Supporting information to:

Control of Lipid Bilayer Phases of Cell-Sized Liposomes by Surface-Engineered Plasmonic Nanoparticles

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Supporting Materials and Methods

General Information

General reagents were purchased from Nakalai Tesque (Kyoto, Japan). Gold(III) chloride and sodium borohydride ($NaBH_4$) were obtained from Sigma-Aldrich (Saint Louis, MO, USA). Silver nitrate, L-(+)-ascorbic acid, and sodium oleate were purchased from Wako (Osaka, Japan). 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) was obtained from NOF (Tokyo, Japan). 1,2-Dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-*N*-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-PE), and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) (Rhodamine-PE) were purchased from Avanti Polar Lipids, Inc (Alabaster, AL, USA). Methyl-beta-cyclodextrin (MβCD) and cholesterol (chol) were obtained from Sigma-Aldrich (Saint Louis, MO, USA). ITO glasses were purchased from Sigma, and the fluorescent ganglioside probe (ATTO-GM3) was obtained from Prof. Hiromune Ando[1]. Visible/near-infrared (Vis/NIR) spectra were measured using the V-630 spectrometer (JASCO Cooperation, Tokyo, Japan). Giant unilamellar vesicles (GUV) images were obtained using Zeiss LSM 800 confocal fluorescent microscope or Nikon Ti eclipse fluorescent microscope. The concentration of the cationic high-density lipoprotein mutant (catHDL) on a protein basis was measured with a DC Protein Assay kit (Bio-Rad, CA, USA). The hydrodynamic diameter of catHDL was measured utilizing Nanotrack UPA-UT151 apparatus (MicrotracBEL, Osaka, Japan).

catHDL preparation

The preparation of catHDL was described previously.[2,3] Briefly, POPC and DOTAP were mixed in ethanol in a round-table flask at a molar ratio of 7:3. A 6 mol% solution of NBD-PE in ethanol was added if necessary. The reaction mixture was concentrated and dried under reduced pressure to remove all solvents. The lipid film formed at the bottom of the flask was dispersed using 30 mg/mL sodium cholate solution in phosphate-buffered saline (PBS, pH 7.4). Following incubation at 37 °C for at least 2 h, a recombinant apoA-I mutant with a deleted N terminal 43 amino acid, and a cell-penetrating TAT peptide fused at the C terminus were mixed at a protein-to-lipid molar ratio of 100 in PBS containing 4 M urea. The reaction mixture was incubated at room temperature (r.t.) overnight. The mixture containing catHDL was dialyzed against 3 L PBS for at least 4 h, changing the dialysate three times to remove urea, sodium cholate, and unreacted proteins. Subsequently, the sample was centrifuged to remove debris. The hydrodynamic diameter of catHDL was determined at approximately 37 nm using Nanotrack UPA-UT151 apparatus.

Synthesis of CTAB-coated AuNRs

AuNRs were synthesized utilizing the seedless method.[4] Typically, 1200 μL of a 4 mM aqoueous

solution of $AgNO_3$, 400 μL of 50 mM aqoueous solution of $HAuCl_4$, and 258 μL of 100 mM solution of aqueous L-ascorbic acid were added to 40 mL of 0.1 M aqueous solution of hexadecyltrimethylammonium bromide (CTAB). After the color of the reaction mixture turned from yellow to colorless, 48 μL of 35% HCl and 30 μL of a freshly prepared ice-cold aqueous solution of $NaBH_4$ were added while stirring. AuNRs formed in the mixture were pelletted by centrifuging at 20,000 × *g* for 40 min at 25 ˚C, followed by incubation at 30 ˚C overnight. Finally, the AuNRs were redispersed in 0.1 M CTAB at ca. 1 mg/mL.

