Supporting Information

Photo-responsive materials with strong cell trapping ability for light-guided manipulation of nonadherent cells

Natalia Teresa Jarzębska,[a] Satoshi Yamaguchi,[b,c]* Shin Izuta,[a] Takahiro Kosaka,[a] Shinya Yamahira,[d] Teruyuki Nagamune,[a] and Akimitsu Okamoto[a,b]*

[a] *Department of Chemistry and Biotechnology, Graduate School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan*

[b] *Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan*

[c] *PRESTO, Japan Science and Technology Agency (JST), 4-1-8 Hon-cho, Kawaguchi, Saitama 351-0198, Japan*

[d] *Laboratory for Joint Research & Development, St. Luke's International University, 10-1 Akashi-Cho, Chuo-ku, Tokyo, 104-0044, Japan*

*Corresponding authors:

Satoshi Yamaguchi, E–mail: yamaguchi@bioorg.rcast.u-tokyo.ac.jp

Akimitsu Okamoto, E–mail: okamoto@chembio.t.u-tokyo.ac.jp

Materials and Methods

1. General procedures and materials

Fmoc-Lys-OH was purchased from Watanabe Chemical Industries, Ltd. (Hiroshima, Japan). Oleylamine was from Acros Organics (New Jersey, USA). 4-[4-(1-Hydroxyethyl)-2-methoxy-5-nitrophenoxy]-butyric acid was from Sigma–Aldrich Japan KK (Tokyo, Japan). SUNBRIGHT PA-034HC and SUNBRIGHT BO-020HC was from NOF Co. (Tokyo, Japan). **(**Boc-amino)-PEG4-carboxylic acid was from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Fmoc-NH-PEG2-propionic acid and Fmoc-NH-PEG8-propionic acid were from AAPPTec (Louisville, USA).

All other reagents were commercially available and used as supplied without further purification. Column chromatography was performed on a silica gel provided by Kanto Chemical Co. Inc. (60N spherical, 40–50 μm). NMR chemical shifts are reported in ppm downfield of tetramethylsilane using a residual solvent as an internal reference. NMR spectra were recorded using Avance 600 (600 MHz; Bruker, Germany). ESI mass spectra were recorded with a microTOF II (Bruker, Germany).

2. Synthesis of PEG-PL-Me_3K-Oleyl

A new photo-cleavable poly(ethylene glycol) (PEG)-lipid with a cationic trimethyl lysine linker between the oleyl and the photo-cleavable linker (PL) moieties was synthesized, based on a previously reported ‘prototype’ photo-cleavable PEG-lipid[1] (Fig. S1).

