**Supplementary methods**

**Preparation of (9Z,12Z)-Octadecadien-1-ol**

 This compound was prepared by a method previously reported with minor modifications.1 To a suspension of lithium aluminum hydride (375 mg, 20 mmol) in THF (50 mL) at 40C, was added linoleic acid (1.65 mL, 10 mmol). The mixture was stirred for 30 min and then heated at reflux for 2 hours. The reaction was quenched with 5 mL of water and 50 mL of 1 M NaOH (aq). The reaction suspension was diluted with EtOAc and washed with brine twice, dried with Na2SO4, and concentrated to dryness. The crude product was purified by silica gel column chromatography (hexane:EtOAc, sequential gradient) to give the desired product as a pale yellow oil (2.4 g, 9.0 mmol, 90% yield). 1H NMR (500 MHz)  0.88 (t, 3H, *J* = 7.2Hz), 1.25–1.36 (m, 16H), 1.53–1.58 (m, 2H), 2.02–2.06 (m, 4H), 2.76 (t, 2H, *J* = 6.9 Hz), 3.62 (t, 2H, *J* = 6.9 Hz), 5.29–5.40 (m, 4H).

**Preparation of YSK05**

 To a solution of (9Z,12Z)-Octadecadien-1-ol (2.2 g, 8.2 mmol) in benzene (50 mL) was added 1-methyl-4-piperidine (455 µL, 3.7 mmol) and *p*-toluenesulfonic acid (780 mg, 4.1 mmol) and mixed well. The reaction mixture was heated to reflux with a Dean-Stark apparatus and a reflux condenser for 16 hours. After cooling, the reaction mixture was washed with NaHCO3 (aq) and brine, dried with Na2SO4 and concentrated. The crude product was applied to silica gel column chromatography (CH2Cl2: MeOH, sequential gradient) to give the desired product as a pale yellow oil (1.55 g, 2.4 mmol, 62% yield). 1H NMR (400 MHz) 0.89 (t, 6H, *J* = 7.1 Hz), 1.23-1.38 (m, 36H), 1.50–1.55 (m, 4H), 1.77–1.82 (m, 4H), 2.02–2.07 (m, 8H), 2.28 (s, 3H), 2.40 (br, 3H), 2.77 (t, 2H, *J* = 6.6 Hz), 3.36 (t, 2H, *J* = 6.9 Hz), 5.29–5.39 (m, 8H). MS (FD): (M)+ Calcd for C42H77NO2: 627, found 627.

***In vitro* PLK1 gene silencing**

HeLa-dluc cells and OS-RC-2 cells were seeded at a density of 1 x 105 cells per well in 6-well plates in growth medium 24 hours prior to transfection and incubated overnight at 370C in 5% CO2 to allow the cells to become attached. For transfection, media containing MENDs at the indicated doses of siRNA were added to the cells after aspiration of the spent media. The MENDs were allowed to incubate with the cells for 24 hours and the cells were then washed twice with PBS. Total RNA (1 g) was isolated with the TRIZOL reagent and reverse transcribed using a High Capacity RNA-to-cDNA kit (ABI) according to manufacturer‘s protocol. A quantitative PCR analysis was performed as described in Materials and Methods 2.11.

**Cytotoxicity**

 HeLa-dluc cells and OS-RC-2 cells were seeded at a density of 4 x 104 cells per well in 24-well plates in growth medium 24 hours prior to transfection and incubated overnight at 370C in 5% CO2 to allow the cells to become attached. For transfection, media containing the MENDs at the indicated doses of siRNA were added to the cells after aspiration of the spent media. The MENDs were allowed to incubate with the cells for 24 hours and the cells were then washed twice with PBS and lysed with PBS containing 1 w/v% TritonX-100. Protein concentrations were determined using a BCA Protein Assay Kit (Pierce, Rockford, IL). The amount of cellular protein is represented as the % of untreated control.

**Supplementary figure**



**Supplementary Fig. S1. Measurement of the apparent pKa of MENDs containing pH-sensitive cationic lipid using TNS fluorescence assay**

TNS, an anionic fluorochrome exhibiting high fluorescence in a hydrophobic environment, was added to (a) DODAP-, (b) YSK05- or (c) optimized YSK05-MEND in buffers with various pH. The TNS fluorescent intensity was measured by a fluorescent micro plate reader with ex=321 nm, em=447 nm following temperature adjustment at 370C. The pKa values were determined as a pH giving rise to half-maximal fluorescent intensity. Data points are represented as the mean ± SD (n=3).