Preparation of pm-AuNR

pm-AuNRs were synthesized according to our previous report.[2,3] Briefly, 1 mL of CTAB-coated AuNR (ca. 1 mg/mL) was centrifuged at 20,000 × *g* for 20 min at 25 ˚C followed by removal of 900 μL of supernatants. The sample was redispersed with the resting 100 μL supernatants, diluted with 900 μL of deionized water, then centrifuged at 20,000 × *g* for 20 min at 25 ˚C. Subsequently, 750 μL of the supernatant was removed and 250 μL of 16 mg/mL sodium oleate solution was added to the samples. This mixture was heated for 1 h at 50 ˚C and the products (oleate-coated AuNRs) were purified using the Nap5 gel column (GE Healthcare). Oleate-coated AuNRs were mixed with catHDL in a 1:0.4 weight ratio and then heated for 1 h at 50 ˚C in a 1.5 mL microtube. The products (pm-AuNRs) were centrifuged at 20,000 × *g* for 20 min at 25 ˚C and all supernatants were carefully removed. pm-AuNRs were suspended in a 200 mM sucrose solution at ca. 1 mg/mL.

Preparation of GUVs

GUVs were prepared using the electro-formation method.[5] 40 mM of DPPC, DOPC, and cholesterol in chloroform were mixed in a Durham tube. 1 mg/mL solution of Rhodamine-PE or ATTO594-GM3 in ethanol was added if necessary. A portion of the mixture (5.7 mmol lipids) was dropped and spread on an ITO glass (8–12 Ω/sq, Sigma-Aldrich, MO). The ratios of lipids and cholesterol for Lo, Ld, Lo/Ld, and So/Ld GUVs were DPPC:DOPC:Chol = 2:0:1, 0:1:0, 2:2:1 or 1:1:0, respectively. After heating for 5 min at 50 ˚C, the lipid film was further spread by adding a single drop of hot chloroform, then drying *in vacuo* for ≥2 h. The sample glass was covered with another ITO glass using a silicon sheet (ca. 1 mm thickness) with a 1.5 cm square-shaped hole to create a small chamber on the lipid film. 0.3×10^2 μL of 200 mM sucrose solution was added into the chamber and a home-made electric circuit was made of thin foil, the chamber, and an arbitrary function generator AFG-2005 (GW Instek). For So/Ld GUVs, the procedure was slightly modified. The lipid film for So/Ld GUVs was heated at 70 ˚C for 5 min instead of 50 ˚C, and the 200 mM sucrose solution and other apparatus were heated at 70 ˚C prior to electrification. Electric power (10 Hz, 1.4 vpp, 0.35 mV) was applied to the chamber with the arbitrary function generators at 50 ˚C in an ICI-1 incubator (AS

One) overnight. After cooling the chamber to room temperature, the dispersion of GUVs was recollected from the chamber with a disposable syringe and stored in 1.5 mL tubes at room temperature.

Observation of GUVs

A chamber for GUV observation was prepared by sandwiching a single 0.1 mm thick silicon sheet with a ca. 6 mm hole between two 0.13–0.17 mm thick cover glasses (24 mm × 50 mm and 18 mm × 18 mm, respectively). Typically, after mixing 5 μL of the GUV dispersion and 5 μL of the pm-AuNRs dispersion in a microtube, 3.5 μL of the mixture was dropped within the hole on one cover glass. After the hole was covered with another cover glass to close the chamber, the phase separation pattern of GUVs was observed at room temperature with the following two types of microscopes. For a Nicon ECLIPSE Ti epifluorescence microscope (Nikon, Tokyo, Japan), the excitation/emission wavelengths were 455/485 nm for NBD-PE and 535/575 nm for Rhodamin-PE, and a ×60 objective lens (Nikon) was used. For a Zeiss LSM-800 confocal microscopy (Zeiss, Oberkochen, Germany), the excitation wavelength was 488 nm for NBD-PE and 560 nm for Rhodamin-PE. The acquired range of the emission wavelengths was adjusted with a Zen software (Zeiss), and a ×40 objective lens was used. The exposure time was adjusted so as to observe the images allowing to identify the domain structure most clearly but for <1 s to avoid photobleaching.