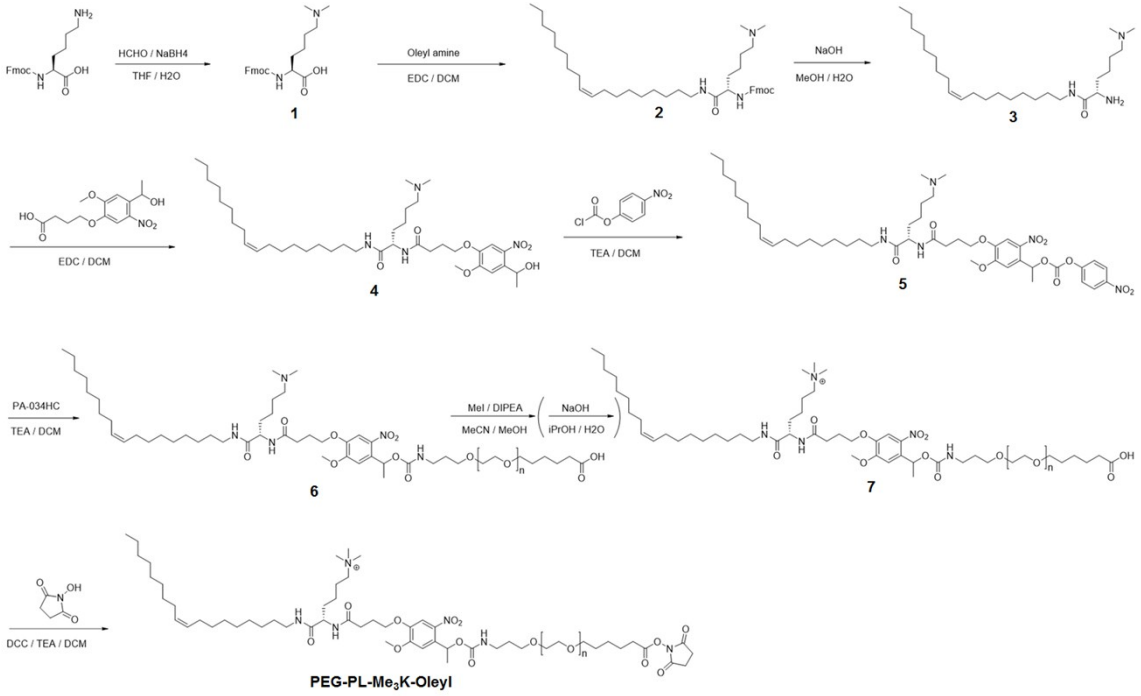

Figure S1. Synthetic scheme of **PEG-PL-Me$_3$K-Oleyl**

Synthesis of 1. Fmoc-Lys-OH (505 mg, 1.37 mmol) was dissolved into 5 ml of 1,4-dioxane on ice. Then, acetic acid (1.5 ml), formaldehyde (37 % solution, 1.5 ml, 20.2 mmol) and $NaBH_4$ (417 mg, 11.0 mmol) was added to start reaction. After reacted for 25 min, H_2O was added to dissolve precipitates, and dioxane was removed by evaporator. After adding acetic acid to mixture, product was extracted with $CHCl_3$ for 4 times. Organic layer was washed with brine once, then concentrated and precipitated by adding 80 mL of diethyl ether, followed by centrifugation at 10000 *g* for 10 min at -10 °C. The supernatant was removed by decantation, and the product was dried in a vacuum. White viscoid solid of **1** was obtained (403 mg, 79 %).
^{1}H-NMR of one (600 MHz, $CDCl_3$) δ: 7.78 (m, 2H), 7.64 (t, 2H), 7.41 (t, 2H), 7.33 (t, 2H), 5.99 (bs, 1H), 4.36 (d, 2H), 4.24 (t, 1H), 3.51 (br, 2H), 2.98 (d, 1H), 2,78 (s, 6H), 1.99-1.51 (br, 6H). ESI-MS calculated $[M+H]^+$: 397.21, observed: 397.24.

Synthesis of 2. Compound **1** (356 mg, 962 μmol), EDC (297.6 mg, 1.55 mmol) and HOBt (136.7 mg, 1.01 mmol) were dried up and then dissolved into 10 ml of dry DCM in N_2 atmosphere. Then, oleylamine (396 μl, 1.20 mmol) were added to start reaction. After reacted for 2 h, the reaction mixture was concentrated by evaporator, and the product was purified with silica column chromatography (DCM/MeOH: 7/1). Yellow

oil of **2** was obtained (639 mg, 90.5 %).
^{1}H-NMR of one (600 MHz, $CDCl_3$) δ: 7.78 (m, 2H), 7.64 (t, 2H), 7.41 (t, 2H), 7.33 (t, 2H), 6.16 (bs, 1H), 5.34 (t, 2H), 4.33 (m, 2H), 4.24 (t, 1H), 3.23 (m, 2H), 3.07 (d, 1H), 2.98 (br, 2H), 2,82 (d,d, 6H), 1.99 (br, 4H), 1.76 (m, 2H), 1.57 (m, 4H), 1.25 (m, 22H), 0.87 (t, 3H). ESI-MS calculated $[M+H]^+$: 646.49, observed: 646.59.

Synthesis of 3. Compound **2** (150 mg, 193 μmol) was dissolved into 6 ml of methanol and 1 ml of 2 M NaOH aq was added to start reaction. After reacted overnight, methanol was removed by evaporator, and then, product was extracted with $CHCl_3$ for 6 times (3 times with brine). The organic layer was dried with Na_2SO_4, then concentrated and dried in a vacuum. This crude product of **3** was used in next reaction without further purification.

Synthesis of 4. 4-[4-(1-Hydroxyethyl)-2-methoxy-5-nitrophenoxy]-butyric acid (56.9 mg, 218 μmol), EDC (71.5 mg, 372 μmol) and HOBt (30.7 mg, 227 μmol) were added to the crude product of **3**, dried up and dissolved into 10 ml of dry DCM in N_2 atmosphere. After reacted overnight, the reaction mixture was concentrated by evaporator, and the product was purified with silica column chromatography (DCM/MeOH: from 7/1 to 1/1). Yellow solid of **4** was obtained (65.7 mg, 46 %).
^{1}H-NMR of one (600 MHz, DMSO-d_6) δ: 7.52 (s, 1H), 7.36 (s, 1H), 5.52 (d, 1H), 5.33 (br, 2H), 5.25 (t, 1H), 4.21 (m, 2H), 4.02 (t, 2H), 3.90 (m, 1H+3H), 3.00 (m, 2H+2H), 2,75 (d,d, 6H), 2.30 (br, 2H), 1.96 (br, 6H), 1.60 (m, 2H), 1.50 (m, 2H), 1.37-1.22 (m, 27H), 0.85 (t, 3H). ESI-MS calculated $[M+H]^+$: 705.51, observed: 705.70.

Synthesis of 5. Compound **4** (201 mg, 284 μmol) and 4-Nitrophenyl chloroformate (496 mg, 2.46 mmol) were dried up and then dissolved into 10 ml of dry DCM in Ar atmosphere. 300 μl of distilled TEA (2.15 mmol) was added to start reaction. After reacted for 3 h, the reaction mixture was concentrated by evaporator and dried in a vacuum overnight, and the product was purified with silica column chromatography (DCM/MeOH: 6/1). Orange oil of **5** was obtained (37.8 mg, 15.3 %).
^{1}H-NMR of one (600 MHz, DMSO-d_6) δ: 8.30 (d, 2H), 7.59 (s, 1H), 7.53 (d, 2H), 7.20 (s, 1H), 6.27 (bs, 1H), 5.31 (br, 2H), 4.20 (m, 2H), 4.06 (t, 2H), 3.96 (s, 3H), 3.90 (t, 1H), 3.00 (m, 2H+2H), 2,71 (s, 6H), 2.31 (br, 2H), 1.97 (br, 6H), 1.73 (d, 3H), 1.59 (m, 2H), 1.50 (m, 2H), 1.35 (br, 2H), 1.21 (m, 22H), 0.84 (t, 3H). ESI-MS calculated $[M+H]^+$: 870.52, observed: 870.65.

