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1 *Supplementary Information*

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3 Accumulation of Supramolecular Nanoparticles 4 Self-Assembled from a Bola-Shaped Cytidylic Acid-Appended 5 Fluorescein Dye into Cell Nucleus

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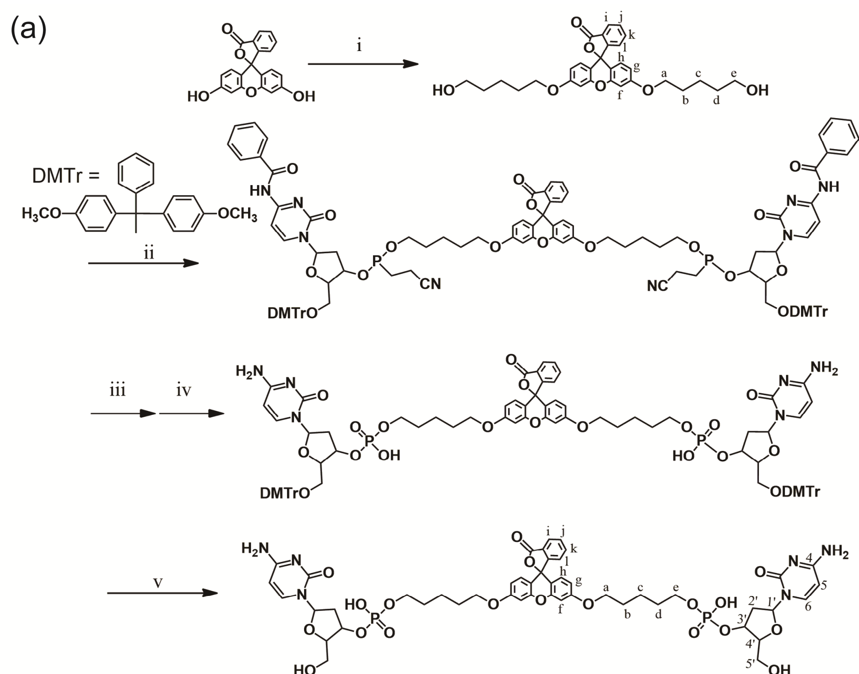
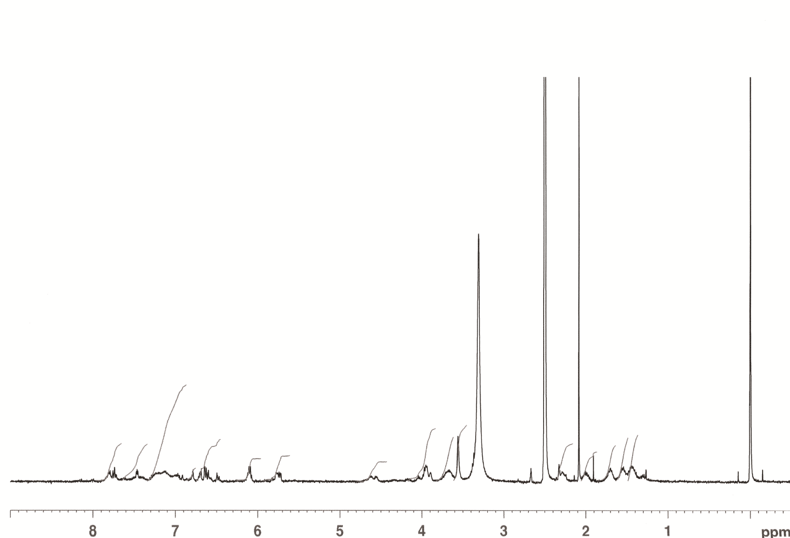
18 **Materials and general methods:** Fluorescein was purchased from Kishida Chemical
19 Co. (Osaka, Japan), and 5-bromo-1-pentanol, potassium carbonate, and sodium
20 borohydride were purchased from Tokyo Chemical Industry (Tokyo, Japan).
21 *N*-Benzoyl-3′-*O*-[2-cyanoethoxy(*N*,*N*-diisopropylamino)phosphino]-5′-*O*-(4,4′-dimetho
22 xytrityl)cytidine was purchased from Applied Biosystems (Tokyo, Japan). The
23 structures of the intermediates and the final products were confirmed by Fourier
24 transform infrared (FTIR) spectroscopy, proton nuclear magnetic resonance
25 spectroscopy (^{1}H NMR), and electrospray ionization Fourier-transform ion cyclotron
26 resonance mass spectrometry (ESI FTICR-MS) or electrospray ionization
27 Fourier-transform orbitrap mass spectrometry (ESI FTMS). ^{1}H NMR spectra were
28 recorded on a Bruker Advance 400 spectrometer (Bruker BioSpin, MA, U.S.A.). FTIR
29 measurements were performed with a Spectrum One spectrometer (Perkin-Elmer, MA,
30 U.S.A.). Mass experiments were performed with an Apex II 70e mass spectrometer
31 (Bruker Daltonics, MA, U.S.A.) or an Orbitrap Veros Pro ETD mass spectrometer
32 (Thermo Fisher Scientific, MA, U.S.A.). Fluorescence spectra were recorded on an
33 RF-5300 spectrofluorometer (Shimadzu, Kyoto, Japan).
34
35 **Synthesis of bis-(5-hydroxypentyloxy)fluorescein:** A mixture of fluorescein (3.2 g, 10
36 mmol) and potassium carbonate (6.9 g) in 200 mL of dimethyl formamide was warmed
37 until complete dissolution had occurred. 5-Bromo-1-pentanol (7.4 g, 45 mmol) was then
38 added over 20 min. The reaction mixture was refluxed for 12 h and cooled to room
39 temperature, and the solvent was evaporated under reduced pressure. Water was added
40 to the remaining solid, and the aqueous layer was extracted with dichloromethane. The
41 collected dichloromethane extract was dried with magnesium sulfate overnight and