The contents of GUVs with a specific phase pattern in Figures 5, S3, S6, S7, S9, S12 were calculated with ≥30 independent images of more than 30 individual GUVs [6] [7]. GUVs with partially circular and partially noncircular domains and/or very small domains were classified as "unclear".

Observation of the phase transition process

2 μL of pm-AuNRs and 2 μL of Lo/Ld GUVs (DPPC:DOPC:chol = 2:2:1) were dropped within the above-mentioned hole on one cover glass. Placing another cover glass on top allowed mixing the two substances. The area near the boundary was then observed using an LSM 800 microscope.

Cholesterol depletion using MβCD

MβCD was dissolved in a 200 mM sucrose solution at 20 mM and stored at 4 ˚C. The MβCD solution was warmed at r.t. and mixed with the GUV dispersant. Microscopic observation was performed at r.t.

Preparation of pm-AuNR-chols

pm-AuNRs dispersion was mixed with 1/10 volume of 32 mg/mL chol in 2-propanol and centrifuged at 20,000 × *g* for 20 min at 25 ˚C. All supernatants were removed carefully and the pellet

was re-dispersed in a 200 mM sucrose solution at ca. 1 mg/mL.

Observation of the heating-induced So-to-Lo phase transition

pm-AuNR-chols and So/Ld GUVs (DPPC:DOPC:Chol = 1:1:0), in which the Ld phase was labeled with 0.1 mol % Rhodamine-DPPE, were mixed in a 1:1 volume ratio and heated at 50 ˚C for 10 min in a microtube. The mixture was placed into the chamber and the domain patterns of GUVs were observed using the ECLIPSE Ti microscope.

Observation of the NIR laser-induced So-to-Lo phase transition

The chamber containing the above-mentioned mixture was set on a glass-bottom dish (IWAKI) and illuminated at 852 nm with a Chameleon femtosecond-pulsed laser (Coherent). Then, the chamber was cooled to r.t. for at least 10 min prior to the analysis.

Observation of the phase transition induced by saturated chol-loaded MβCD (MβCD-chol)

MβCD-chol preparation was described previously.[8] MβCD and chol were dissolved in 200 mM sucrose solution at 5 mM and in a 1:1 chloroform:2-propanol solution at 64.7 mM, respectively. 1 mL of the MβCD solution and 19.3 μL of the chol solution were mixed, then rotated at 37 ˚C overnight to obtain a clear solution. The mixture was then filtrated with a 0.45 μm pore size filter (Millipore) and mixed with GUVs in a 1:1 volume ratio.

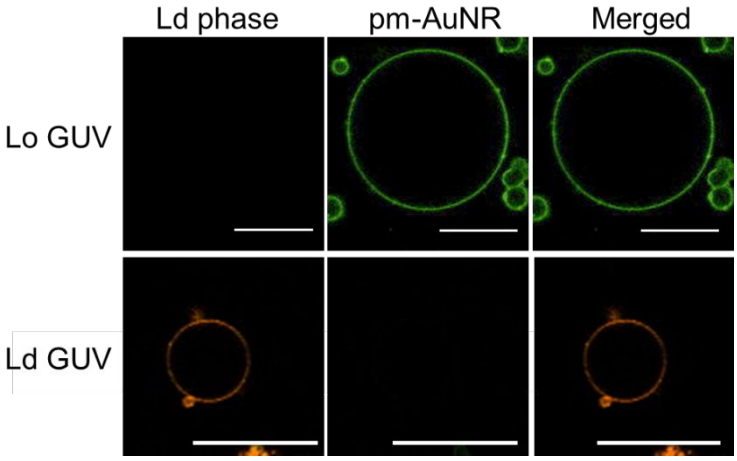

Figure S1. Lo phase selective adhesion of NBD-PE labeled pm-AuNRs. Lo-phase GUVs were prepared with DPPC and cholesterol in a 2:1 molar ratio, and Ld-phase GUVs were prepared with DOPC with 1% Rhodamine-PE, a fluorescent lipid selectively located on the Ld phase. *Scale bar,* 20 μm.

