HOKKAIDO UNIVERSITY

Title	Therapeutic Assessment of Cytochrome C for the Prevention of Obesity Through Endothelial Cell-targeted Nanoparticulate System
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Supplementary Figure Legends

Supplementary Fig. 1. Cell specific apoptosis of the CytC-PTNP system.

pcEC-IWAT and NIH3T3 cells (5 x10^4 cells/well) were treated with the CytC-loaded PTNP and the physical mixture [empty-PTNP (0.65 μmol) + CytC (6 μg/ml)] or remaining non-treated control cells for 3h to induce apoptosis. A mixture of equal volumes of Caspase-Glo 9 reagent and cell lysate was incubated for approximately 1h and caspase 9 activity was measured based on luminescence (RLU) (n=4). The ratio of caspase 9 activation of apoptotic cells versus non-treated cells is shown. *P<0.05 vs. NT controls, n=4, ANOVA followed by Dunnett's test.

Supplementary Fig. 2. Confocal observation of plasma fractions for the confirmation of intact PTNP system in the circulation.

Mice were intravenously injected with a total lipid dose of 0.1mmol/kg body weight of the double-labeled PTNP system and the double-labeled NTNP system and incubated for 6h or remained untreated. (**a**) The plasma fractions of both treated and untreated mice were collected and then observed by CLSM. Yellow dots indicated the intactness of the NPs. Scale bar indicates 20 μm. (**b**) The spectra of NBD-DOPE and rhodamine are shown.

Supplementary Fig. 3. *In vivo* apoptosis of vascular endothelial cells with a single dose of the CytC- PTNP.

Mice (6-wks-old male *wt* C57BL/6J) were intravenously injected with a single dose of a physical mixture [empty-PTNP (0.1 mmol/kg) + CytC (6 mg/kg)] and the CytC-PTNP (6 mg/kg) or remain untreated. At 24h postinjection, tissues from several organs (inguinal region, heart, liver, spleen, lung and kidney) were collected. We identified endothelial cells (red) and apoptotic

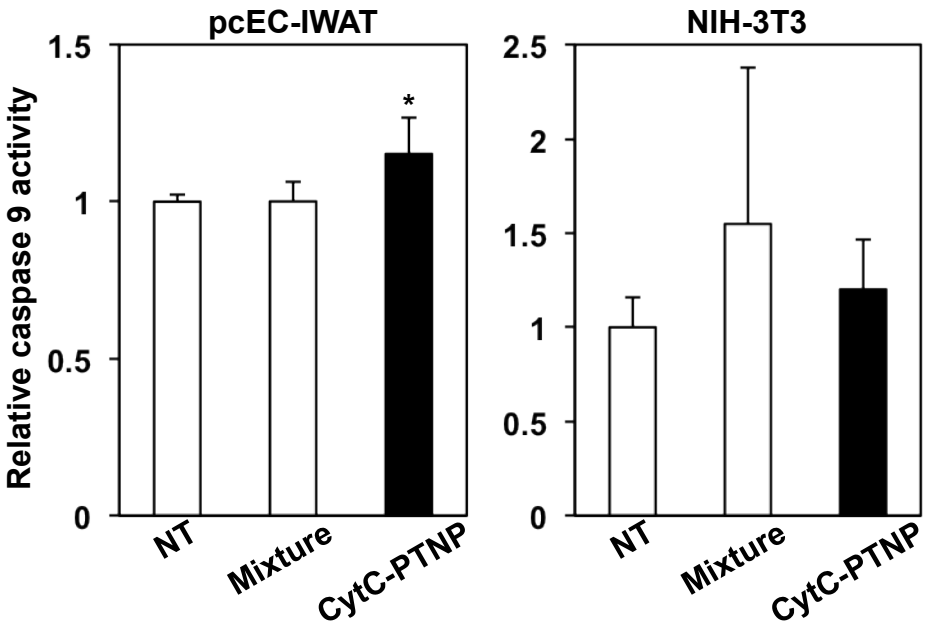
cells (green) in unfixed tissues. Cells stained with both markers appear yellow indicate endothelial cell apoptosis. Scale bar = 50 μm.

