Supproting Information for the article:

A Novel Gemini-like Cationic Lipid for Efficient Delivery of siRNA

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Supplementary date 1 (Fig. S1)

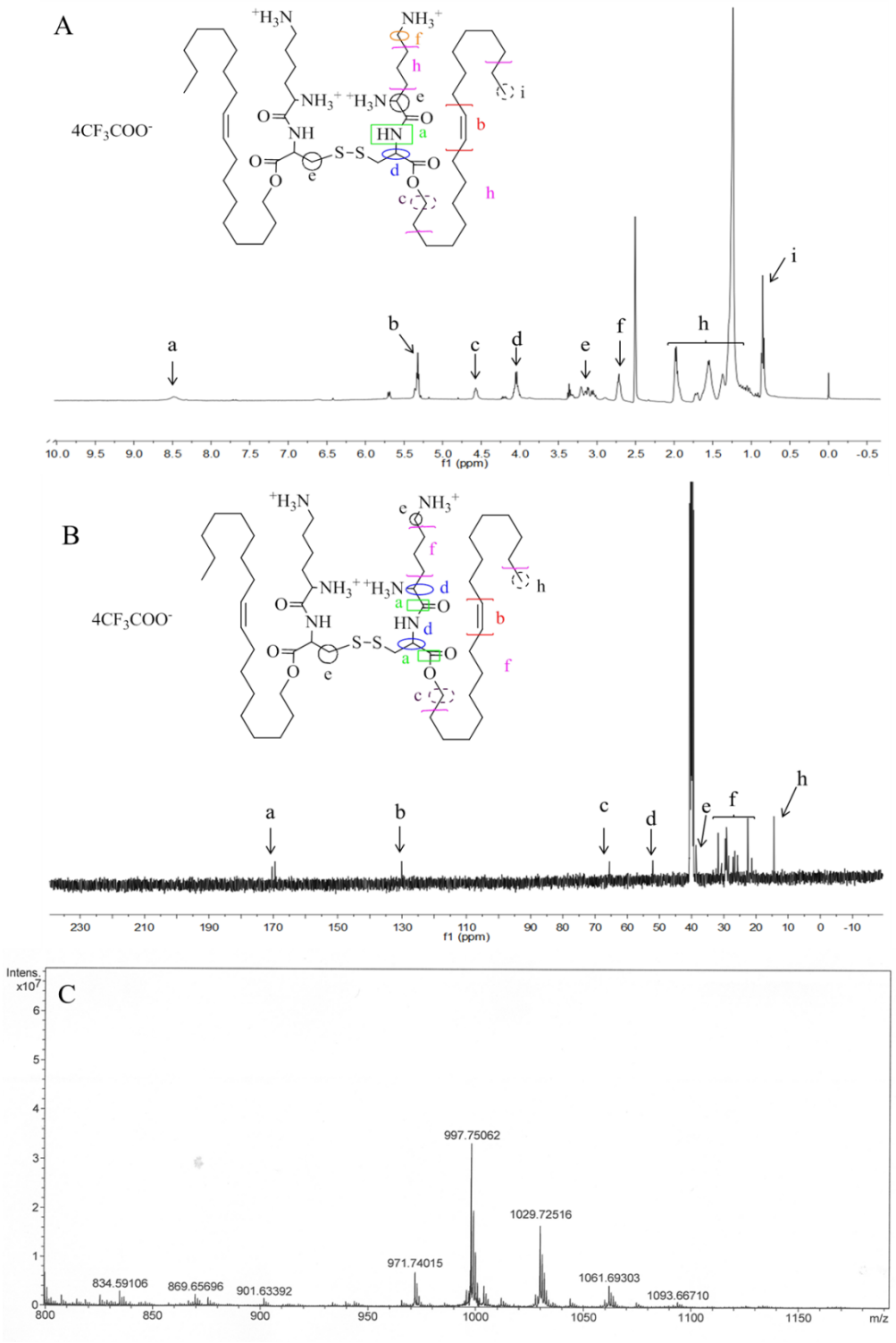

Fig. S1 ^{1}H NMR spectra of **CLD** (A), ^{13}C NMR spectra of **CLD** (B) and MS spectra of **CLD** (C).

