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Supplementary information

Zn(II)-cyclen complex-based liposomes for gene delivery: the advantage of Zn coordination

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Table S1 Zinc concentration of lipid solution measured by ICP-AES.

	ZnL4	**ZnL5**
Theoretical zinc concentration (μg/L)	10	20
Measured zinc concentration (μg/L)	9.85	16.5

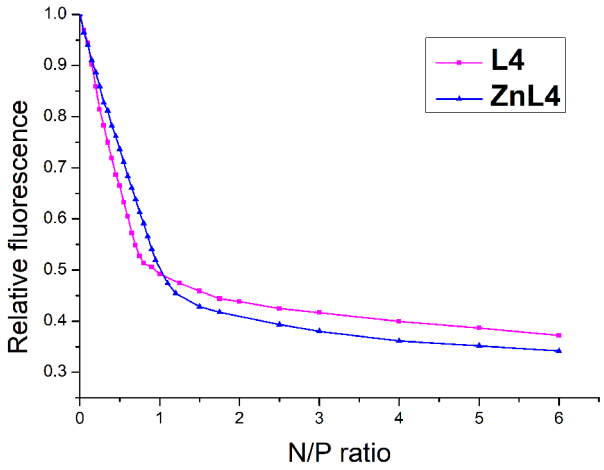

Fig. S1 Florescent quenching assay of EB/DNA by the addition of lipids **L4 & L4(Zn).** The samples were excited at 520 nm and the emission were measured at 600 nm. The pure EB solution and DNA-EB solution without the liposome were measured as negative and positive controls.

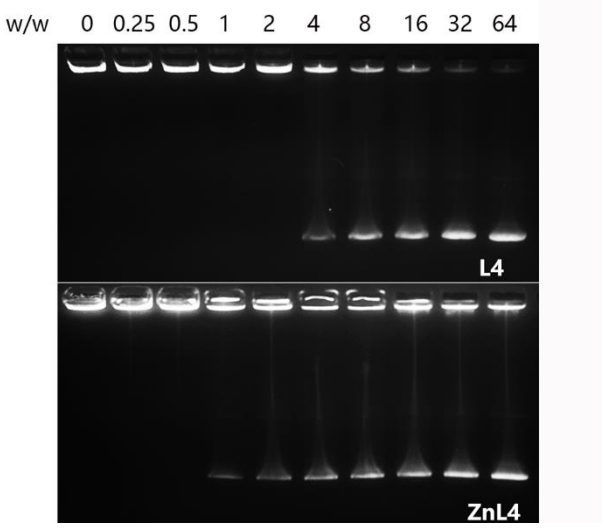

Fig. S2 Release of DNA from the lipoplex (N/P = 8 with 0.125 μg of pUC-19 DNA) with the addition of heparin with various heparin/DNA mass ratios.

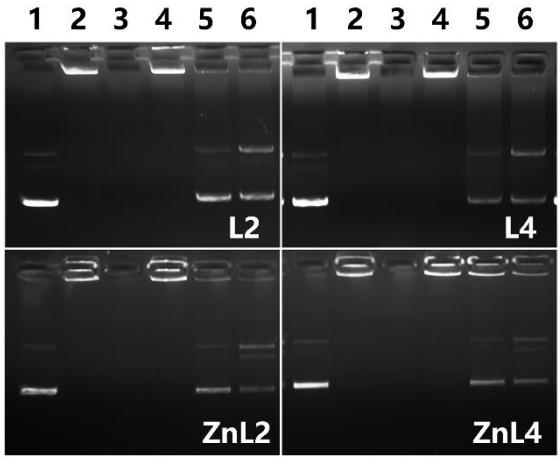

Fig. S3 DNA protection by liposomes against DNase. Lane 1: naked DNA; lane 2: lipoplex (N/P = 8); lane 3: DNA with DNase for 2 h; lane 4: lipoplex (N/P = 8) with DNase for 2 h; lane 5: lipoplex (N/P = 8) treated with heparin; lane 6: lipoplex (N/P = 8) with DNase for 2 h followed by heat-inactivation of DNase and then treated with heparin. 0.125 μg of pUC-19 DNA was used in each well.