42 filtered. Fractionation by gel permeation chromatography with chloroform and
43 subsequent evaporation of the solvent gave bis-(5-hydroxypentyloxy)fluorescein as an
44 orange oil (2.5 g, 50% pure, 5 mmol). Thin layer chromatography (TLC): (silica gel,
45 chloroform/methanol = 4/1, v/v) R_f = 0.78. ^{1}H NMR: (400 MHz in $CDCl_3$ at 25 °C) δ
46 1.5–1.6 (m, 4 H, c), 1.6–1.7 (m, 4 H, d), 1.8–1.9 (m, 4 H, b), 3.6–3.7 (t, 4 H, e), 6.6 (dd,
47 *J* = 9.7 and 1.9 Hz, 1 H, g), 6.75 (d, *J* = 1.8 Hz, 1 H, f), 6.9 (d, *J* = 6.6 Hz, 1 H, h), 7.3
48 (dd, *J* = 7.6 and 1.1, 1 H, l), 7.7 (dt, *J* = 7.6 and 1.4 Hz, 1 H, j), 7.7 (dt, *J* = 7.5 and 1.5
49 Hz, 1 H, k), 8.3 (dd, *J* = 7.7 and 1.4 Hz, 1 H, i). ESI-FTMS (*m*/*z*): Calcd for $C_{30}H_{33}O_7^+$
50 $[M+H]^+$, 505.2221; Found, 505.2217.
51
52 **Synthesis of bis[5-(3′-cytidylyloxy)pentyloxy]fluorescein (C-FLU-C): C-FLU-C** was
53 synthesized as previously described with slight modifications (R. Iwaura et al. *Chem.*
54 *Mater*. **2002**, *14*, 3047.). Dried tetrahydrofuran (THF, 60 mL) was added to 1.0 g (1.2
55 mmol) of
56 *N*-benzoyl-3′-*O*-[2-cyanoethoxy(*N*,*N*-diisopropylamino)phosphino]-5′-*O*-(4,4′-dimetho
57 xytrityl)cytidine, and the solution was mixed with
58 3,6-bis-(5-hydroxypentyloxy)fluorescein (250 mg, 0.5 mmol) and 1*H*-tetrazole (0.2 g,
59 2.8 mmol) and stirred for 3 days. To this reaction mixture, 0.3 mL of 70%
60 *t*-butylhydroperoxide aqueous solution was added and the mixture was stirred for 1 h.
61 The solution in the reaction mixture was evaporated under reduced pressure, the residue
62 was purified by gel permeation chromatography with chloroform as the eluent followed
63 by the evaporation of the solvent. The protecting cyanoethyl group of the residue was
64 removed by mixing with 4 mL of 25% aqueous ammonia and 20 mL of methanol for
65 1.5 h at 50 °C and 24 h at room temperature and then evaporating the solvent. The