Supplementary Fig. 4. Measurement of cumulative energy intake and blood glucose level after treatment with the CytC-loaded PTNP system.

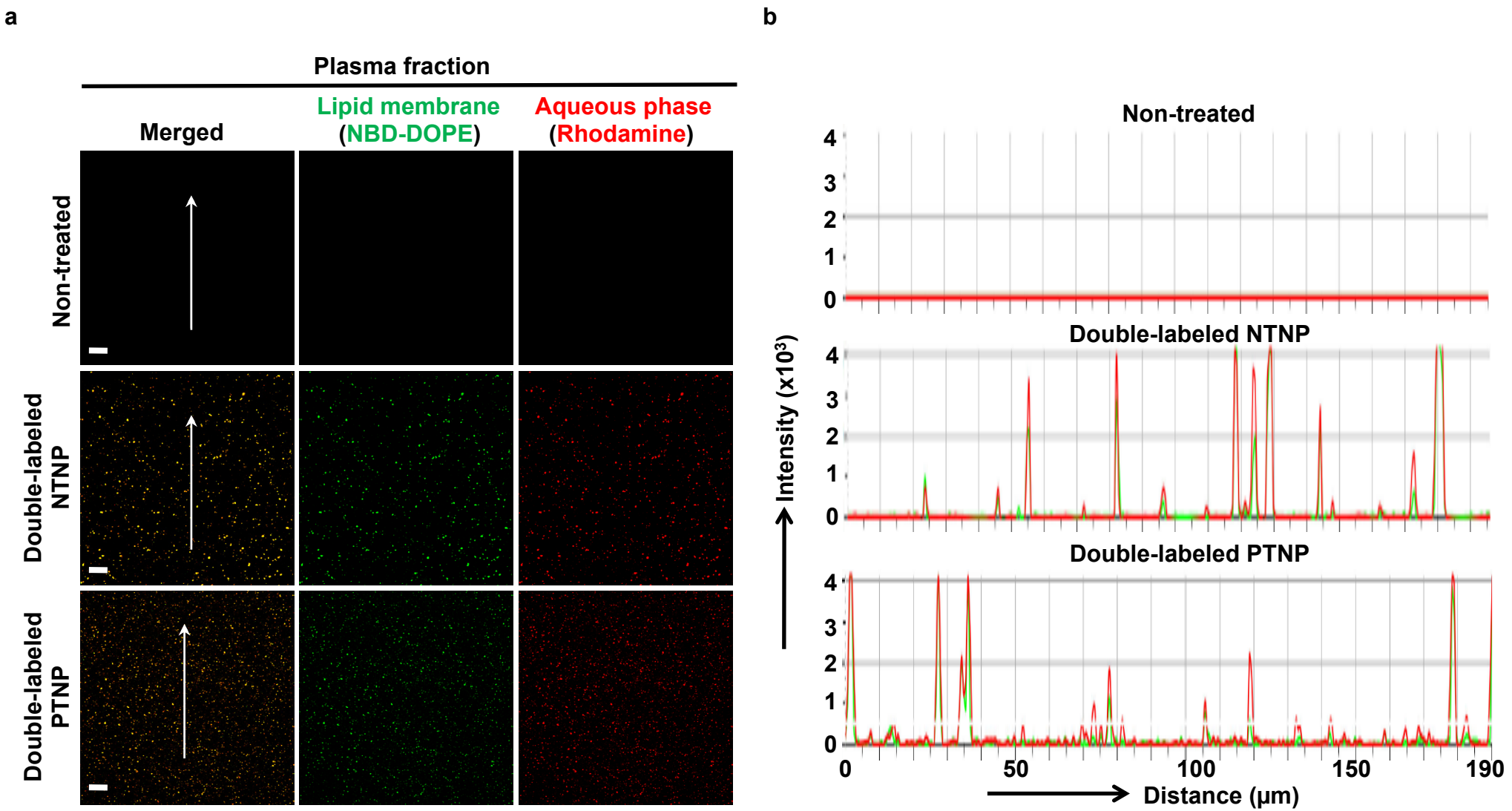
(**a**) Cumulative energy intake. Mice that were fed by HFD were treated with the CytC-PTNP system at the highest dose (6.0 mg/kg). The physical mixture [empty-PTNP (0.1 mmol/kg) + CytC soln. (6.0 mg/kg)]-treated (HFD), non-treated (HFD) and non-treated (ND) were also taken as controls. The amount of food intake of the CytC-PTNP-treated mice was measured every 24 h during a 3-day internal. The cumulative energy intake was calculated by metabolizable energy of HFD (5.10 kcal/g) and ND (3.12 kcal/g), respectively. (**b**) The blood glucose concentration of all groups in feeding condition was also measured every 24 h. *P<0.05, ANOVA followed by Tukey-Kramer's HSD test (n=3). NS means non-significant.