**Supplementary Fig. S2. Effect of phospholipids for stability in mouse serum and gene silencing activity *in vitro* of YSK05-MEND**

(a) The influence of the chemical structure of phospholipid for the stability of YSK05-MEND in mouse serum. YSK0-MENDs containing different phospholipids were mixed with mouse serum and incubated for various times at 370C. After extracting the siRNA with a phenol-chloroform-isoamylalcohol mixture, siRNAs were run on 20% polyacrylamide gel, stained and visualized with 1 g/mL ethidium bromide. The degraded siRNAs are seen as the broad bands below the narrow bands of intact siRNAs. (b) The influence of a kind of phospholipids for gene silencing activity of YSK05-MEND *in vitro*. HeLa-dluc cells were treated with anti-luciferase siRNA formulated in YSK05-MEND incorporating different phospholipids for 24 hours. Data points are represented as the mean ± SD (n=1-3).



**Supplementary Fig. S3. Observation of intracellular pattern and distribution of siRNA using confocal microscopy**

HeLa-dluc cells were treated with Cy5-labeled siRNA formulated in DODAP- (left) or optimized YSK05- (right) MEND at a dose of 100 nM siRNA for 1 hour. Unincorporated MENDs were washed out and siRNA distribution was visualized after 0 hour (upper) or 6 hours (lower) using a confocal microscopy. Scale bars indicate 50 m.



**Supplementary Fig. S4. Durability of gene silencing activity *in vitro* of optimized YSK05-MEND**

HeLa-dluc cells were treated with anti-luciferase siRNA formulated in optimized YSK05-MEND for 24 hours. HeLa-dlluc cells were re-seeded on day 3, 5 and 8, and luciferase activities were analyzed on day 1, 2, 3, 5, 7 and 9. Data points are represented as the mean ± SD (n=3).



**Supplementary Fig. S5. Blood concentration of MENDs**

MENDs composed of DOTAP or DODAP/DOPE/Chol (30/40/30 mol%) of around 120 nm in diameter were modified with various amounts of PEG from 1 to 13.5 mol% of total lipid. MENDs were intravenously injected and blood concentration was determined at 6 hr. Data points are represented as the mean (n=2-3) ± SD.



**Supplementary Fig. S6. Effect of the incorporation of PEG-DSG into optimized YSK05-MEND for the stability in mouse serum**

Optimized YSK05-MENDs before and after incorporation of PEG-DSG were mixed with mouse serum and incubated for various times at 370C. Free siRNAs, and the mixture of siRNA/protamine complex and empty MEND were used as negative controls. 0.05 w/v% TritonX-100 was used for disruption of MEND lipid bilayer. After extracting the siRNAs with a phenol-chloroform-isoamylalcohol mixture, siRNAs were run on 20% polyacrylamide gel, stained and visualized with 1 g/mL ethidium bromide. It was observed that 0.05 w/v% TritonX-100 didn’t influence the RNase activity from negative control results but completely disrupt the lipid bilayer. PEG-DSG incorporation significantly increased the stability of optimized YSK05-MEND in serum and no evidence for the presence of degraded siRNA was observed.



**Supplementary Fig. S7. PLK1 gene silencing and cytotoxicity *in vitro* of optimized YSK05-MEND**

(a) PLK1 gene silencing of optimized YSK05-MEND. OS-RC-2 cells and HeLa-dluc cells were treated with anti-PLK1 siRNA and anti-luciferase siRNA formulated in optimized YSK05-MEND for 24 hours. Data points are represented as the mean ± SD (n=3-4). (b, c) Cytotoxicity as the result of PLK1 gene silencing. (b) OS-RC-2 cells and (c) HeLa-dluc cells were treated with anti-PLK1 siRNA and anti-luciferase siRNA formulated in optimized YSK05-MEND for 24 hours. The amounts of cellular protein were quantified. Data points are represented as the mean ± SD (n=4).