66 resulting residue was treated with a chloroform solution containing 5% trifluoroacetic
67 acid to remove the protecting dimethoxytrityl group. After the removal of the solvent,
68 the residual solid was reprecipitated from acetone, and the precipitate was filtered and
69 dried. The final compound, **C-FLU-C**, was obtained as a yellow powder (110 mg, 20%
70 pure). TLC: (silica gel, chloroform/methanol = 4/1, v/v) R_f = 0.1. ^{1}H NMR: (400 MHz
71 in dimethyl sulfoxide-d_6 at 25 °C) δ 1.5–1.6 (m, 4 H, c), 1.6–1.7 (m, 4 H, d), 1.8–1.9 (m,
72 4 H, b), 2.0–2.3 (br, 4 H, 2′-H), 3.5 (d, *J* = 3.8 Hz, 4 H, 5′H), 3.6–3.7 (br, 4 H, e),
73 3.8-3.9 (br, 4H, a) 4.5 (br, 2 H, 4′), 5.7 (br, 2 H, 5-H), 6.1 (t, *J* = 6.5 Hz, 2 H, 1′-H), 6.5
74 (dd, *J* = 9.7 and 1.9 Hz, 2 H, g), 6.6 (d, *J* = 1.8 Hz, 2 H, f), 6.7 (br, 2 H, h), 6.8 (br, 1 H,
75 l), 7.0 (br, 1 H, j), 7.2 (br, 1 H, k), 7.4 (m, 1 H, i) 7.7 (d, *J* = 7.8 Hz, 2 H, 6-H), 7.8 (m,
76 4-NH_2). IR: ν (cm^{-1}) = 3191, 3069, 2945, 1701, 1645, 1600, 1496, 1471, 1427, 1372,
77 1330, 1281, 1177, 1103, 1056, 1001, 951, 827, 782, 759. ESI FTICR-MS (*m*/*z*): Calcd
78 for $C_{48}H_{55}N_6O_{19}P_2^-$ $[M-H]^-$, 1081.3003; Found, 1081.3015. mp = 177 °C.
79





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81 Fig. S1 (a) Synthesis of bis[5-(3′-cytidylyloxy)pentyloxy]fluorescein (**C-FLU-C**): (i)
82 K_2CO_3, Br-$(CH_2)_5$OH; (ii) *N*-benzoyl-3′-*O*-[2-cyanoethoxy(*N*,
83 *N*-diisopropylamino)phosphino]-5′-*O*-(4,4′-dimethoxytrityl)cytidine, 1*H*-tetrazol, THF;
84 (iii) *t*-butylhydroperoxide; (iv) 25% aqueous ammonia solution; (v) trifluoroacetic acid.
85 (b) ^{1}H NMR spectrum of **C-FLU-C**.

Atomic force and transmission electron microscopic (AFM and TEM) observations of the nanoparticles: For the AFM observation, 1 μL of the aqueous solution containing **C-FLU-C** ([C] = 2 × 10^{-3} mol/L) was placed on a sheet of highly ordered pyrolytic graphite and dried in air for a few minutes. The specimen was then washed with excess cold Milli-Q water and blotted with filter paper. Tapping-mode AFM was carried out with a NanoNavi station and an S-image system (1024 × 1024 pixels; SII Nanotechnology, Tokyo, Japan) and a silicon microcantilever (spring constant = 40 Nm^{-1}, frequency = 120 kHz, aluminum coated, SII Nanotechnology). For the TEM observation, the aqueous solution containing **C-FLU-C** was diluted 100 times. A drop of the diluted solution was put on an elastic carbon-supporting film, dried at room temperature, and then subjected to TEM observation with an H-9500 300 kV transmission electron microscope (Hitachi High Technologies, Tokyo, Japan) and a CCD camera (AMT Corp. ER-B, Woburn, MA).