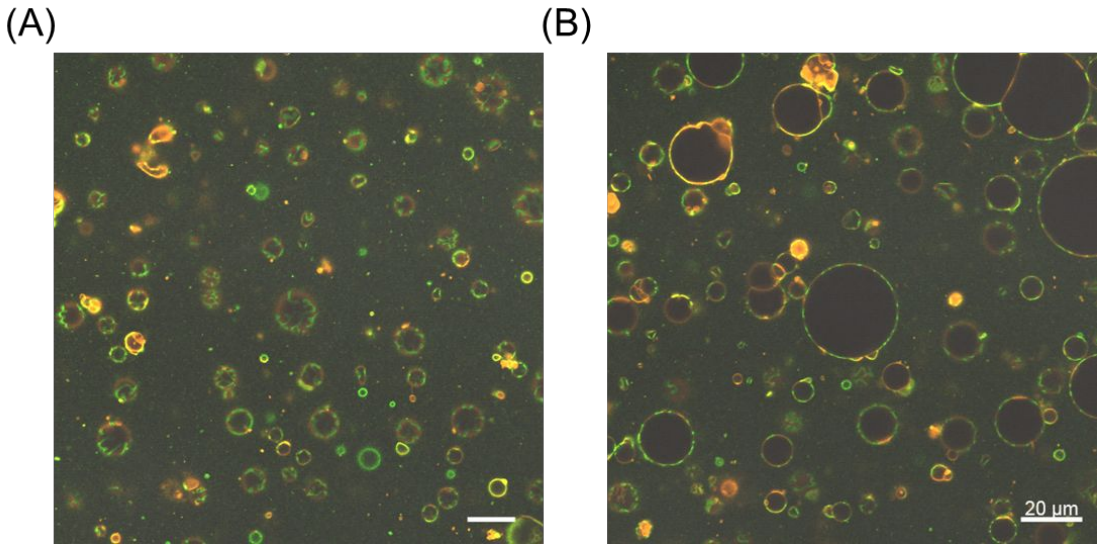

Figure S2: Typical confocal fluorescence images of GUVs following the pm-AuNRs treatment. The Ld phase domains were labeled with Rhodamine-PE (red) and pm-AuNRs were labeled with NBD-PE (green). The focus was set around the upper surfaces of many GUVs to observe their domain patterns clearly (A) and around the equatorial plane of many GUVs to detect their inside fluorescence (B). In (A), non-circular (string-shaped) domain structures in green are observed in GUVs throughout the image, while the pattern looks differently in much smaller GUVs for some unknown reason. In (B), their inside fluorescence in not significantly detected. Scale bar, 20 µm.