Supplementary Fig. 5. Physicobiological properties of the CytC after treatment with organic solvent mixture.

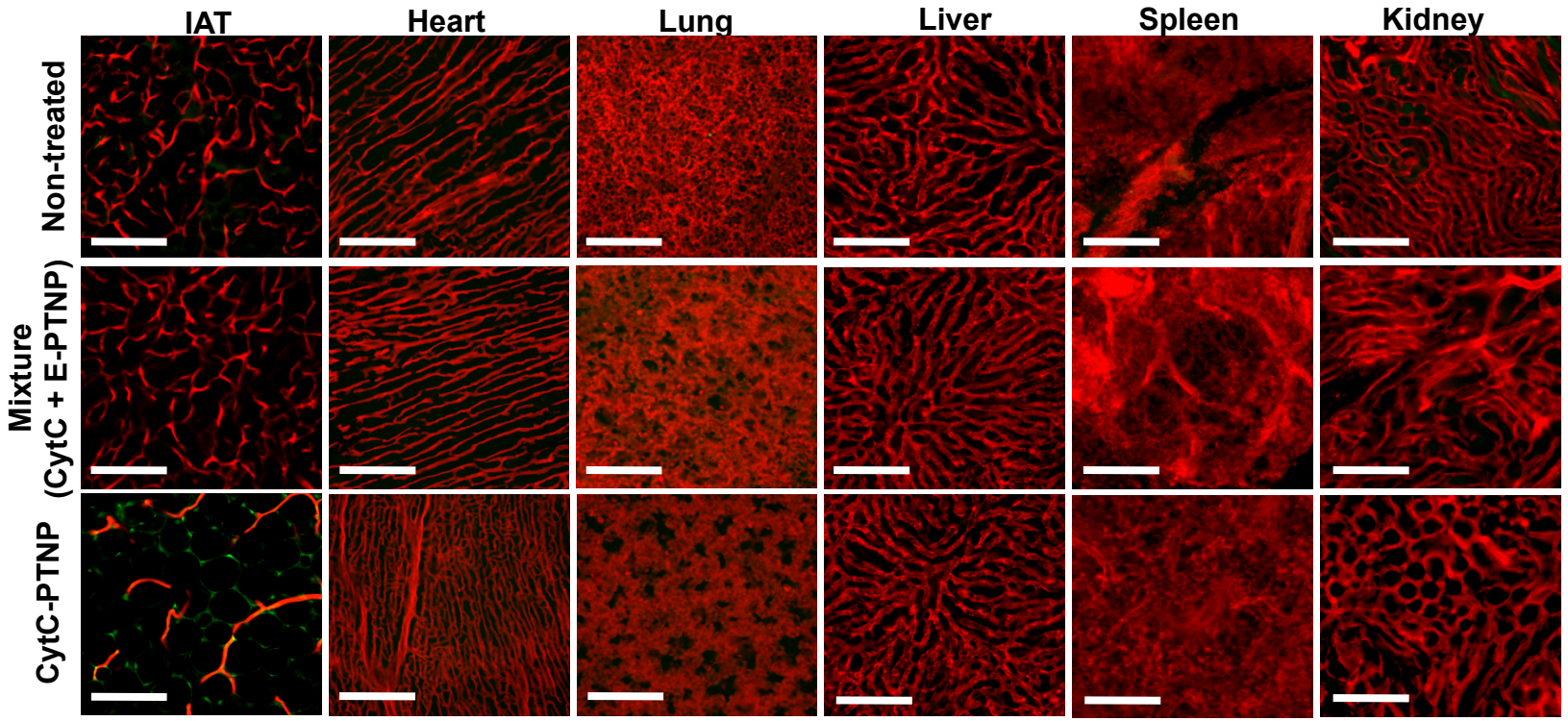
(**a**) Appearance of phase separation of the treated CytC after probe sonication. Snaps of the CytC and HEPES buffer (control) with or without treatment with a 1:1 solvent mixture (Chloroform and Diisopropyl ether) before or after probe sonication were taken using a digital camera. (**b and c**). Photometric analysis of the solvent treated CytC. The absorbance of the CytC with or without treatment with organic solvent mixture (n=3) was measured at 280 (b) and 400 nm (c). (**d**) The ratio of the abs. of the CytC with or without treatment with solvent at 400 and 280 nm is shown. **P<0.01, Student's *t*-test.