Supplementary date 2 (Fig. S2)

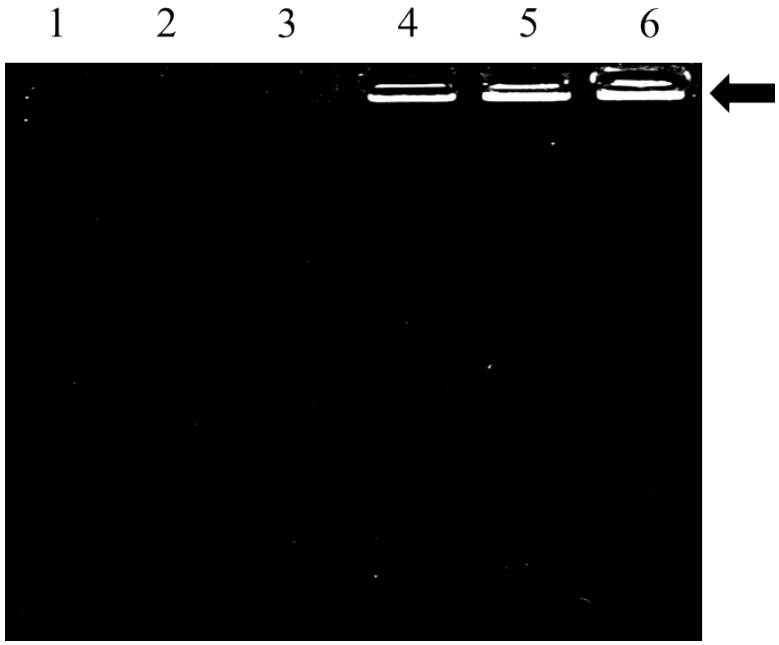

Fig. S2 Serum stability of **CLD**/**siMek1** lipoplexes. Samples of siRNA in aqueous solution were mixed in a 1:1 ratio with fresh serum to give 50% serum concentration and incubated at 37 ^{o}C for 24 h. After 24 h, each sample was loaded on a 1% agarose gel as described above and the electrophoresis was performed to visualize the siRNA. The lane numbers correspond to different cationic lipid/**siMek1** N/P ratios: 1. 0:1(**siMek1** only), 2. 4:1, 3. 6:1, 4. 8:1, 5. 10:1, 6. 12:1. Each lane contains 1.33 μg **siMek1**. The **siMek1** was visualized with Goldenview^{TM} dye staining. The brightness band indicated the free **siMek1**.

Supplementary date 3 (Fig. S3)

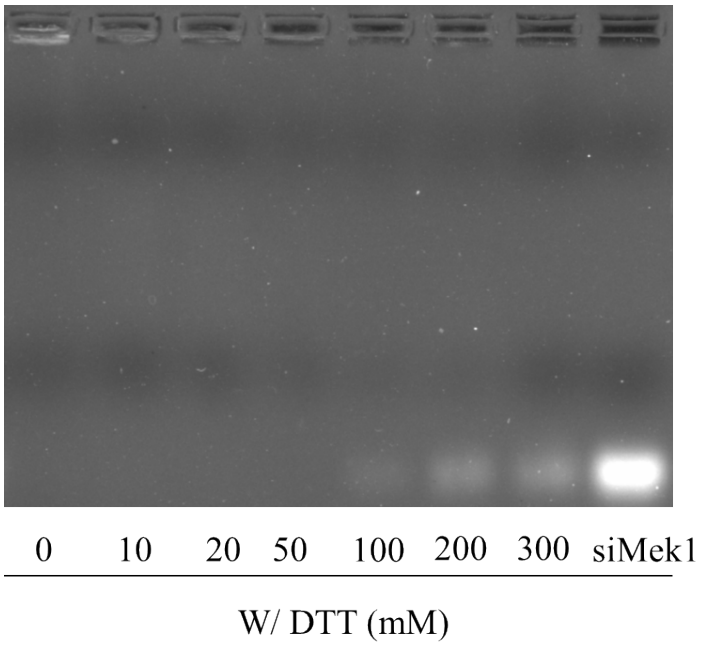

Fig. S3 DTT decomplexation assay of **CLD/siMek1** complexes. Lipoplexes of **CLD/siMek1** were prepared to ensure complete binding of **siMek1** by **CLD** liposomes at N/P = 12, and then incubated with 0, 10, 20, 50, 100, 200, 300 mMof DTT in RNase free water at 37 temperature for 40 min. The samples were analyzed on a 1% agarose gel as described above. Results were presented as the average of three independent experiments at least