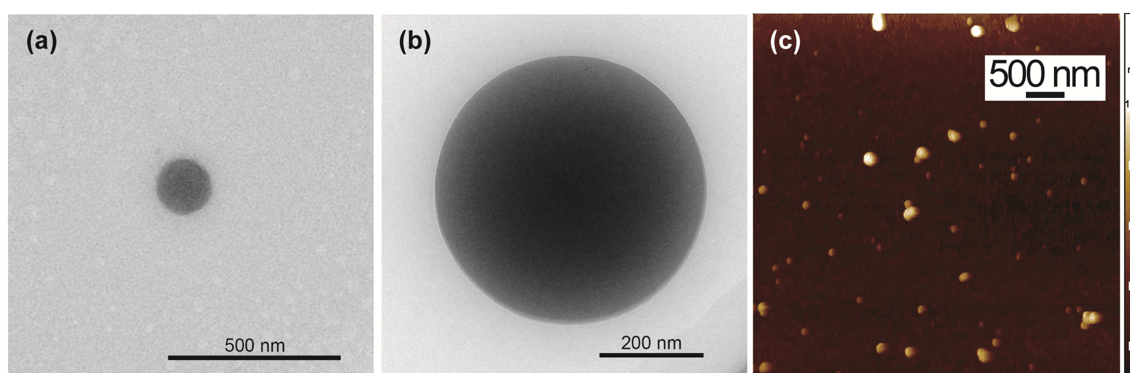
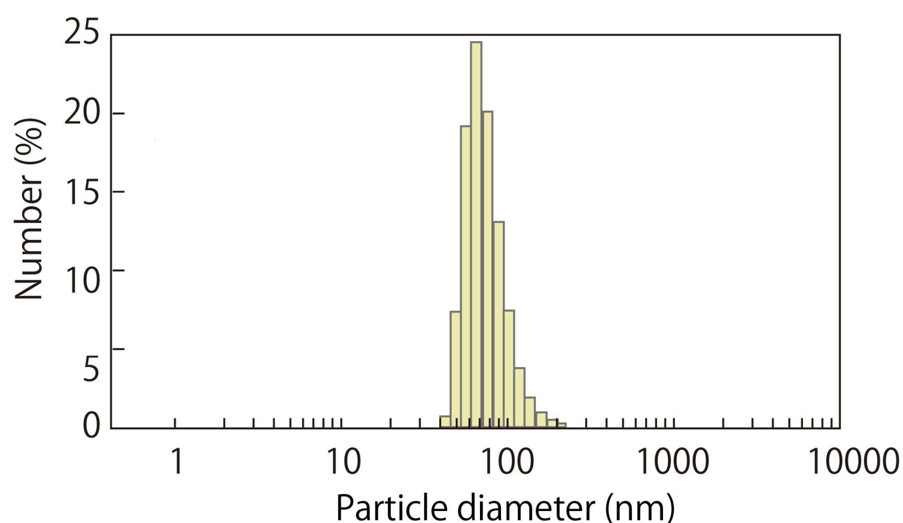


Fig. S2 (a,b) Transmission electron microscopic images and (c) atomic force microscopic images of self-assembled **C-FLU-C** nanoparticles in aqueous solution.

Particle size distribution measurement: The particle size distribution of self-assembled **C-FLU-C** nanoparticles in aqueous solution ([C] = 4 × 10^{-4} mol/L) was analyzed with a Zetasizer (Malvern Instruments, UK) at 20 °C.