Synthesis of 6. Compound **5** (31.3 mg, 35.6 μmol) and SUNBRIGHT PA-034HC (110 mg, 32.3 μmol) were dried up and then dissolved into 10 ml of dry DCM in N_2 atmosphere. 100 μl of distilled TEA was added to start reaction. After reacted for 4 days, the reaction mixture was concentrated by evaporator, and the product was precipitated by adding 80 mL of cold diethylether (-20 °C), followed by centrifugation at 10000 *g* for 10 min at -20 °C. The supernatant was removed by decantation, and the product was dried in a vacuum. Yellow solid f **6** was obtained (94 mg, 70.5 %).
^{1}H-NMR of one (600 MHz, DMSO-d_6) δ: 8.08 (bs, 1H), 7.91 (bs, 1H), 7.58 (s, 1H), 7.37 (bs, 1H), 7.12 (s, 1H), 6.08 (d, 1H), 5.31 (br, 2H), 4.23 (m, 2H), 4.03 (br, 2H), 3.92 (s, 4H), 3.68-3.39 (br, PEG), 2.98 (m, 2H+2H), 2,65-2.45 (br, 6H+DMSO), 2.30 (br, 2H), 2.19 (t, 2H), 2.10 (m, 2H), 1.97 (m, 4H), 1.67 (m, 2H), 1.56 (t, 2H), 1.54-1.45 (m, 7H), 1.35 (br, 2H), 1.31-1.17 (m, 24H), 0.84 (t, 3H).

Synthesis of 7. Compound **6** (34.1 mg, 8.25 μmol) was dried up and then dissolved into the mixture of 2 ml of dry MeOH and 4 ml of dry MeCN in N_2 atmosphere. Then, MeI (6 ml, 96 μmol) and DIPEA (1.7 ml, 9.73 μmol) were added to start reaction. After reacted overnight, the product was precipitated by adding 80 mL of cold diethylether (-20 °C), followed by centrifugation at 10000 *g* for 10 min at -20 °C. The supernatant was removed by decantation, and the precipitate was dried in a vacuum and then, dissolved in water, followed by dialysis with MWCO=2,000 membrane. Freeze-drying of the water solution gave pale yellow solid of **7** (7.53 mg, 19 %).
^{1}H-NMR of one (600 MHz, DMSO-d_6) δ: 8.06 (bs, 1H), 7.91 (bs, 1H), 7.56 (s, 1H), 7.37 (bs, 1H), 7.12 (s, 1H), 6.08 (d, 1H), 5.30 (m, 2H), 4.22 (m, 2H), 4.03 (br, 2H), 3.91 (s, 3H), 3.83 (m, 1H), 3.64-3.28 (br, PEG+H_2O), 3.03 (s, 9H), 2.98 (m, 2H+2H), 2.80 (s, 2H), 2.30 (t, 2H+2H), 2.01-1.90 (br, 4H+2H), 1.67 (m, 2H), 1.58-1.47 (br, 9H), 1.42-1.19 (br, 26H), 0.84 (t, 3H).

Synthesis of PEG-PL-Me$_3$K-Oleyl. Compound **7** (16.9 mg, 1.32 μmol), NHS (4.70 mg, 40.8 μmol) and DCC (55.3 mg, 268 μmol) were dried up and then dissolved into 10 ml of dry DCM in N_2 atmosphere. 100 μl of dry TEA was added to start reaction. After reacted for 1 day, the reaction mixture was filtered, concentrated and then precipitated by adding 80 mL of diethyl ether, followed by centrifugation at 10000 *g* for 10 min at -10 °C. The supernatant was removed by decantation, and the product was dried in a vacuum. Yellow solid of **PEG-PL-Me$_3$K-Oleyl** was obtained (5.50 mg, 89 %).
^{1}H-NMR of one (600 MHz, DMSO-d_6) δ: 8.07 (bs, 1H), 7.93 (bs, 1H), 7.58 (s, 1H), 7.38 (bs, 1H), 7.11 (s, 1H), 6.10 (d, 1H), 5.36 (m, 2H), 4.22 (m, 2H), 4.03 (br, 2H), 3.91

(s, 3H), 3.83 (m, 1H), 3.70-3.20 (br, PEG+H_2O), 3.03 (s, 9H), 2.98 (m, 2H+2H), 2.89 (s, 4H), 2.80 (s, 1H), 2.30 (t, 2H+2H), 2.01-1.90 (br, 4H+2H), 1.73 (m, 2H), 1.58-1.47 (br, 9H), 1.42-1.19 (br, 26H), 0.84 (t, 3H).

