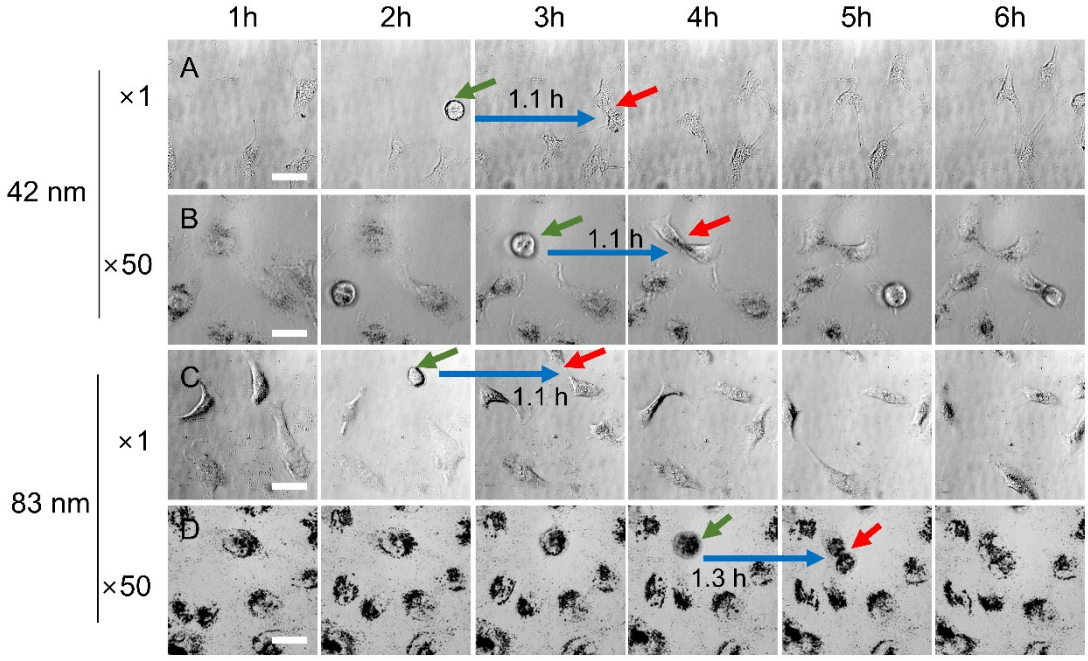

Fig. S6 Time-lapse snapshots of HeLa cells cultured in 4-chamber dishes and exposed to (A-B) 42-nm and (C-D) 83-nm N-AuNSs at concentrations of ×1 and ×50. These six snapshots, taken at 1 h intervals, were extracted from Video S6 (42 nm N-AuNS, ×1), S7 (42 nm N-AuNS, ×50), S8 (83 nm N-AuNS, ×1), and S9 (83 nm N-AuNS, ×50) over a total duration of 24 h.

Green arrows indicate the start point of metaphase and red arrows indicate the end point of telophase. Blue arrows indicate M-phase durations. Scale bar, 50 μm.

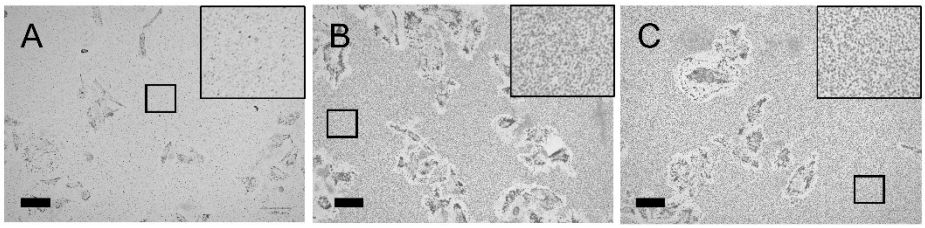

Fig. S7 Bright-field optical microscopy images of HeLa cells exposed to (A-B) 83-nm N-AuNSs at concentration of ×50 in (A) 4-chamber dishes and (B) 96-well plates, and (C) 42-nm N-AuNSs at ×200 in 96-well plates for 1 h. Scale bar, 100 μm. The insets in A-C show an enlarged image of the square areas.

Table S1 Numbers of NH_2-PEG molecules on 83- and 111-nm AuNS surfaces from UV-Vis absorbance of N-AuNSs-treated FITC solution (standard curve shown in Fig. S1).

Diameter (nm)	Number of AuNSs	Absorbance (456 nm)	NH_2-PEG /AuNS	Footprint (/nm^2)	Number of NH_2-PEG at ×1	Number of NH_2-PEG at ×50
83	4.2×10^{9}	0.88	2.6×10^{4}	1.2	1.1×10^{13}	4.6×10^{21}
111	4.2×10^{9}	0.86	4.7×10^{4}	1.2	2.0×10^{13}	8.3×10^{21}

Table S2 Concentrations of N-AuNSs.

Name	×1	×50	×100	×200
Concentration (NSs/mL)	4.2×10^{7}	2.1×10^{9}	4.2×10^{9}	8.4×10^{9}

Table S3 Numerical values of cell proliferation rates in HeLa cells after a 24 h exposure to 42, 83, and 111 nm N-AuNSs at concentration of ×1, ×50, ×100, and ×200, corresponding to Fig. 3.

Concentration	Control	42 nm	83 nm	111 nm
×1	100	104	102	83
×50	100	105	73	35
×100	100	101	73	28
×200	100	102	79	18

Video S1 Time-lapse live-cell imaging of HeLa cells cultured on fibronectin coated 96-well plates and incubated without N-AuNSs for 24 h

Video S2 Time-lapse live-cell imaging of HeLa cells cultured on fibronectin coated 96-well plates and incubated with 42-nm N-AuNSs at concentration of ×50 for 24 h.

Video S3 Time-lapse live-cell imaging of HeLa cells cultured on fibronectin coated 96-well plates and incubated with 83-nm N-AuNSs at concentration of ×50 for 24 h.

Video S4 Time-lapse live-cell imaging of HeLa cells cultured on fibronectin coated 96-well plates and incubated with 111-nm N-AuNSs at concentration of ×50 for 24h.

Video S5 Time-lapse live-cell imaging of apoptotic HeLa cells exposed to 111-nm N-AuNSs

Video S6 Time-lapse live-cell imaging of HeLa cells cultured on fibronectin coated 4-chamber dish and incubated with 42-nm N-AuNSs at concentration of ×1 for 24 h.

Video S7 Time-lapse live-cell imaging of HeLa cells cultured on fibronectin coated 4-chamber dish and incubated with 42-nm N-AuNSs at concentration of ×50 for 24 h.

Video S8 Time-lapse live-cell imaging of HeLa cells cultured on fibronectin coated 4-chamber dish and incubated with 83-nm N-AuNSs at concentration of ×1 for 24 h.

Video S9 Time-lapse live-cell imaging of HeLa cells cultured on fibronectin coated 4-chamber dish and incubated with 83-nm N-AuNSs at concentration of ×50 for 24 h.