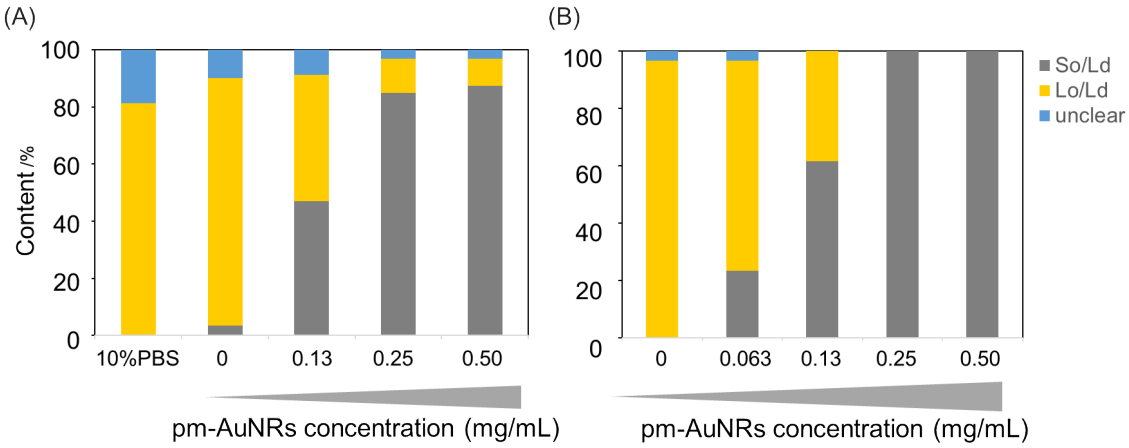

Figure S3. pm-AuNRs concentration dependency of the phase transition judged by the fluorescence signal pattern of Rhodamine-DPPE (A) (see Figure 2(B), (C)) or ATTO-GM3 fluorescence (B) (see Figure 4(B), (C)). The right-hand bar in (A), indicated as "10% PBS", means the GUV content in the presence of 10% PBS. This control experiment is to eliminate the effects of the salt involved in pm-AuNRs dispersion on the phase transition.

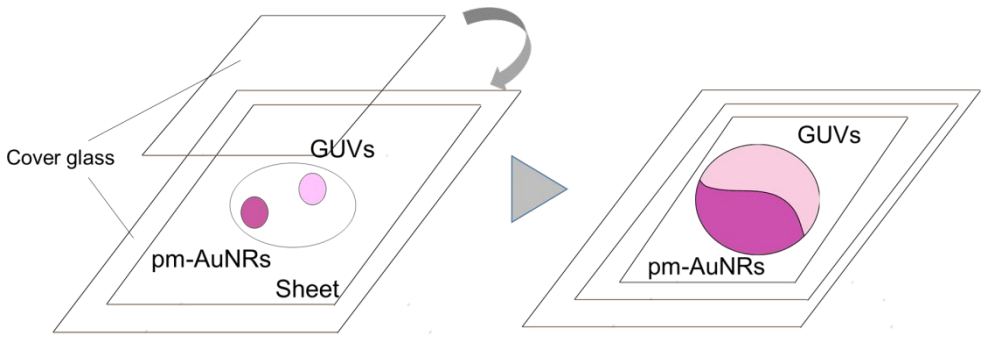

Figure S4. The schematic image of the observation of the boundary of pm-AuNRs and GUVs dispersants. A droplet of each GUVs and pm-AuNRs dispersants were loaded on a cover glass chamber, as shown on the left. A boundary was formed on the cover glass chamber, as shown on the right.

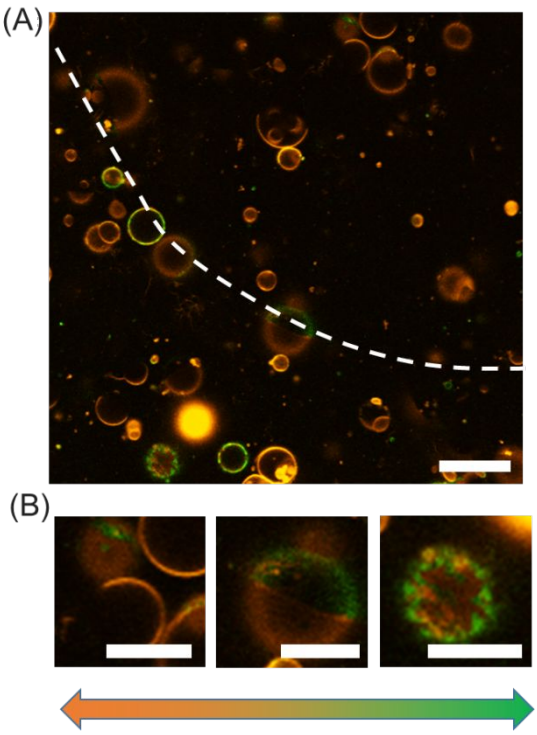

Figure S5. Typical microscopic image of GUVs near the boundary of GUVs and pm-AuNRs rich regions. (A) Fluorescent image near the contact area between the pm-AuNRs dispersion and the Lo/Ld GUVs dispersion. The white line indicates the boundary between the two areas. (B) Magnified GUV images of (A), which is in the GUVs rich region (left), on the boundary (middle), and in the pm-AuNRs rich region (right). *Scale bar*, 10 μm.

