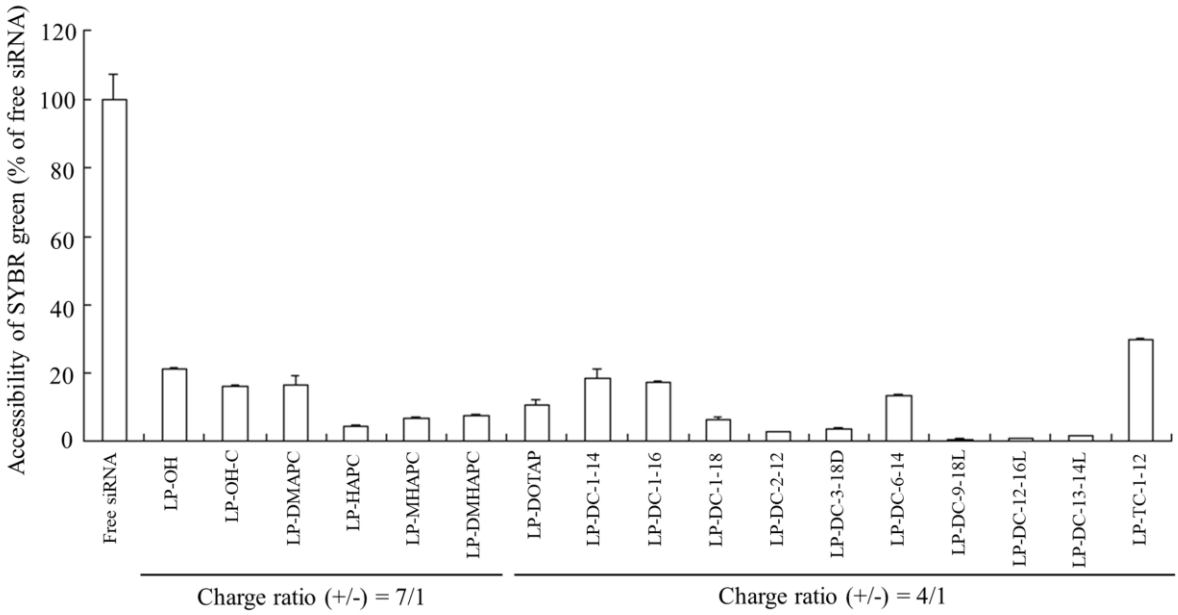
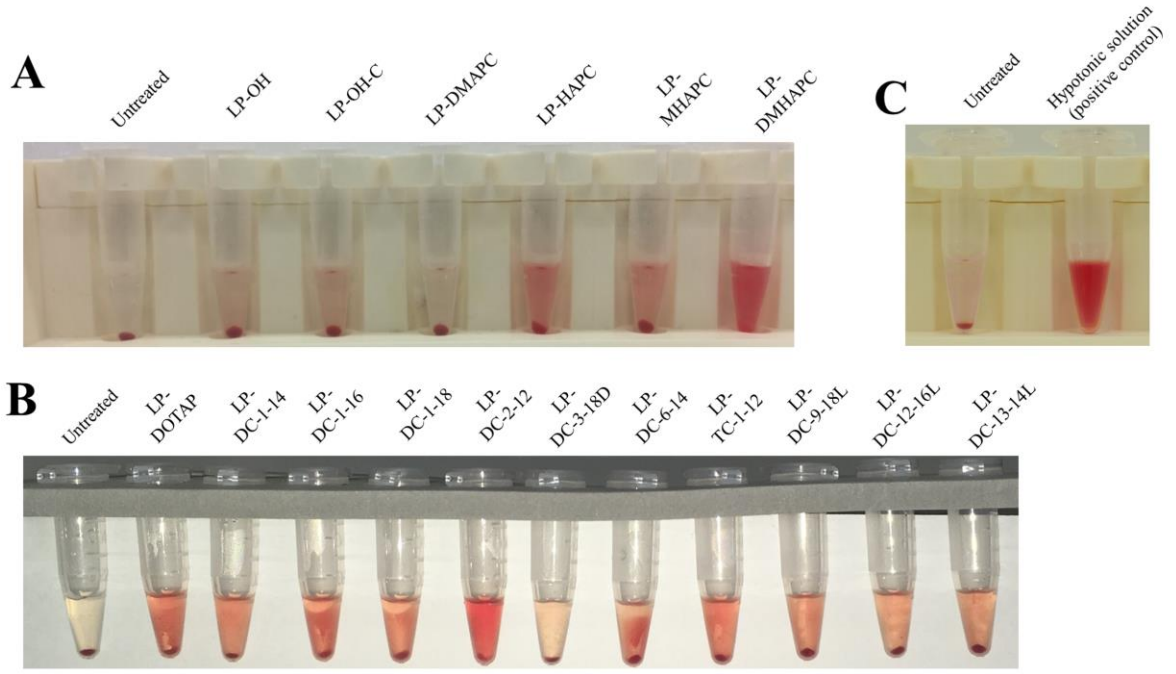
SUPPLEMENTAL DATA



Supplemental Figure S1

Supplemental Figure S1. Association of siRNA with cationic liposome by SYBR® Green assay. siRNA association by cationic lipoplexes was analyzed by exclusion assay using an SYBR® Green I Nucleic Acid Gel Stain (Takara Bio Inc., Shiga, Japan) [20]. Cationic lipoplexes were formed at charge ratios (+:-) of 7/1 for cationic liposomes composed of cationic cholesterol derivatives and 4/1 for those of dialkyl or trialkyl cationic lipids. The cationic lipoplexes of 1 μg of siRNA in a volume of 100 μL of Tris-HCl buffer (pH 8.0) were mixed with 100 μL of 2500-fold diluted SYBR® Green I Nucleic Acid Gel Stain solution with Tris-HCl buffer, and then incubated for 30 min. The fluorescence was measured at an emission wavelength of 535 nm with an excitation wavelength of 485 nm using a fluorescence plate reader (ARVO X2, Perkin Elmer, Waltham, MA, USA). As a control, the value of fluorescence obtained upon addition of free siRNA solution was set as 100%. The amount of siRNA available to interact with the SYBR® Green I is expressed as a percentage of the control.



Supplemental Figure S2

Supplemental Figure S2. Hemolysis of mouse erythrocytes by addition of cationic lipoplex. Erythrocytes were collected from blood of female BALB/c mice (8 weeks of age; Sankyo Lab. Service Corp., Tokyo, Japan) at 4°C by centrifugation at 300 *g* for 3 min and resuspended in PBS as a 2% (v/v) suspension of erythrocytes. Cationic lipoplexes with 2 μg siRNA were added to 100 μl of 2% (v/v) erythrocyte suspension. As a positive control for hemolysis (100% hemolysis), erythrocytes were suspended in hypotonic solution (water). After incubation for 15 min at 37°C, the sample was centrifuged at 200 *g* for 3 min and hemolysis of erythrocytes was observed. In A, cationic liposomes composed of cationic cholesterol derivatives and DOPE, in B, cationic liposomes composed of dialkyl or trialkyl cationic lipids and DOPE, and in C, hypotonic solution were used.