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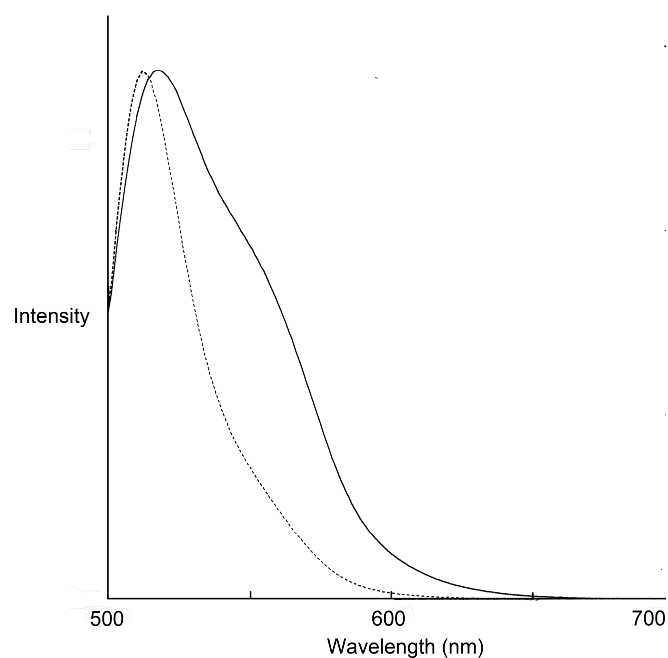
106 Fig. S3 Particle size distribution of self-assembled **C-FLU-C** nanoparticles in aqueous

107 solution.



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109 Fig. S4 Normalized emission spectra of fluorescein (---) and **C-FLU-C** (—) in Tris-HCl

110 buffer (pH = 8) at 25 °C ([C] = 2×10^{-6} M, Ex. = 480 nm).

111

112 **Cell cultures:** Caco-2 cells (human colon carcinoma cell line) and BALB/3T3 cells

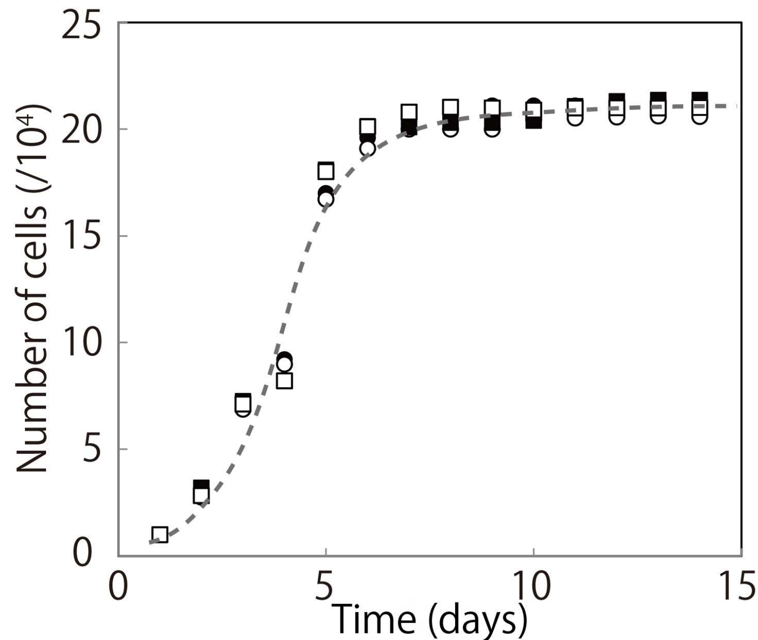
113 (contact-inhibited seminormal cell line: mouse embryo cell line) were obtained from