Supplementary date 4 (Fig. S4)

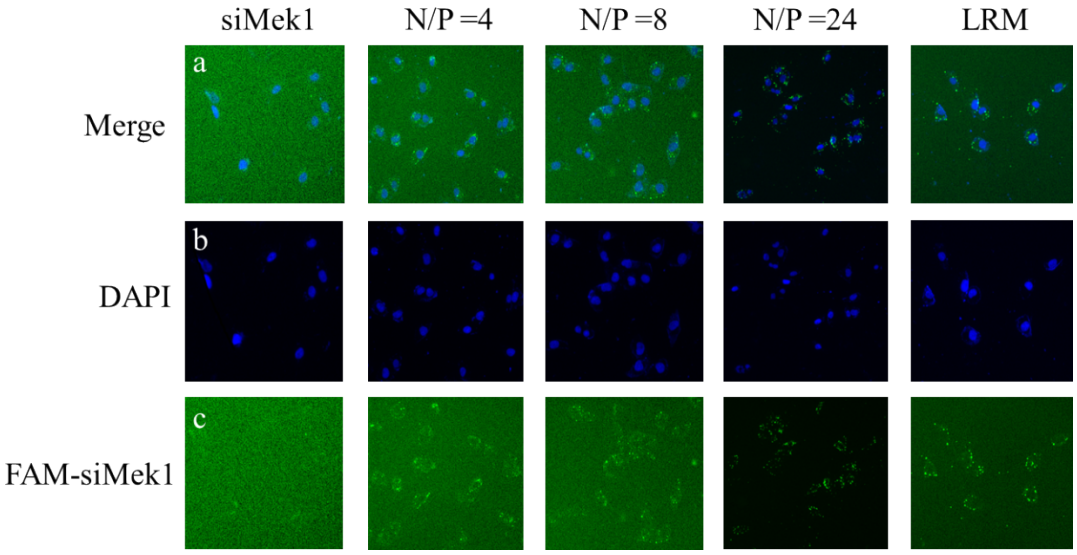

Fig. S4 *In vitro* **siMek1** uptake study by High Content Screening (**HCS**) reader. A375 cells were incubated with liposomes entrapping **FAM-siMek1** for 5 h at 37 ºC. The final concentration of **FAM-siMek1** was 50 nM. After washing with **PBS**, the cells were fixed with 4% **PFA**. Cells were ten incubated with **DAPI** for nuclear staining. The fluorescence of the cells was visualized with **HCS**. Row a: the merge images of **DAPI** and **FAM-siMek1**; Row b: the images of **DAPI** in the cell nuclei; Row c: the images of **DAPI** in the cytoplasm.

Supplementary date 5 (Fig. S5)