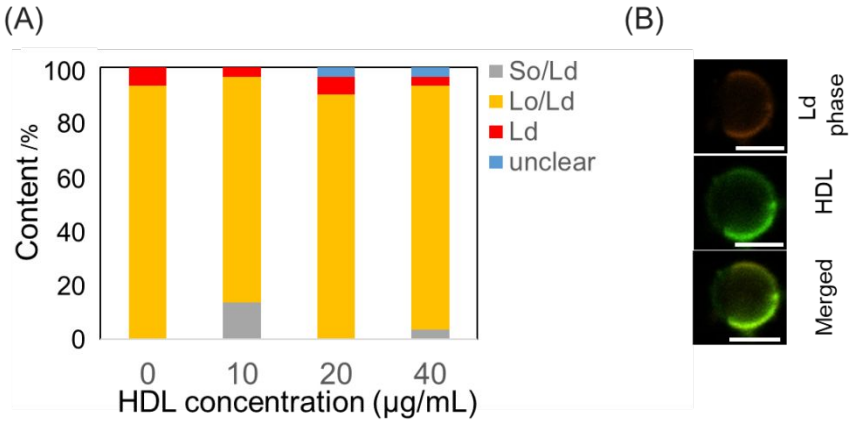

Figure S6. (A) Cationic HDL dependency of the Lo-to-So phase transition. HDLs were previously diluted in 200 mM sucrose solutions. (B) Representative image of HDL attached Lo/Ld GUV. HDL concentration was 40 μg/mL on a protein basis. *Scale bar*, 5 μm.

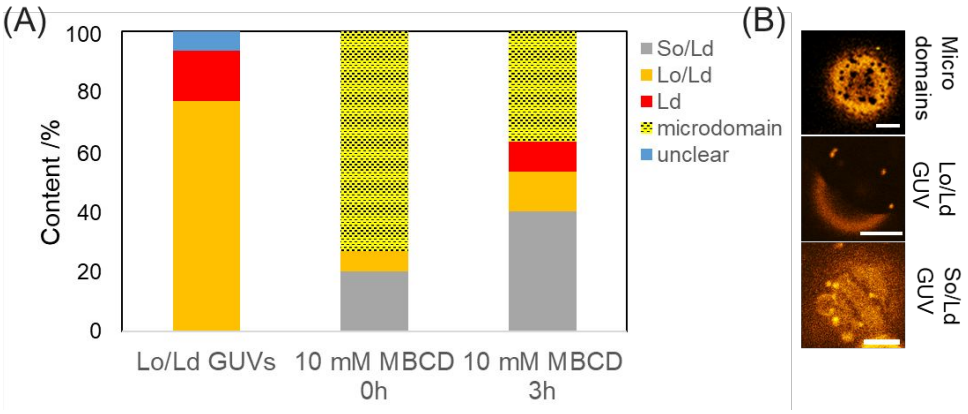

Figure S7. Effect of MβCD on the Lo-to-So phase transition in Lo/Ld-mixed GUVs (Lo/Ld-GUVs). (A) The ratio of the GUV domain patterns. Observations were performed 0 (middle) or 3 h (left) after the MβCD treatment. (B) Representative image of each domain pattern. 'Micro domains' were defined as GUVs with domains moving and larger than those involved in 'unlcear'. *Scale bar*, 5 μm.