114 RIKEN Cell Bank (Tsukuba, Japan) and were used to examine the uptake
115 characteristics of self-assembled **C-FLU-C** nanoparticles. An aqueous solution of
116 **C-FLU-C** was added to both cell lines after 1 day of culture, and the concentration was
117 adjusted to 2×10^{-6} mol/L. Both cell lines were cultured in culture medium containing
118 antibiotics (Wako Pure Chemical Industries, Tokyo, Japan), non-essential amino acids
119 (GIBCO Life Technologies, CA, U.S.A.), and 10% fetal bovine serum (FBS, Sigma
120 Aldrich, MO, U.S.A.). Dulbecco's modified Eagle's medium and Eagle's minimal
121 essential medium (both GIBCO Life Technologies) were used as the culture medium for
122 the Caco-2 and BALB/3T3 cells, respectively. The culture medium was changed at 3, 7,
123 and 10 days of cell culture. Cells were grown in T25 tissue culture flasks (Falcon,
124 Corning Incorporated Life Sciences, MA, U.S.A.) at 37 °C in 100% humidity and 5%
125 CO_2 and were passaged when they reached near confluency.
126 As references, the experiments were repeated by using fluorescein instead of
127 **C-FLU-C**. Fluorescein was dissolved in a trace amount of dimethyl sulfoxide, and the
128 concentration of fluorescein was adjusted to 5×10^{-6} mol/L by using water.
129
130 **Measurements of cell growth curves:** Caco-2 or BALB/3T3 cells were cultured in
131 **C-FLU-C**–containing medium in 12-well plates and were treated with 0.025% trypsin
132 solution after 1–14 days incubation. Cultured cell suspensions were collected by
133 centrifugation (100 ×*g*, 3 min) and washed with phosphate buffered saline (PBS) several
134 times before the cells were counted.
135 As references, both cell lines were cultured, and the cells were counted without the
136 addition of **C-FLU-C**. Cytotoxicity and the median lethal dose (LD_{50}) of **C-FLU-C** on

137 Caco-2 cells were determined with a WST-1 cell proliferation assay system (Takara,

138 Shiga, Japan).

139



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141 Fig. S5 Cell growth curves of Caco-2 cells (○), Caco-2 cells + **C-FLU-C** (●),

142 BALB/3T3 cells (□), and BALB/3T3 cells + **C-FLU-C** (■). [**C-FLU-C**] = 2×10^{-6}

143 mol/L. Median lethal dose (LD_{50}) = 3.5×10^{-3} mol/L.

144

145 **Optical and laser-scanning confocal microscopic observations and flow cytometric**

146 **analysis of cells:** Cells cultured with **C-FLU-C** nanoparticles were washed with PBS

147 and harvested by adding a trypsin/ethylenediaminetetraacetic acid mixture (Wako).

148 Trypsin was inactivated with a mixture of culture medium and FBS, and cells were

149 resuspended in PBS and collected by centrifugation (100 ×*g*, 3 min). The collected cells

150 were washed three times with PBS and resuspended in PBS, and the suspension was

151 subjected to optical microscopic measurements and flow cytometric analysis.

152 Fluorescence microscopic images were obtained with an optical microscope (BX51;
153 Olympus, Tokyo, Japan) equipped with a 40× objective lens, a 10× ocular lens
154 (Olympus), band-pass filters (510–550 nm Olympus), and a 3-CCD video camera
155 (CS520MD; Olympus). Laser-scanning confocal microscopic observations of cells were
156 performed on a TCS SP8 system (Leica Microsystems, Tokyo, Japan) with a visible
157 semiconductor laser operating at a wavelength of 488 nm and a fluorescence filter cube
158 (I3). Flow cytometry experiments were carried out on an EC800 flow cytometry
159 analyzer (Sony, Tokyo, Japan). Cells containing **C-FLU-C** were excited at 488 nm, and
160 fluorescence signals from individual cells were collected through a band-pass filter
161 (500–550 nm). Flow cytometric histograms were constructed for 1×10^4 cells in each
162 experiment.
163

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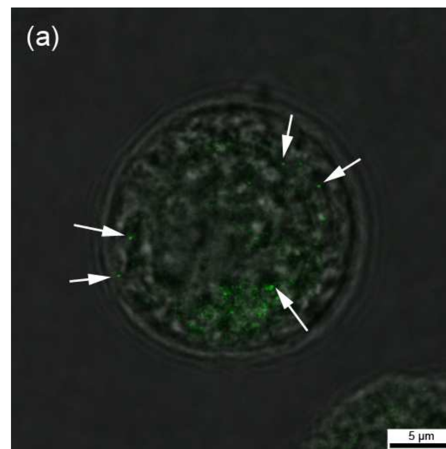