3. Synthesis of PEG-PL-nEG-Oleyl

A series of photo-cleavable PEG-lipid with an origo(ethylene glycol) (EG) linker (n = 2, 4, 8 and 40) between the oleyl and the PL moieties was synthesized in almost the same synthetic scheme as that of **PEG-PL-4EG-Oleyl** (Fig. S2).

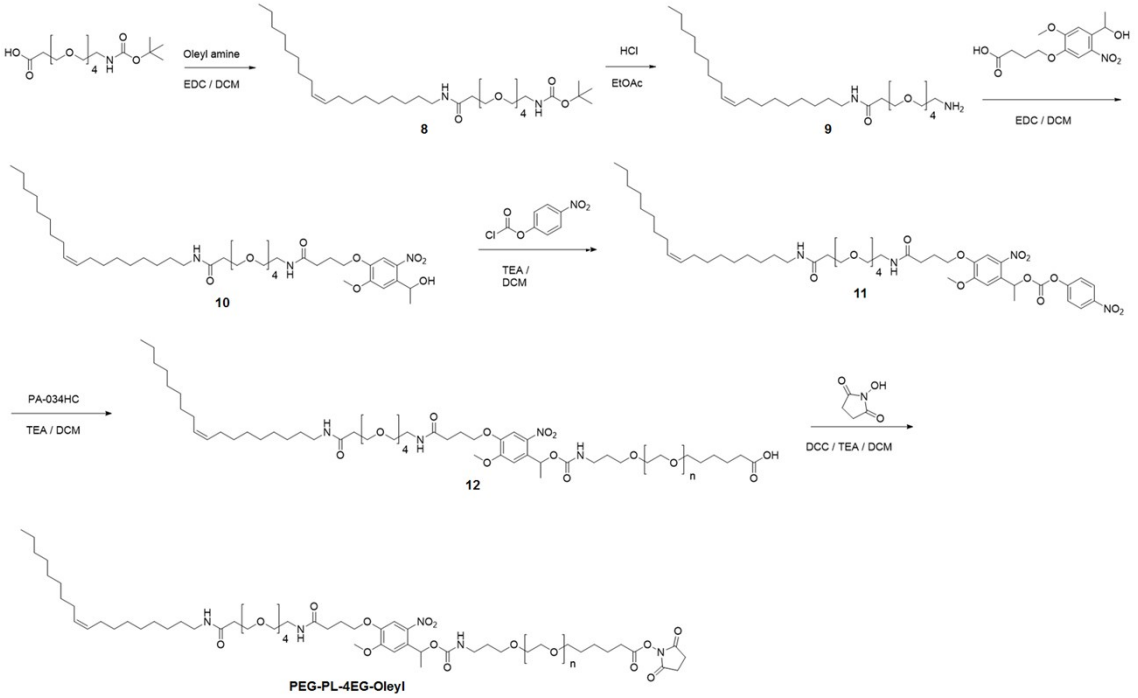

Figure S2. Synthetic scheme of **PEG-PL-4EG-Oleyl**

Synthesis of 8. (Boc-amino)-PEG4-carboxylic acid (388 mg, 1.06 mmol), EDC (443 mg, 2.30 mmol) and HOBt (155 mg, 1.14 mmol) were dried and then dissolved into 20 ml of dry DCM. Then, Oleylamine (450 μl, 1.36 mmol) was added to start reaction. After reacted for 6 days, the reaction mixture was concentrated by evaporator, and the product was purified with silica column chromatography (DCM/MeOH: 9/1). Yellow oil of **8** was obtained (573 mg, 84.5 %).

^{1}H-NMR of one (600 MHz, DMSO-d_6) δ: 7.78 (s, 1H), 6.77 (s, 1H), 5.33 (m, 2H), 3.57 (t, 2H), 3.48 (s, 8H), 3.03 (m, 2H), 3.01 (t, 2H), 2.28 (t, 2H), 1.98 (m, 4H), 1.37 (m, 11H), 1.35-1.23 (br, 22H), 0.84 (t, 3H). ESI-MS calculated $[M+Na]^+$: 637.89, observed: 637.64.

Synthesis of 9. Compound **8** (101 mg, 163 μmol) was dissolved into 3 ml of ethylacetate, and 1.5 ml of 12 M HCl aq was added to start reaction. After reacted overnight, ethylacetate was removed by evaporator, and then, product was dried in a

vacuum. This crude product of **9** was used in next reaction without further purification.

Synthesis of 10. 4-[4-(1-Hydroxyethyl)-2-methoxy-5-nitrophenoxy]-butyric acid (61.9 mg, 206 μmol) and EDC (65.2 mg, 340 μmol) were added to the crude product of **9** (100.7 mg, 163 μmol), dried up and dissolved into 10 ml of dry DCM in N_2 atmosphere. Then, distilled TEA (1.43 mmol) was added to start reaction. After reacted overnight, the reaction mixture was concentrated by evaporator, and the product was purified with silica column chromatography (DCM/MeOH: 7/1). Brown oil of **10** was obtained (138 mg, 87 %).
^{1}H-NMR of one (600 MHz, DMSO-d_6) δ: 7.85 (s, 1H), 7.78 (s, 1H), 7.51 (s, 1H), 7.36 (s, 1H), 5.50 (d, 1H), 5.32 (br, 2H), 5.26 (t, 1H), 4.02 (t, 2H), 3.90 (s, 3H), 3.57 (t, 2H), 3.48 (s, 8H), 3.20 (m, 2H), 3.00 (m, 2H), 2,25 (m, 4H), 1.94 (br, 6H), 1.37 (m, 5H), 1.34-1.23 (m, 22H), 0.84 (t, 3H). ESI-MS calculated $[M+H]^+$: 818.52, observed: 818.59.

