Figure S1. Effect of CHS on cell viability. (A) MCF-7-Luc and HeLa-Luc cells were treated with CHS at concentrations ranging from 1.56 to 50 μg/ml. Cell viability was assessed 48 h after treatment. Data are represented as the mean + standard deviation (n=3). ***P<0.001 vs. untreated cells. Cationic and anionic liposomes without siRNA were prepared by the modified ethanol injection method and subsequently added to (B) MCF-7-Luc and (C) HeLa-Luc cells. Cell viability was assessed 48 h after transfection. Data are represented as the mean + standard deviation (n=5 for B; n=4 for C). **P<0.01 and ***P<0.001 vs. untreated cells. CHS, cholesteryl hemisuccinate; Luc, luciferase; LP, liposome; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane methyl sulfate salt; DDAB, dimethyldioctadecylammonium bromide; DC-1-16, *N*-hexadecyl-*N*, *N*-dimethylhexadecan-1-aminium bromide; TC-1-12, 11-((1,3-bis(dodecanoyloxy)-2-((dodecanoyloxy)methyl)propan-2-yl)amino)-*N*,*N*,*N*-trimethyl-11-oxoundecan-1-aminium bromide; CHS, cholesteryl hemisuccinate.

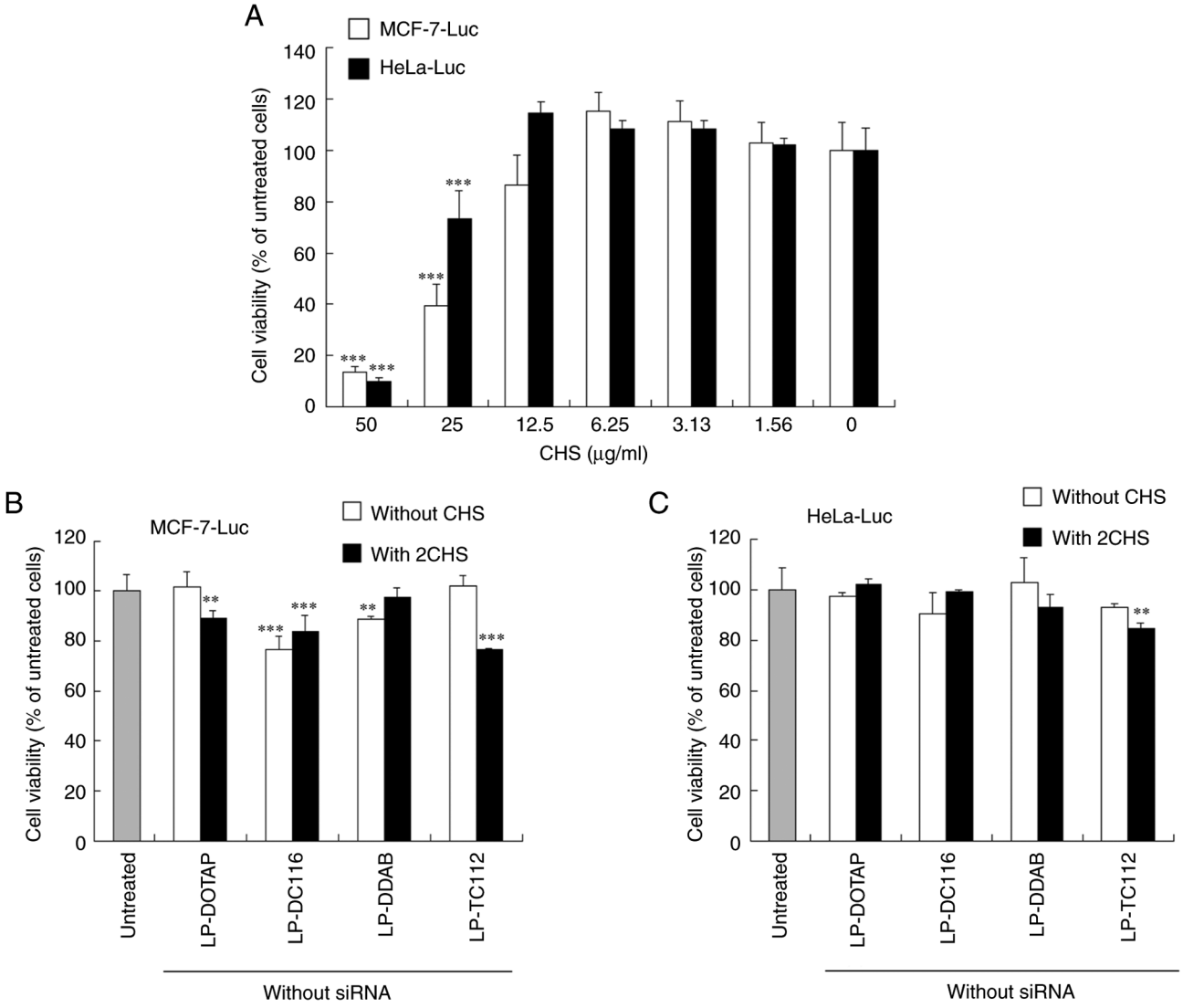

Figure S2. siRNA localization after transfection with anionic lipoplexes. Anionic lipoplexes with Cy5-siRNA were prepared using the modified ethanol injection method and added to MCF-7-Luc cells at a final siRNA concentration of 50 nM. As a control, Cy5-siRNA solution was added to the MCF-7-Luc cells. The cells were stained with Hoechst 33342 to label the cell nuclei 24 h after incubation. Localizations of Cy5-siRNA (green) and nuclei (blue) were observed by fluorescence microscopy. Scale bar, 100 μm. LP, liposome; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane methyl sulfate salt; DDAB, dimethyl-dioctadecylammonium bromide; DC-1-16, *N*-hexadecyl-*N*, *N*-dimethylhexadecan-1-aminium bromide; TC-1-12, 11-((1,3-bis (dodecanoyloxy)-2-((dodecanoyloxy)methyl)propan-2-yl)amino)-*N*,*N*,*N*-trimethyl-11-oxoundecan-1-aminium bromide; CHS, cholesteryl hemisuccinate.