

## **$\alpha$ -Tocopherol-anchored gemini lipids with delocalizable cationic head groups: Effect of spacer length on DNA compaction and transfection properties**

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100% Methanol

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100% Methanol

**Fig. S16** HPLC spectra of TIM 6

100% Methanol

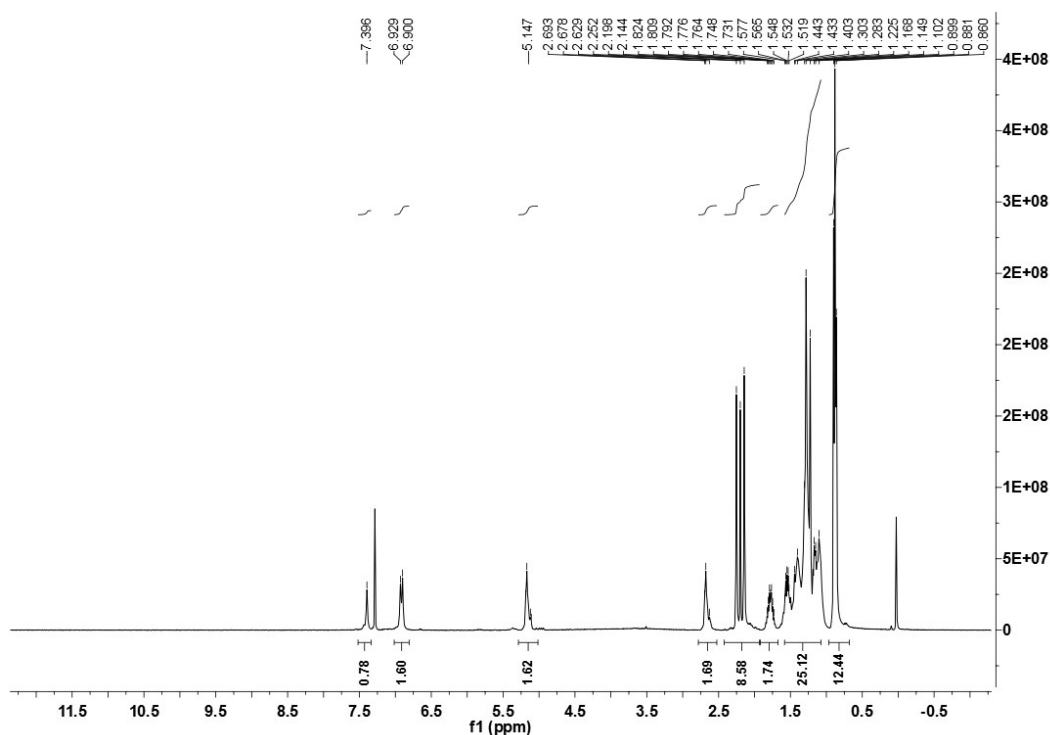
**Fig. S17** HPLC spectra of TIM 10

100% Methanol

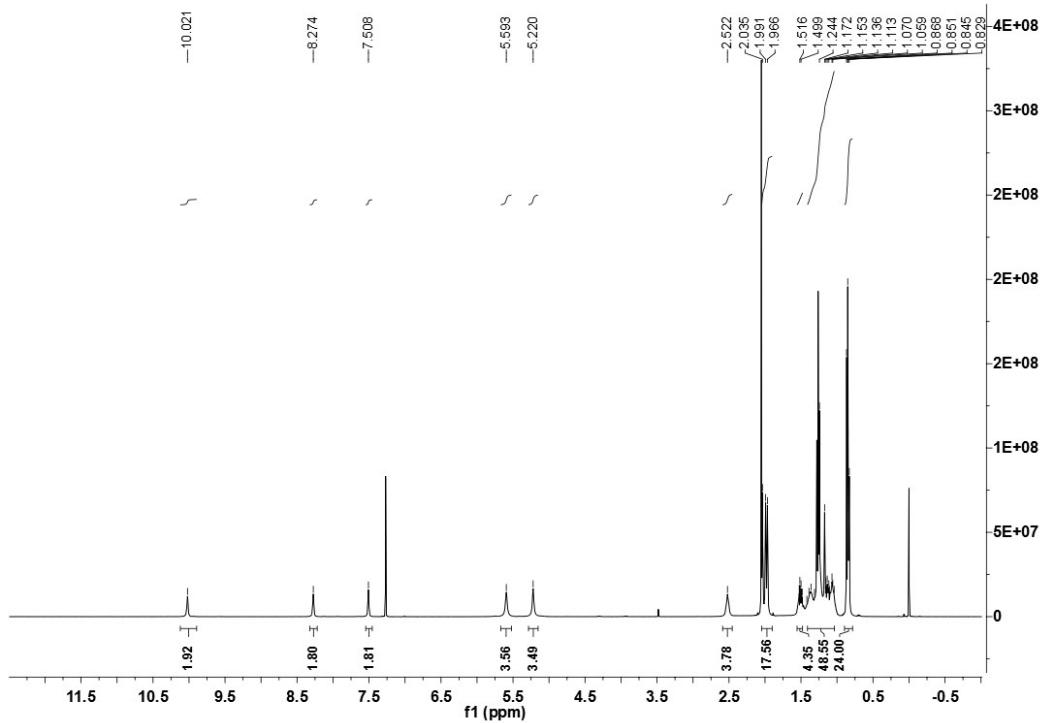
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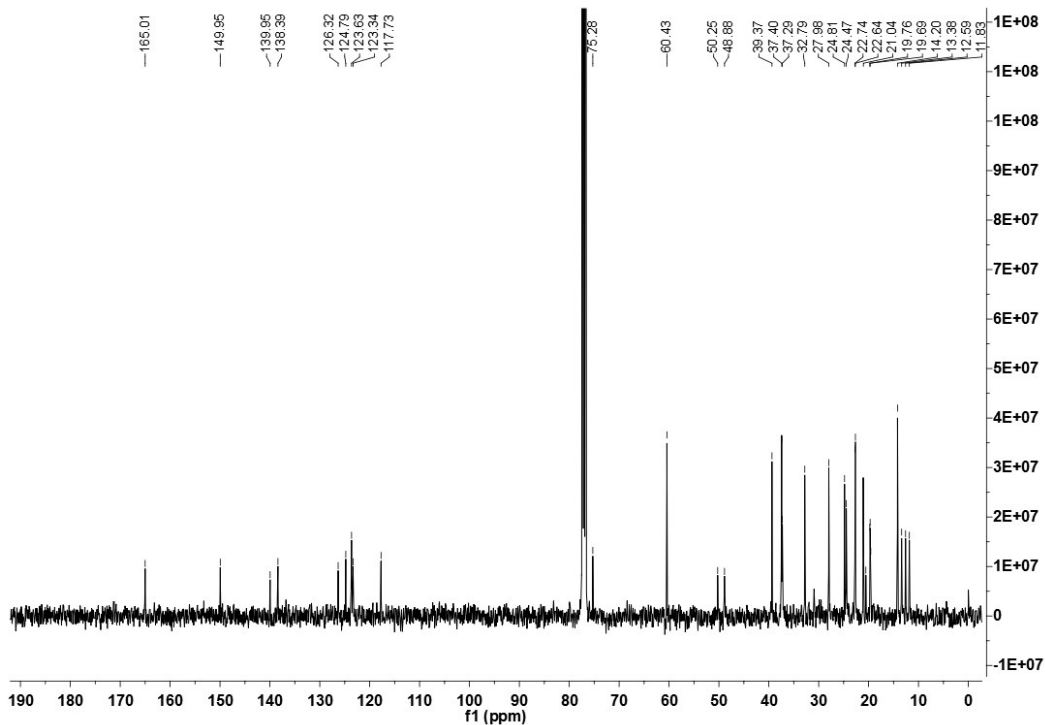
**Fig. S20** Confocal laser scanning microscope images for endosomal co-localization of lipoplexes derived from rhodamine-PE labeled TIM 6 formulation (lipid:DOPE:PE) and pCMV- $\beta$ gal DNA.



**Fig. S1:** <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) spectrum of Toc-Imidazole



**Fig. S2:** <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) spectrum of **TIM 2**



**Fig. S3:** <sup>13</sup>C-NMR (400 MHz, CDCl<sub>3</sub>) spectrum of **TIM 2**

Sample Name	CRR-3	Position	Vial 35	Instrument Name	Instrument 1	User Name	
Inj Vol	-1	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	CRR-3.d	ACQ Method	SCS....m	Comment		Acquired Time	11/28/2016 10:26:03 AM