Synthesis of 11. Compound **10** (46.6 mg, 60.8 μmol) and 4-Nitrophenyl chloroformate (70.0 mg, 348 μmol) were dried up and then dissolved into 10 ml of dry DCM in Ar atmosphere. 100 μl of distilled TEA (718 μmol) was added to start reaction. After reacted for 2 h, the reaction mixture was concentrated by evaporator and dried in a vacuum overnight, and the product was purified with silica column chromatography (DCM/MeOH: 20/1). Orange oil of **11** was obtained (85.2 mg, 85.0 %).
^{1}H-NMR of one (600 MHz, DMSO-d_6) δ: δ: 8.30 (d, 2H), 7.95 (s, 1H), 7.78 (s, 1H), 7.58 (s, 1H), 7.54 (d, 2H), 7.19 (s, 1H), 6.28 (q, 1H), 5.31 (br, 2H), 4.07 (t, 2H), 3.96 (s, 3H), 3.57 (t, 2H), 3.49 (s, 8H), 3.20 (m, 2H), 3.01 (m, 2H), 2,27 (m, 4H), 1.96 (br, 6H), 1.73 (s, 3H), 1.61 (m, 2H), 1.35-1.23 (m, 24H), 0.84 (t, 3H). ESI-MS calculated $[M+Na]^+$: 983.54, observed: 984.64.

Synthesis of 12. Compound **11** (65.3 mg, 40.1 μmol) and SUNBRIGHT PA-034HC (101 mg, 29.8 μmol) were dried up and then dissolved into 10 ml of dry DCM in N_2 atmosphere. 200 μl of distilled TEA was added to start reaction. After reacted for 2 days, the reaction mixture was concentrated by evaporator, and the product was precipitated by adding 80 mL of cold diethylether (-20 °C), followed by centrifugation at 10000 *g* for 10 min at -20 °C. The supernatant was removed by decantation. Then, the precipitate was dissolved into DCM, and the product was precipitated by adding cold ethanol (-20 °C), followed by centrifugation at 10000 *g* for 10 min at -20 °C. The supernatant was removed by decantation, and the precipitate was dried in a vacuum. Yellow solid of **12** was obtained (89 mg, 70.6 %).

^{1}H-NMR of one (600 MHz, DMSO-d_6) δ: 7.93 (bs, 1H), 7.79 (bs, 1H), 7.58 (s, 1H), 7.37 (bs, 1H), 7.12 (s, 1H), 6.08 (d, 1H), 5.31 (br, 2H), 4.03 (br, 2H), 3.92 (s, 3H), 3.68-3.39 (br, PEG), 3.20 (m, 2H), 3.01 (br, 4H), 2,27 (m, 4H), 2.19 (t, 2H), 1.96 (br, 6H), 1.62 (s, 3H), 1.53-1.43 (br, 6H), 1.35-1.23 (m, 26H), 0.84 (t, 3H).

Synthesis of PEG-PL-4EG-Oleyl. Compound **12** (55.5 mg, 13.1 μmol), NHS (16.4 mg, 142 μmol) and DCC (147 mg, 711 μmol) were dried up and then dissolved into 10 ml of dry DCM in N_2 atmosphere. 100 μl of dry TEA was added to start reaction. After reacted for 2 days, the reaction mixture was filtered, concentrated and then precipitated by adding 80 mL of diethyl ether, followed by centrifugation at 10000 *g* for 10 min at -10 °C. The supernatant was removed by decantation, and the product was dried in a vacuum. Yellow solid of **PEG-PL-4EG-Oleyl** was obtained (53.5 mg, 92 %).
^{1}H-NMR of one (600 MHz, DMSO-d_6) δ: 7.93 (bs, 1H), 7.78 (bs, 1H), 7.58 (s, 1H), 7.38 (bs, 1H), 7.11 (s, 1H), 6.10 (d, 1H), 5.32 (m, 2H), 4.03 (br, 2H), 3.92 (s, 3H), 3.63 (m, 2H), 3.61-3.43 (br, PEG), 3.20 (m, 2H), 3.01 (br, 4H), 2.82 (s, 4H), 2.78 (t, 2H), 2,27 (m, 4H), 1.96 (br, 4H), 1.82 (br, 2H), 1.68-1.55 (m, 7H), 1.52 (br, 4H), 1.39 (m, 2H), 1.32-1.22 (m, 24H), 0.84 (t, 3H).