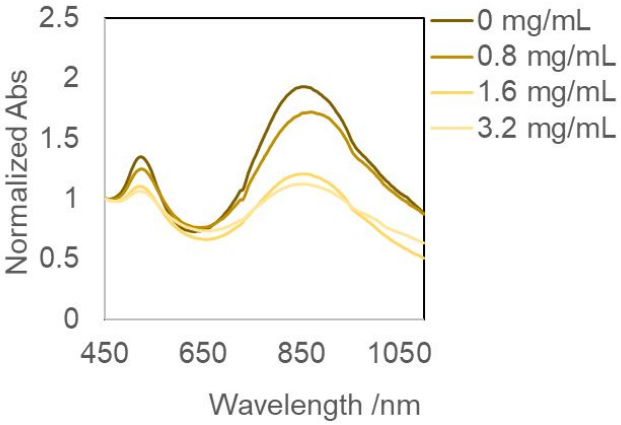

Figure S8. Normalized typical Vis/NIR spectra of cholesterol-loaded pm-AuNRs (pm-AuNRs-chol) prepared at various concentrations of chol.

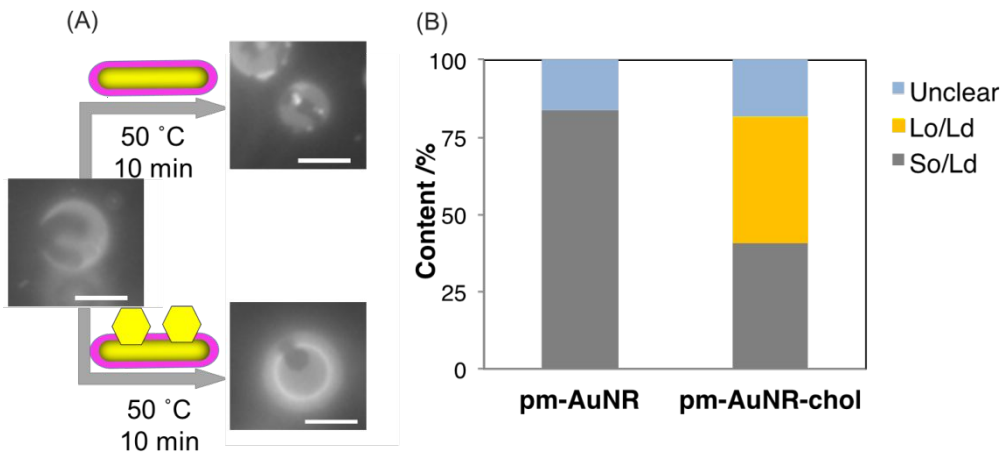

Figure S9. Heat-induced So-to-Lo phase transition with pm-AuNRs-chol. (A) Typical images of So/Ld-mixed GUVs prior to the treatment (left) and after the treatment with pm-AuNRs (top right) or pm-AuNRs-chol (bottom right) at 50 ˚C. *Scale bar*, 10 μm. (B) The ratio of the GUVs phase separation pattern after heating with pm-AuNR (light) or pm-AuNR-chol (left).

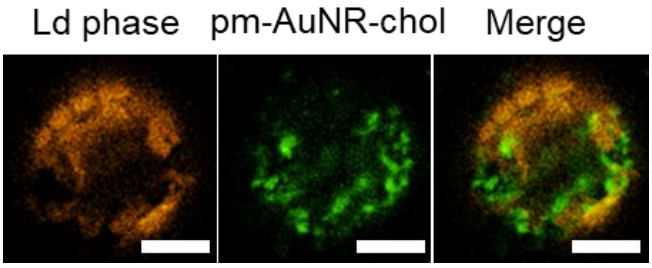

Figure S10. Representative image of a single So/Ld-mixed GUV treated with pm-AuNR-chols. The Ld phase of the GUV and pm-AuNRs-chol were fluorescently labeled with Rhodamine-PE and NBD-PE, respectively. *Scale bar*, 5 μm.