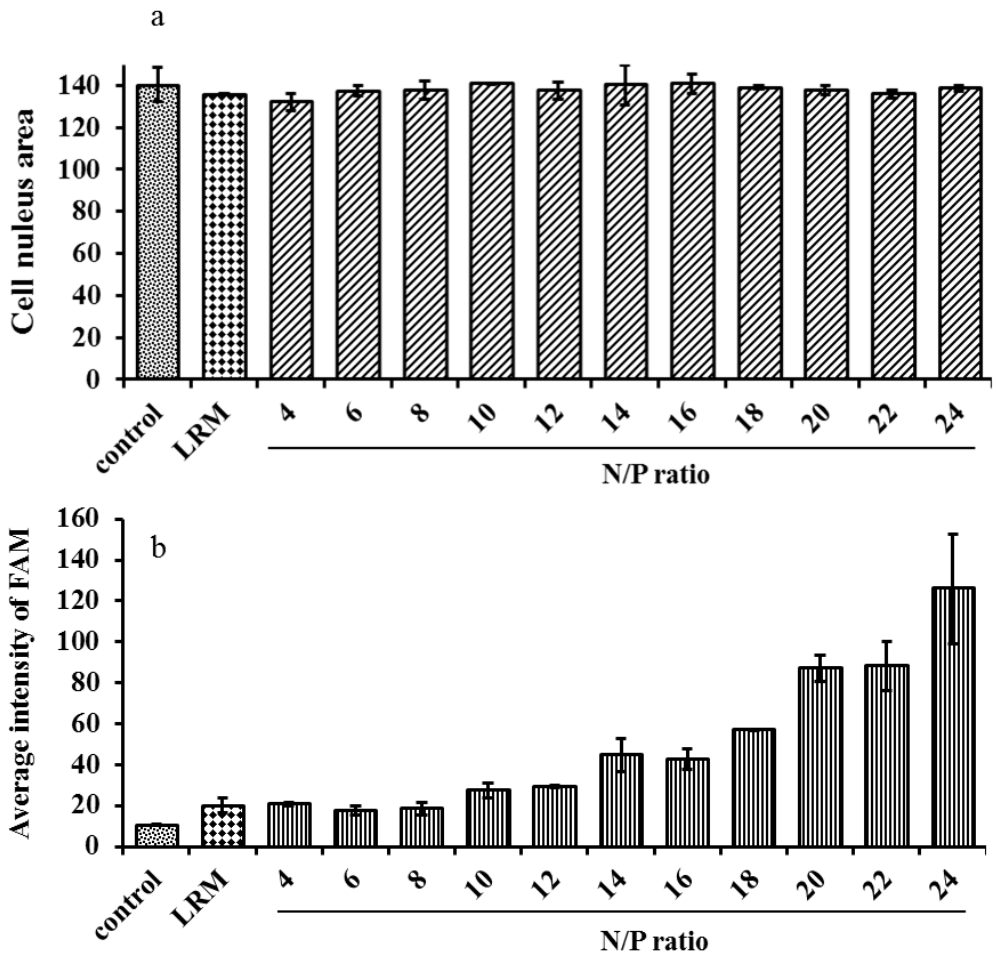

Fig. S5 The average fluorescent intensity of cells nuclear areas study by high-content screening assay (**HCA**, a) and the average FAM fluorescent intensity study by High-content screening assay (**HCA**, b). A375 cells were incubated with incubated with liposomes entrapping **FAM-siMek1** for 5 h at 37 °C. The final concentration of **FAM-siMek1** was 50 nM. After washing with **PBS**, the cells were fixed with 4% **PFA**. Cells were ten incubated with **DAPI** for nuclear staining. The fluorescence of the cells was visualized with **HCS**.

Supplementary date 6 (Figure S6)

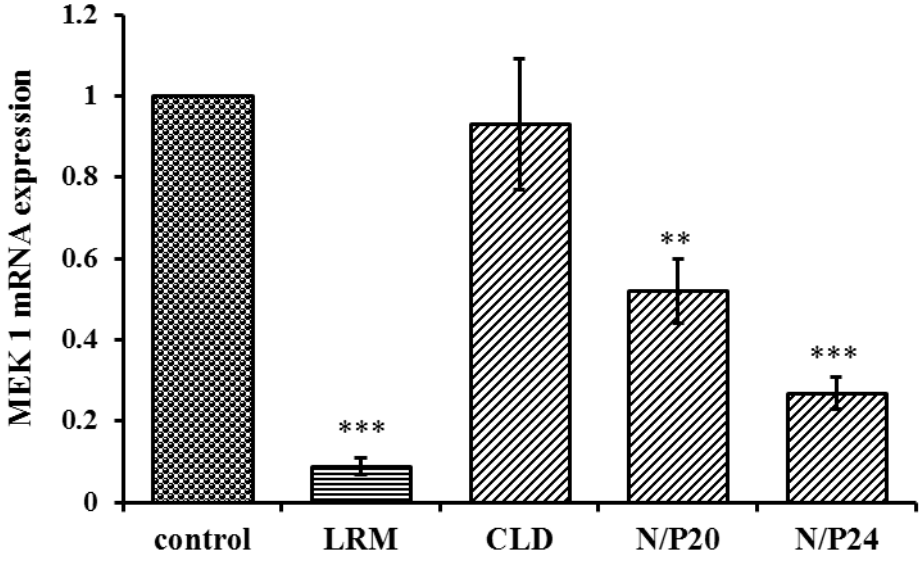

Fig. S6 Gene silence of **CLD/siMek1** lipoplexes *in vitro*. The gene silencing assay was taken in HeLa cells. After 24 h, the MEK1 mRNA expression was analyzed, expressed as percent mRNA expression compared to the untreated control. Date are expressed as mean ± standard error for n = 3. $^{**}P$□0.01, $^{***}P$□0.001.