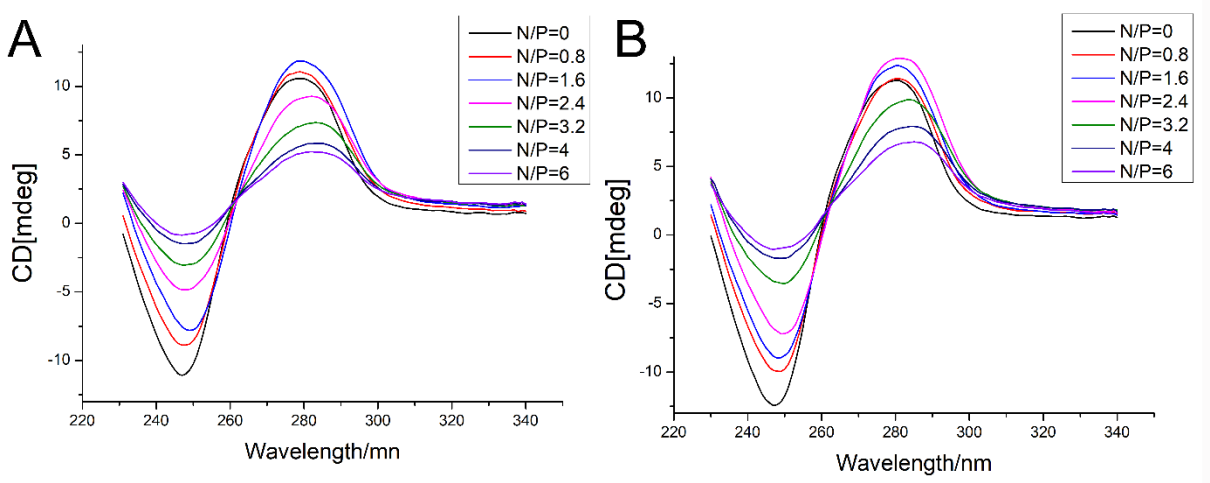

Fig. S4 Circular dichroism spectra of CT-DNA combined with liposomes **L4**(A) or **ZnL4**(B) at different N/P ratio in 10 mM of HEPES (pH 7.2) at 25 ºC.

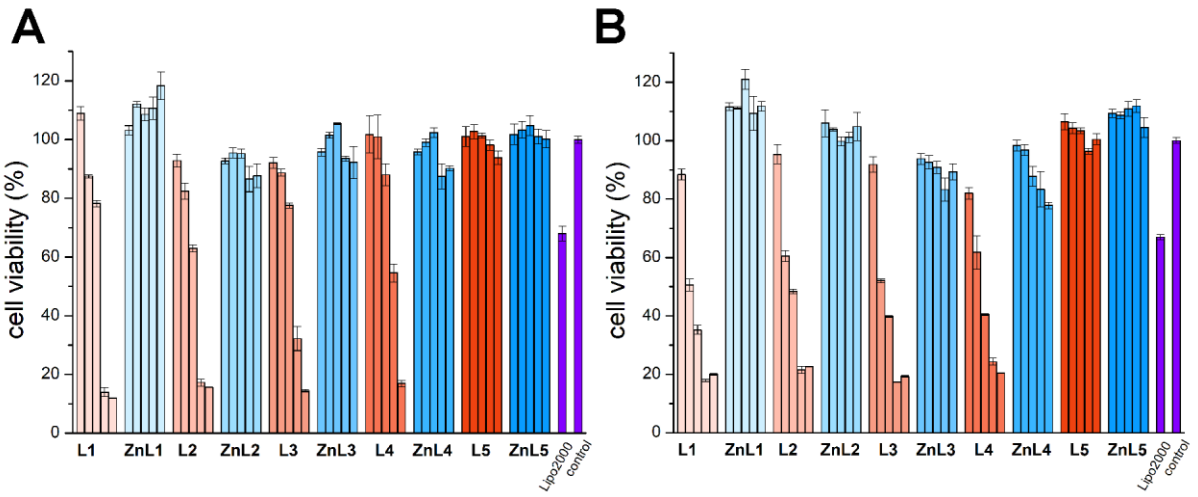

Fig. S5 *In vitro* cytotoxicity of the lipoplexes at various N/P ratios (4, 6, 8, 12 and 16 in each column group, respectively) in 7702 (A) and HEK-293 (B) cells. Lipoplexes were prepared with 0.2 μg of pGL-3 plasmid at various N/P ratios. Data represent mean ± SD (n = 3).