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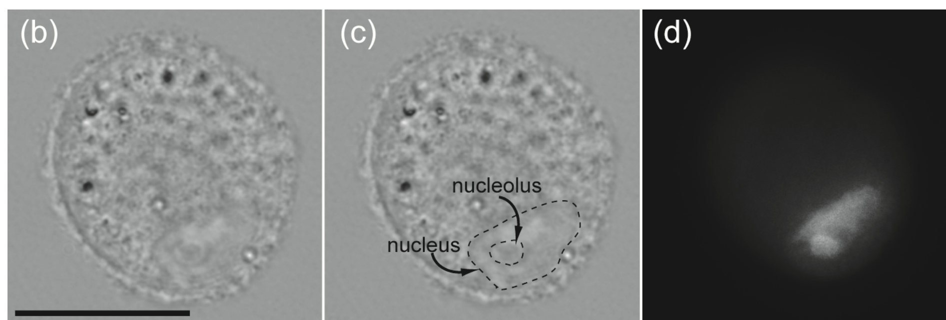


167

168 Fig. S6 (a) Composite image of fluorescence and bright field images of a Caco-2 cell

169 cultured for 4 days with **C-FLU-C** obtained by using a laser-scanning confocal

170 microscope. Arrows indicate fluorescent particles. Microscopic images of a Caco-2 cell

171 cultured for 7 days with **C-FLU-C** for (b, c) a light field image, and (d) a fluorescence

172 microscopic image. Bar = 20 μm.

173

174 **Adsorption of C-FLU-C on nucleosomes:** Cell cultures were treated with colcemid

175 (Sigma; final concentration = 0.04 mg/mL) for 2 h. After colcemid treatment, the cell

176 cultures were trypsinized and suspended in culture medium. The cell suspensions were

177 then collected by centrifugation (100 ×*g*, 5 min), washed several times with PBS, and

178 then, as a hypotonic treatment, stirred in 0.075 mol/L KCl solution containing 2×10^{-6}

179 mol/L **C-FLU-C** for 25 min. Sediments were collected by centrifugation (150 ×*g*, 10

180 min, at 4 °C), fixed in methanol–acetic acid (3/1 = v/v, on ice), and dried on a slide

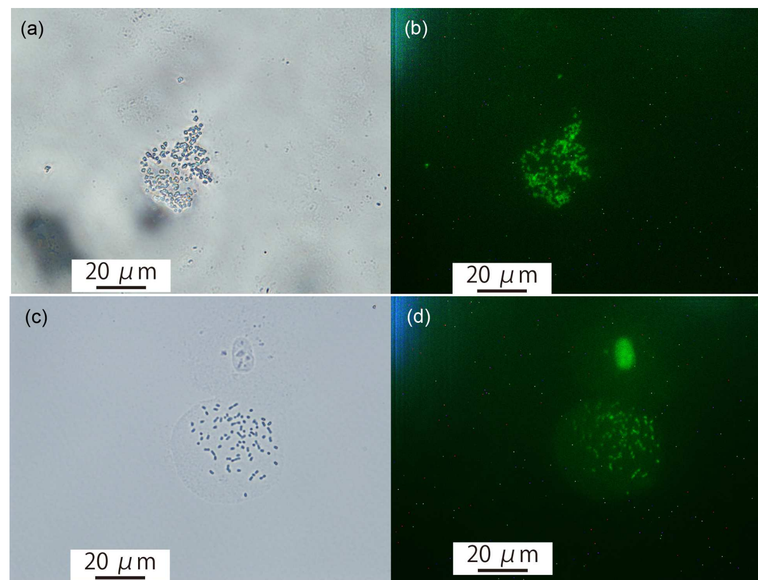
181 glass. Fluorescence microscopic images of the treated nucleosomes were obtained by

182 using the method described above.

183

184

185



186

187 Fig. S7 Light (left) and fluorescence (right) microscopic images of chromosomes of

188 Caco-2 cells (a, b) and BALB/3T3 cells (c, d) cultured for 2 days in the presence of

189 **C-FLU-C**. Prior to observation, cells were treated with colcemid.