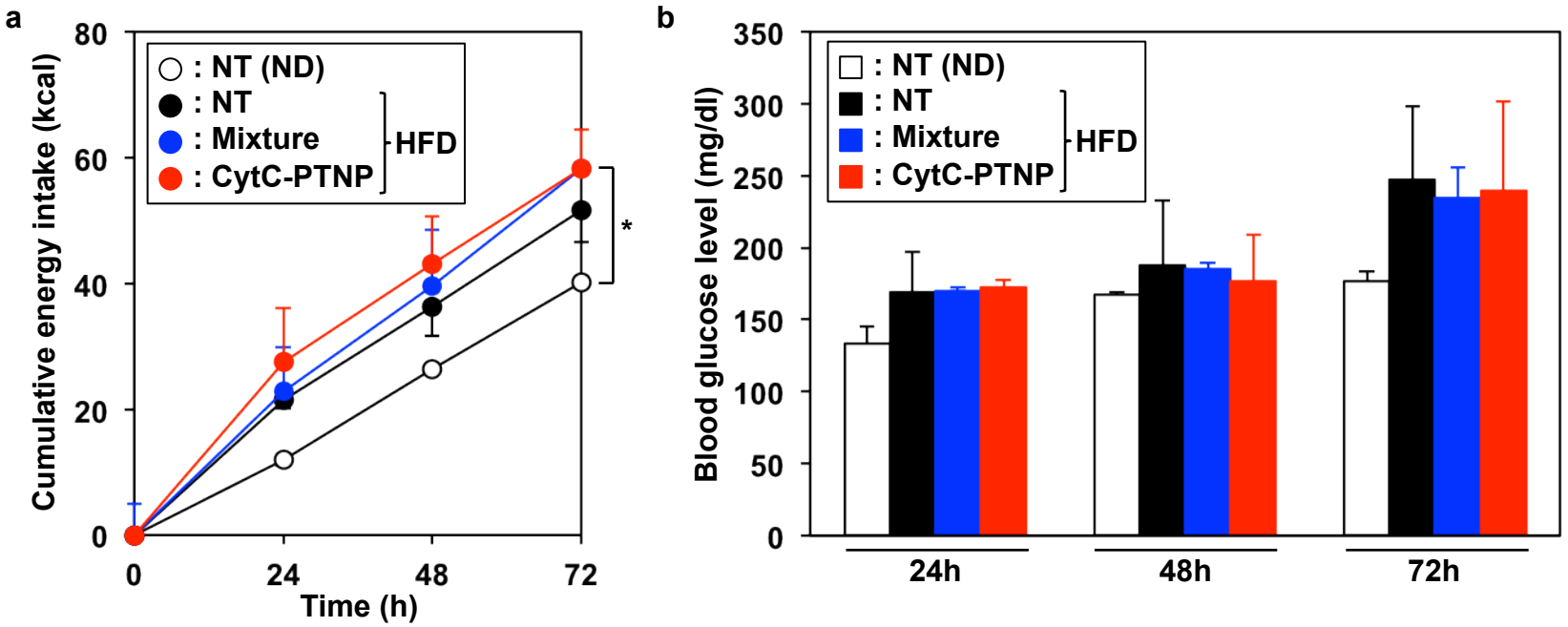
Supplementary Figure 1