Synthesis of PEG-PL-2EG-Oleyl. Fmoc-NH-PEG2-propionic acid (193 mg, 0.501 mmol) and EDC (364 mg, 1.89 mmol) were dried and then dissolved into 15 ml of dry DCM. Then, Oleylamine (200 μl, 0.600 mmol) was added to start reaction. After reacted overnight, the reaction mixture was concentrated by evaporator, and the product was purified with silica column chromatography (DCM/MeOH: 10/1). Yellow oil of Fmoc-NH-PEG2-olyelamide was obtained (200 mg, 63%).
^{1}H-NMR of one (600 MHz, DMSO-d_6) δ: 7.90 (d, 2H), 7.69 (d, 2H), 7.62 (bs, 1H), 7.45 (m, 2H), 7.37 (bs, 1H), 7.33 (m, 2H), 5.32 (m, 2H), 4.29 (d, 2H), 4.21 (t, 1H), 3.85 (t, 2H), 3.57 (m, 4H), 3.43 (t, 2H), 3.16 (t, 2H), 3.06 (m, 2H), 1.95 (m, 4H), 1.37 (t, 2H), 1.31-1.09 (br, 20H), 0.84 (t, 3H).

Fmoc-NH-PEG2-olyelamide (200 mg, 315 μmol) was dissolved into 15 ml of methanol, and 2 ml of 2 M NaOH aq was added to start reaction. After reacted for 1 h, methanol was removed by evaporator, and then, product was extracted into ethylacetate four times. Organic layer was washed with water, dried with Na_2SO_4, concentrated by evaporator and dried in a vacuum. By using this crude product, **PEG-PL-2EG-Oleyl** was synthesized through 4-step reactions in the same synthetic scheme as described above. Finally, yellow solid of **PEG-PL-2EG-Oleyl** was obtained (57.4 mg, 97%).

^{1}H-NMR of one (600 MHz, DMSO-d_6) δ: δ: 7.98 (bs, 1H), 7.60 (bs, 1H), 7.38 (s, 1H), 7.11 (s, 1H), 7.03 (s, 1H), 6.08 (d, 1H), 5.32 (m, 2H), 4.02 (br, 2H), 3.95 (s, 3H), 3.62-3.39 (br, PEG), 3.23 (m, 2H), 3.01 (m, 2H), 2.95 (m, 2H), 2.79 (t, 2H), 2.63 (t, 2H), 2.39 (m, 4H), 1.98 (br, 4H), 1.80 (br, 2H), 1.73 (m, 2H), 1.59, (br, 4H), 1.46 (m, 2H), 1.37 (m, 2H), 1.29-1.16 (m, 24H), 0.80 (t, 3H).

Synthesis of PEG-PL-8EG-Oleyl. Fmoc-NH-PEG8-propionic acid (550 mg, 0.750 mmol) and EDC (433.18 mg, 2.26 mmol) were dried and then dissolved into 10 ml of dry DCM. Then, oleylamine (198 μl, 0.603 mmol) was added to start reaction. After reacted overnight, the reaction mixture was concentrated by evaporator, and the product was purified with silica column chromatography (DCM/MeOH: 10/1). Yellow oil of Fmoc-NH-PEG8-olyelamide was obtained (526 mg, 86%).

^{1}H-NMR of one (600 MHz, DMSO-d_6) δ: 7.90 (bs, 2H), 7.75 (bs, 2H), 7.42 (s 2H), 7.35 (bs, 2H), 5.32 (m, 2H), 4.30 (d, 2H), 4.20 (t, 1H), 4.15 (t, 2H), 3.43 (m, 16H), 3.31-3.19 (m, 4H), 3.01 (t, 2H), 2.26 (d 2H) 1.95 (m, 4H), 1.39 (t, 2H), 1.31-1.09 (br, 20H), 0.84 (t, 3H).

Fmoc-NH-PEG8-olyelamide (526 mg, 0.735 mmol) was dissolved into 7 ml of methanol, and 3 ml of 2 M NaOH aq was added to start reaction. After reacted for 20 min, methanol was removed by evaporator, and then, product was extracted into ethylacetate four times. Organic layer was washed with water, dried with Na_2SO_4, concentrated by evaporator and dried in a vacuum. By using this crude product, **PEG-PL-8EG-Oleyl** was synthesized through 4-step reactions in the same synthetic scheme as described above. Finally, yellow solid of **PEG-PL-8EG-Oleyl** was obtained (46.6 mg, 91%).

^{1}H-NMR of one (600 MHz, DMSO-d_6) δ: 7.99 (br, 1H) 7.82 (bs, 1H), 7.59 (s, 1H), 7.41 (bs, 1H), 7.11 (s, 1H), 6.10 (d, 1H), 5.32 (m, 2H), 4.13 (br, 2H), 3.94 (s, 3H), 3.78-3.43 (br, PEG), 3.21 (m, 2H), 3.01 (br, 4H), 2.81 (s, 4H), 2.62 (t, 2H), 2.23 (m, 4H), 1.98 (br, 4H), 1.80 (br, 2H), 1.77-1.40 (m, 8H), 1.38 (m, 2H), 1.30-1.1 (m, 24H), 0.82 (t, 3H).

Synthesis of PEG-PL-40EG-Oleyl. SUNBRIGHT BO-020HC (320 mg, 16.0 mmol) was dried and then dissolved in 2 ml of dry DCM. Subsequently, oleylamine (105 μl, 32.0 mmol) and DIC (150 μl, 96.0 mmol) were added. After reacted overnight, the reaction mixture was concentrated by evaporator, the product was diluted in small amount of DCM and purified with diethyl ether precipitation, as described above. Yellow solid of Boc-amino-PEG40-olyelamine was obtained (298 mg, 82%).