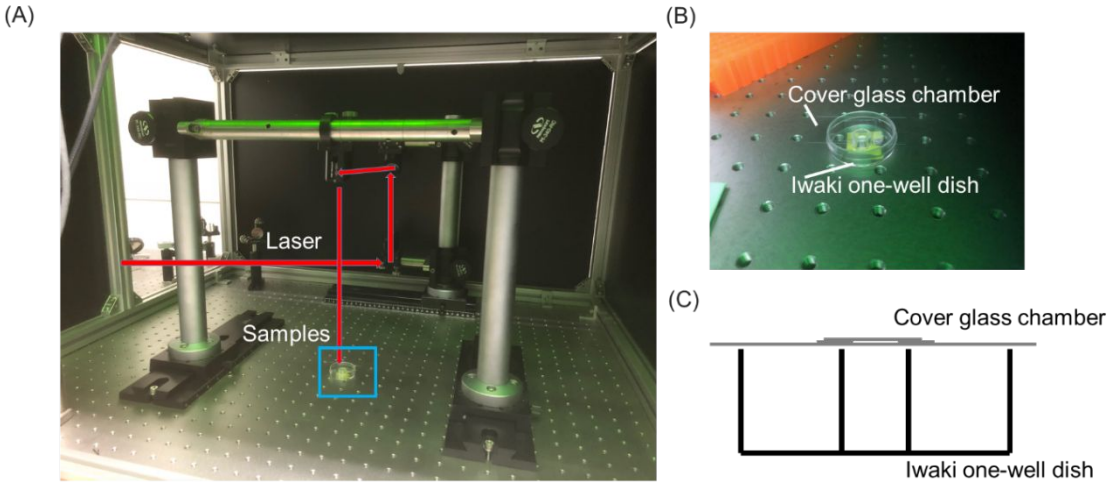

Figure S11. Laser experiment setup. (A) Experimental apparatus for NIR laser irradiation of the mixture of pm-AuNR-chols and So/Ld GUVs. The optical path of the NIR laser is indicated with red arrows. (B) Enlarged view of the blue rectangle in (A). A cover glass chamber is set on a glass-bottom dish (IWAKI). (C) Schematic image of the side view of (B).

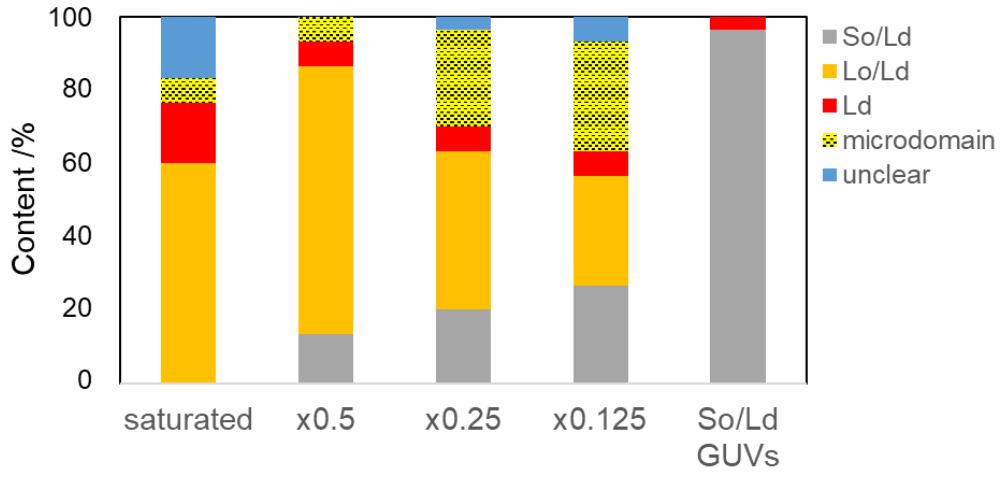

Figure S12. The So-to-Lo phase transition induced with cholesterol-loaded MβCD (MβCD-chol). The MβCD-chol solution used included ca. 1.3 mM cholesterol. The number of horizontal lines indicates the dilution degree of MβCD-chol by a 200 mM sucrose solution.

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