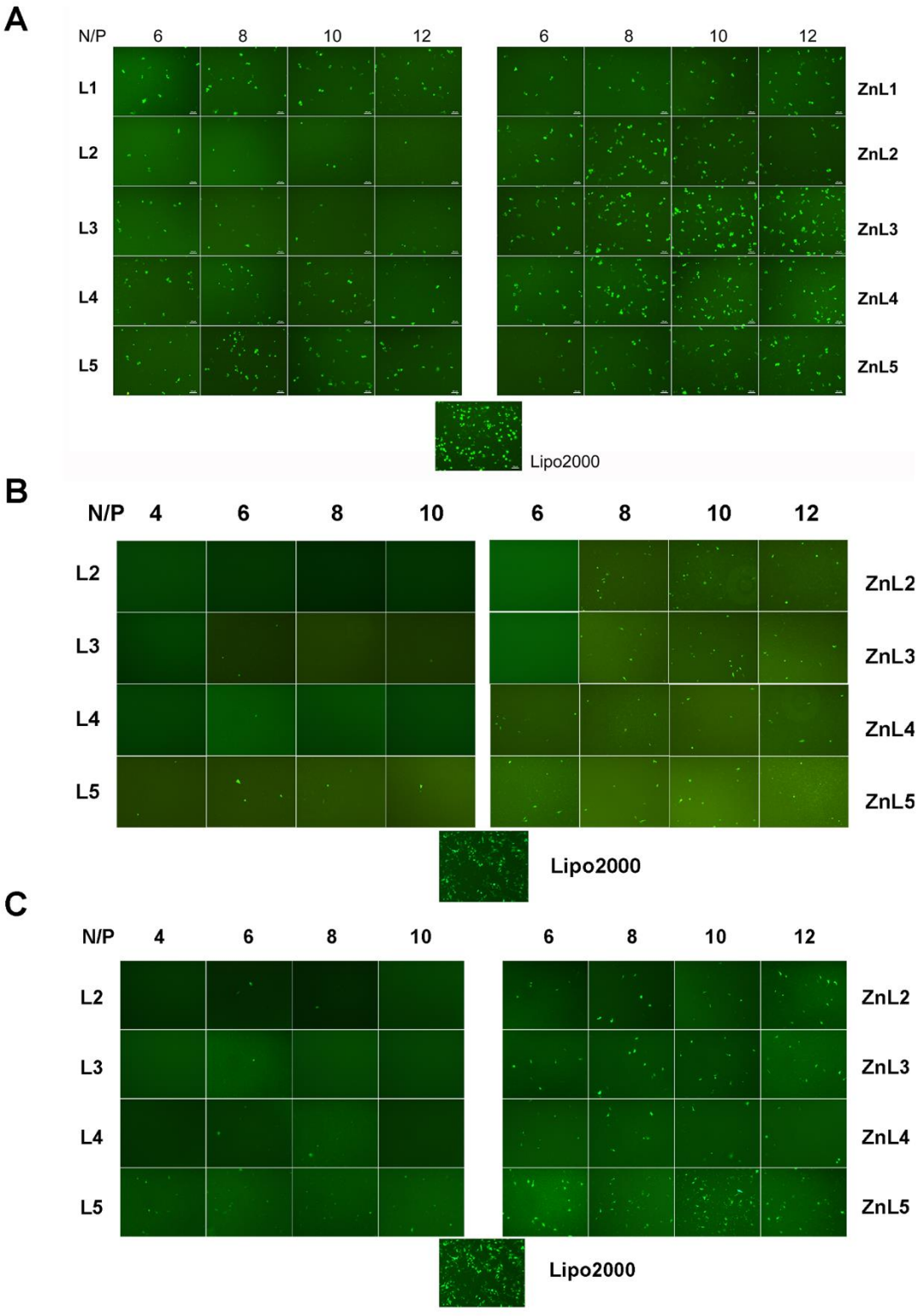

Fig. S6 Fluorescence microscopy images of 7402 (A), 7702 (B) and HEK-293 (C) cells transfected by EGFP (0.4 μg of plasmid in each well) with Zn-free and Zn-contained liposomes at different N/P ratios. (Lipid/DOPE ratio was 1:2, the cells were observed after 24 h transfection).