^{1}H-NMR of one (600 MHz, DMSO-d_6) δ: 7.75 (s, 1H), 6.78 (s, 1H), 5.35 (m, 2H), 4.36 (t, 3H), 4.21-4.35(br, PEG), 3.02-2.95 (m, 4H), 2.23 (t, 2H), 1.59 (m, 2H), 1.42 (m, 4H), 1.40 (s, 9H), 1.30-1.05 (br, 22H), 0.82 (t, 3H).

Boc-amino-PEG40-olyelamine (293 mg 0.132 mmol) was dissolved in 3 mL of DCM, followed by the slow addition of TFA (3 ml, 20.7 mmol). After four hours, the reaction mixture was concentrated by evaporator, the product was purified with diethyl ether precipitation and subsequently dried under vacuum. **PEG-PL-40EG-Oleyl** was synthesized through 4-step reactions in the same synthetic scheme as described above. Finally, yellow solid of **PEG-PL-40EG-Oleyl** was obtained (58.0 mg, 95%).

^{1}H-NMR of one (600 MHz, DMSO-d_6) δ: 8.25 (t 2H), 7.80 (bs, 2H), 7.65 (s, 1H), 7.28 (s, 1H), 5.25 (m, 2H), 4.25-4.33(br, PEG), 4.36 (t, 3H), 3.10 (m, 4H), 2.85 (s, 4H), 2.78 (t, 2H), 2,28 (m, 4H), 1.74 (br, 6H), 1.65-1.56 (m, 6H), 1.55-1.41 (br, 22H) 0.91 (t, 3H).

4. Evaluation of the cell trapping ability in a microfluidic system

The evaluation system based on a micro-flow path was prepared according to the modified procedures in our previous report[2]. A glass substrate (Coverslips for sticky-Slides, catalog number: 10812, ibidi GmbH, Munich, Germany) was washed by sonication in acetone for 10 min and then in isopropanol for 10 min, followed with drying. In this study, collagen coating was selected as the basement coating which presented amine groups for modification of the PEG-lipid via amide-coupling reaction. In our previous works[1,3], bovine serum albumin (BSA) was employed as the basement coating for immobilization of non-adherent cells, but such immobilization on the collagen surface was more reproducible than on the BSA surface probably because collagen coating is stronger against washing the surfaces. In particular, high-speed laminar flow was used on the surface in this study, and therefore, more robust collagen coating was selected. The substrate surface was coated with collagen by immersing in an acidic solution (0.3 mg/mL, pH 3) of collagen (Cellmatrix Type I-A, from Nitta Gelatin, Osaka, Japan) overnight, followed with rinsing with MilliQ water two times and drying.

A NHS-activated reagent of PEG-lipids was dissolved in dry DMSO at 10 mM, and then, the solution was diluted 100-folds by PBS just before use. 3 μL of the PEG-lipid solution (final 100 μM) was dropped at the center position on the collagen-coated substrate and then incubated for 1 hour at 37 ˚C under saturated water vapor. The PEG-lipid-modified surface was washed with MilliQ water six times, followed with drying. The PEG-lipid-modified substrate and a sticky-slide (catalog number: 81128, ibidi GmbH, Munich, Germany) which had a microchannel (width, length and height of 5,

48.5 and 0.1 mm) were fitted together in PBS to prevent trapping of air in the flow path, and then the combined sticky-slide and glass were fixed in place with a set of handmade stainless steel clips[2].

Murine pro-B cell line BaF3 expressing enhanced green fluorescent protein (EGFP) (EGFP-BaF3) was used as a model nonadherent cell. EGFP-BaF3 was kindly gifted from Prof. Masahiro Kawahara in The Univ. of Tokyo, and cultured in RPMI1640 medium supplemented with 10% FBS and 1 ng/ml IL3. 150 μL of a cell suspension (3×10^6 cells/ml) was poured into the flow path, followed by incubation for 30 min to enable immobilization. Then, the flow path was connected to a syringe pump (Legato 100, KD Scientific, Holliston, MA, USA) with tubes (LMT-55, Saint-Gobain, Courbevoie, France) and connectors (VRF106 and VFT106, ISIS Co., Ltd., Osaka, Japan). PBS was poured into the flow path at 0.1 mL/min for 1 min to wash out non-trapped cells. Then, to examine the cell trapping ability, PBS was poured for 1 min at the flow speed from 1 to 40 mL/min. Before and after such microfluidic stress, the florescent images of the remaining cells on the substrate surface was obtained with a confocal laser-scanning microscope (LSM510, Carl Zeiss, Jena, Germany) equipped with 10× objective lenses. Image analysis was performed with ImageJ software (NIH, Bethesda, MD).