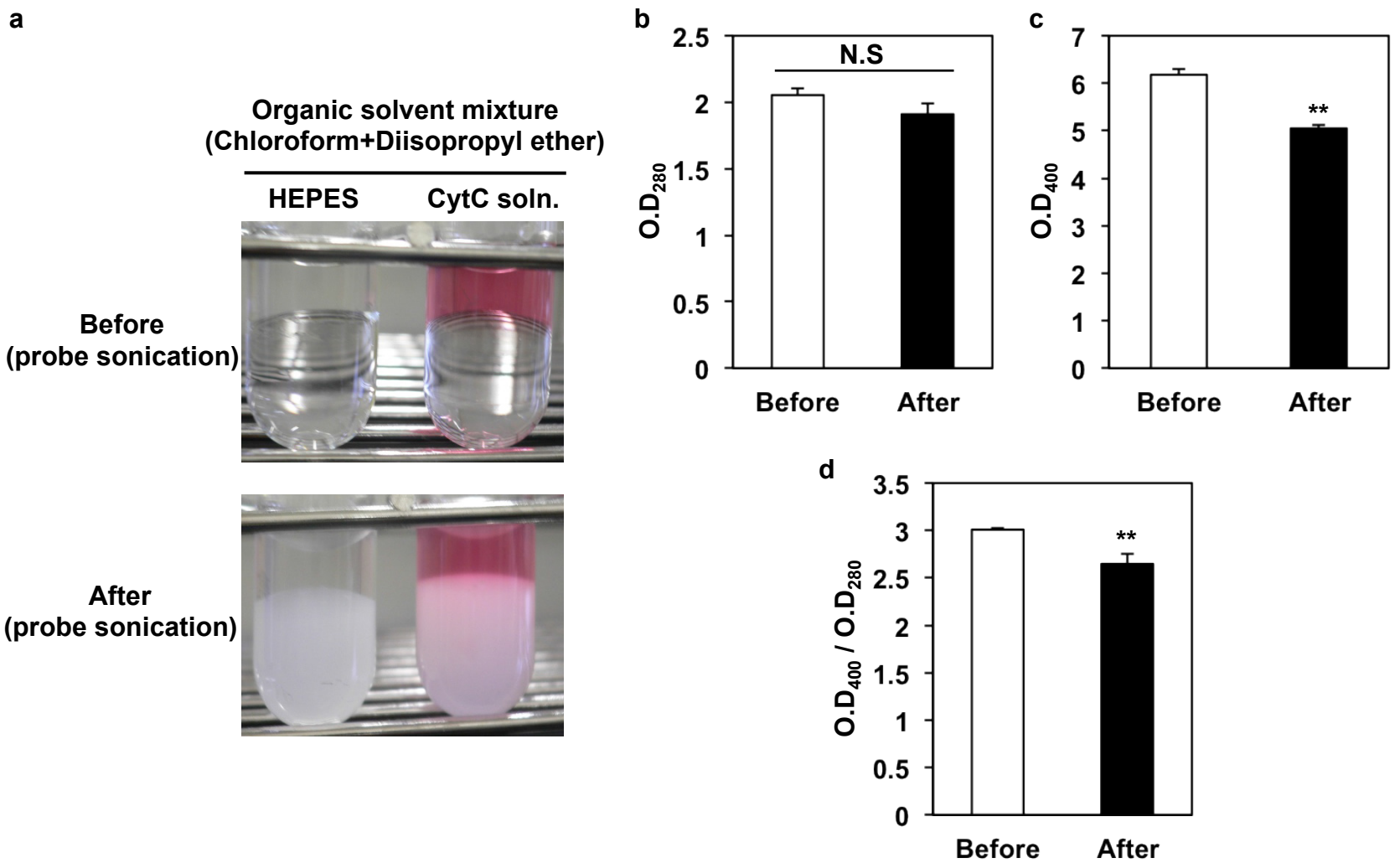
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5