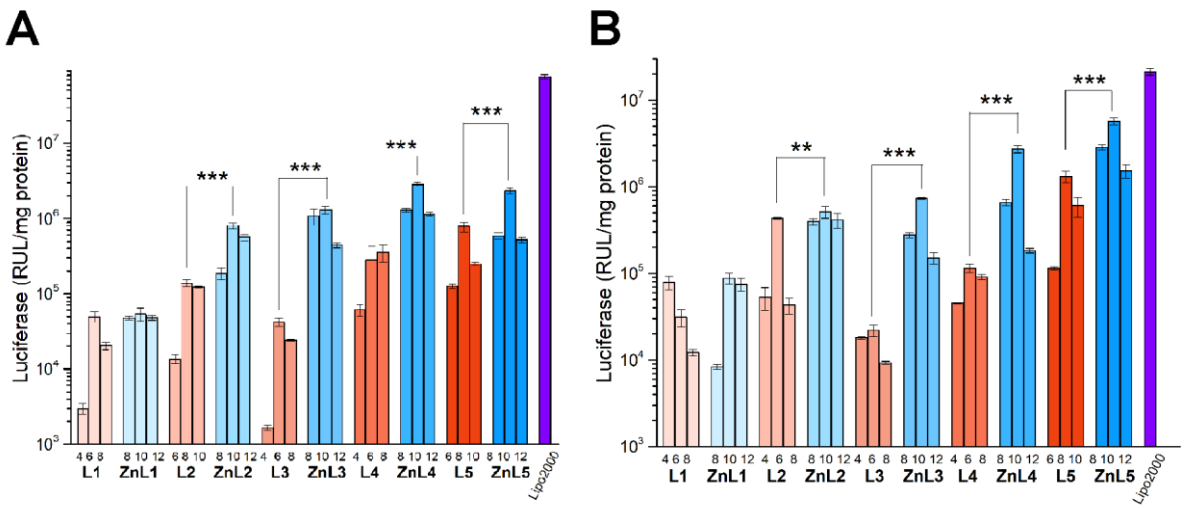

Fig. S7 Luciferase expression in 7702 (A) and HEK-293 (B) cells transfected with liposome/DNA lipoplexes at various N/P ratios at the lipid/DOPE molar ratio of 1:2, while 0.4 μg of pGL-3 plasmid was used in each experiment. Data represent mean ± SD (n = 3). *P < 0.05; **P < 0.01; ***P < 0.001.

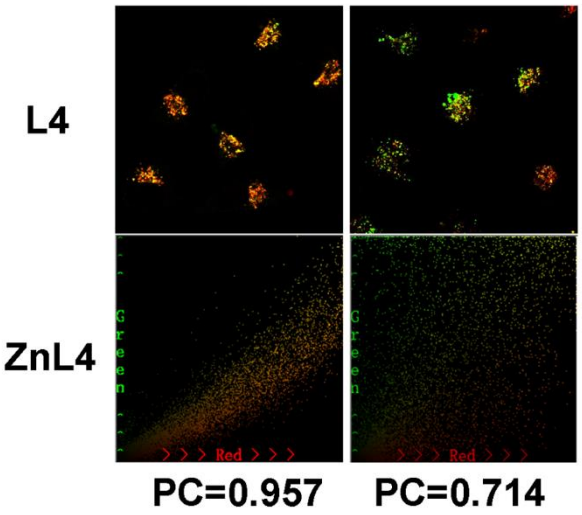

Fig. S8 Color co-localization image and Pearson correlation coefficient of green and red signals in Fig. 7. PC: Pearson correlation coefficient. The result was analyzed by Image-Pro Plus 6.0.