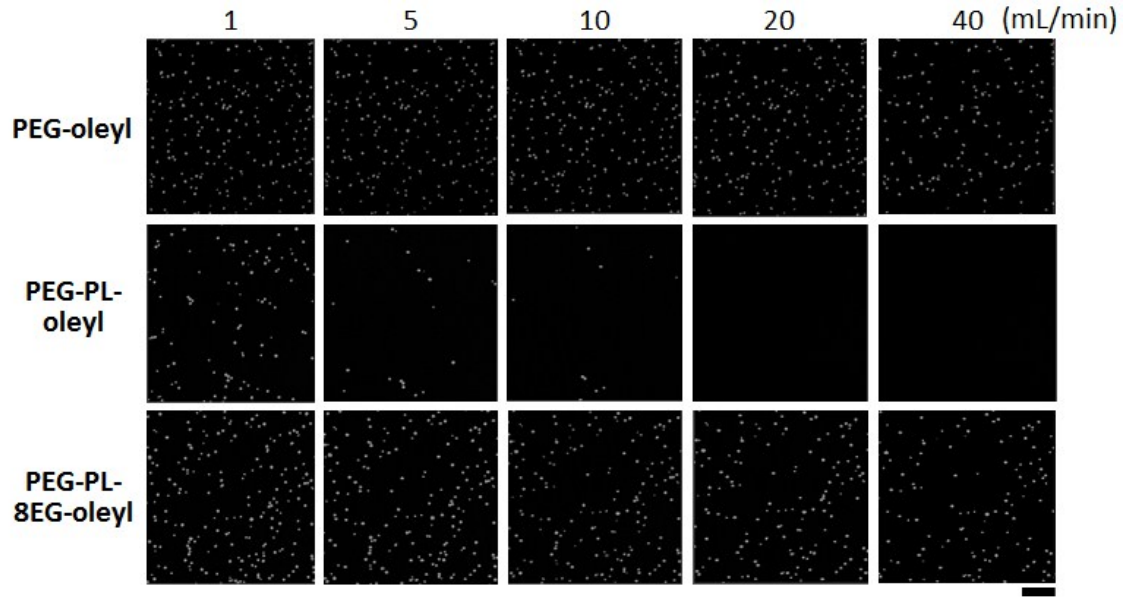

Figure S3. Fluorescence microscopic images of the remaining cells at a certain place on the substrates modified with **PEG-oleyl** (top), **PEG-PL-oleyl** (middle) and **PEG-PL-8EG-oleyl** (botoom) after treatment with laminar flow at 1, 5, 10, 20 and 40 mL/min. Scale bar: 200 μm.

5. Light-guided cell micropatterning

The flow path was prepared according to the following procedures. To irradiate a fine pattern of light, a thin glass film (thickness: 0.03 mm, D263 Teco WD003-50S, from Schott Glaswerke, Mainz, Germany) was employed as the bottom. Before material modification, the sticky-slide with a microchannel was pasted on the thin glass bottom by using the glue on the surface of this sticky-slide. Then, the bottom surface was coated with BSA by loading 1% BSA solution (in PBS) into the path, followed with incubation overnight at 4 °C. The BSA solution was removed from the outlet of the path with a pipette, and the bottom surface was washed by both pouring and removing 1 mL of PBS three times. The BSA-coated surface was modified with **PEG-PL-8EG-oleyl** by loading 100 μM **PEG-PL-8EG-oleyl** solution (in PBS) into the path, followed with incubation for 1 h at 37 °C. Similar to BSA coating, the material solution was removed and washed with PBS three times. This flow path was put on the photomask, which was printed with array patterns (50 μm × 50 μm array grid) of circular spots (spot diameter: 14-22 μm), and light (360 nm, 1.5 J/cm^2) was irradiated from below through the photomask. Light irradiation was performed with a xenon light source through a band-pass filter (± 5 nm; MAX-302, from Asahi Spectra Co. Ltd., Tokyo, Japan). To immobilize cells, 200 μL of an EGFP-BaF3 cell suspension (1 × 10^7 cells/ml) was poured into the flow path, followed by incubation for 30 min. Then, non-trapped cells were washed with 1 mL of PBS three times. The cell pattern was observed with a confocal microscope as described above.

6. Light-guided cell release

EGFP-BaF3 was trapped on the **PEG-PL-8EG-oleyl-**modified bottom of the flow path according to the procedure as described above. The bottom of the flow path was irradiated from below with the xenon light source (360 nm) at various doses of light from 0.5 to 3 J/cm^2. After light irradiation, PBS was poured into the flow path at 1 mL/min for 1 min to wash out the released cells. Before and after light irradiation and washing, the bottom was observed with the confocal microscope as described above. The cell densities were determined by image analysis, and the cell remaining ratios were calculated by dividing the cell density after light irradiation with that before light irradiation.

References

[1] S. Yamaguchi, S. Yamahira, K. Kikuchi, K. Sumaru, T. Kanamori and T. Nagamune, *Angew. Chem. Int. Ed.*, 2012, **51**, 128 –131.

[2] S. Izuta, S. Yamaguchi, R. Misawa, S. Yamahira, M. Tan, M. Kawahara, T. Suzuki, T. Takagi, K. Sato, M. Nakamura, T. Nagamune and A. Okamoto, *Sci. Rep.*, 2017, **7**, 14962.

[3] S. Yamahira, S. Yamaguchi, M. Kawahara, T. Nagamune, *Macromol. Biosci.*, 2014, **14**, 1670-1676.