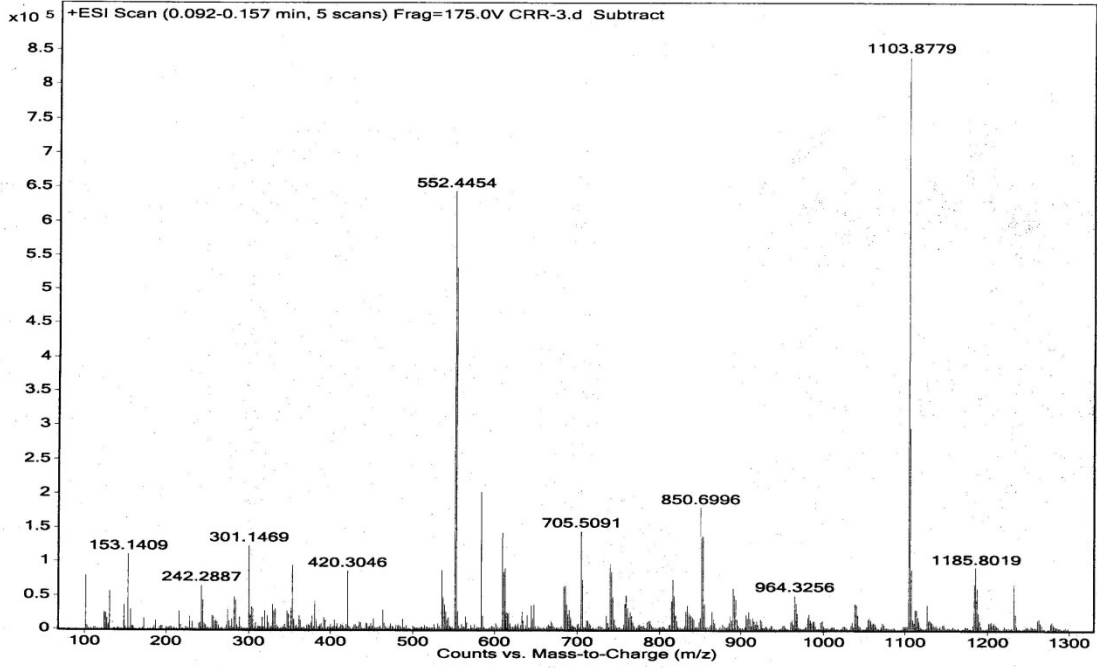


Fig. S4: ESI-HRMS Spectrum of TIM 2

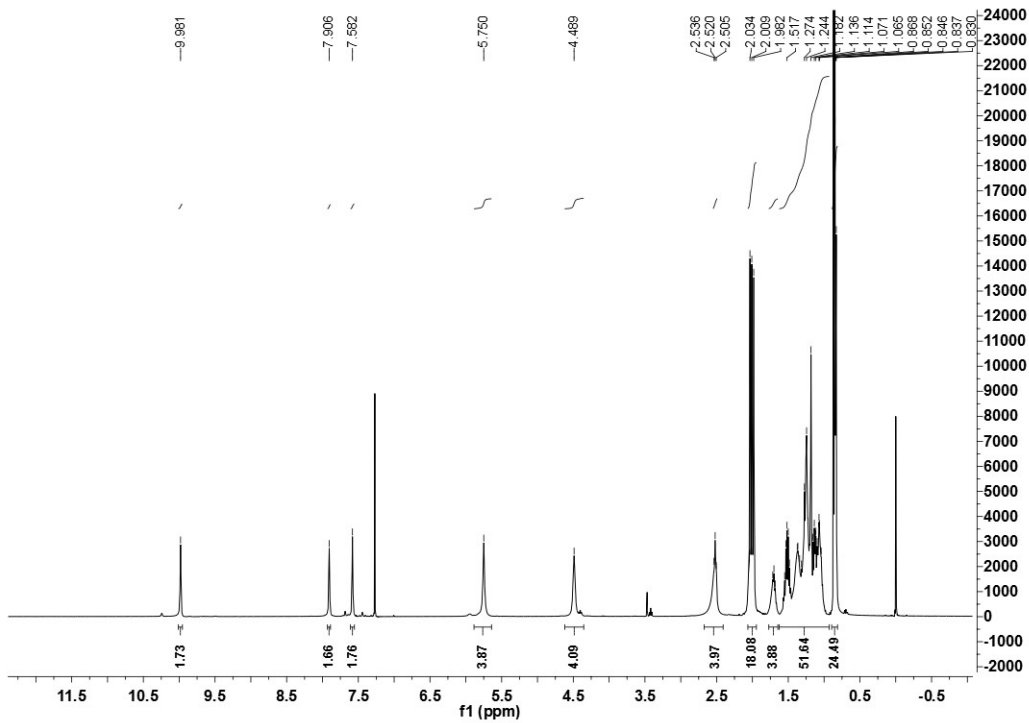


Fig. S5: <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) spectrum of TIM 4

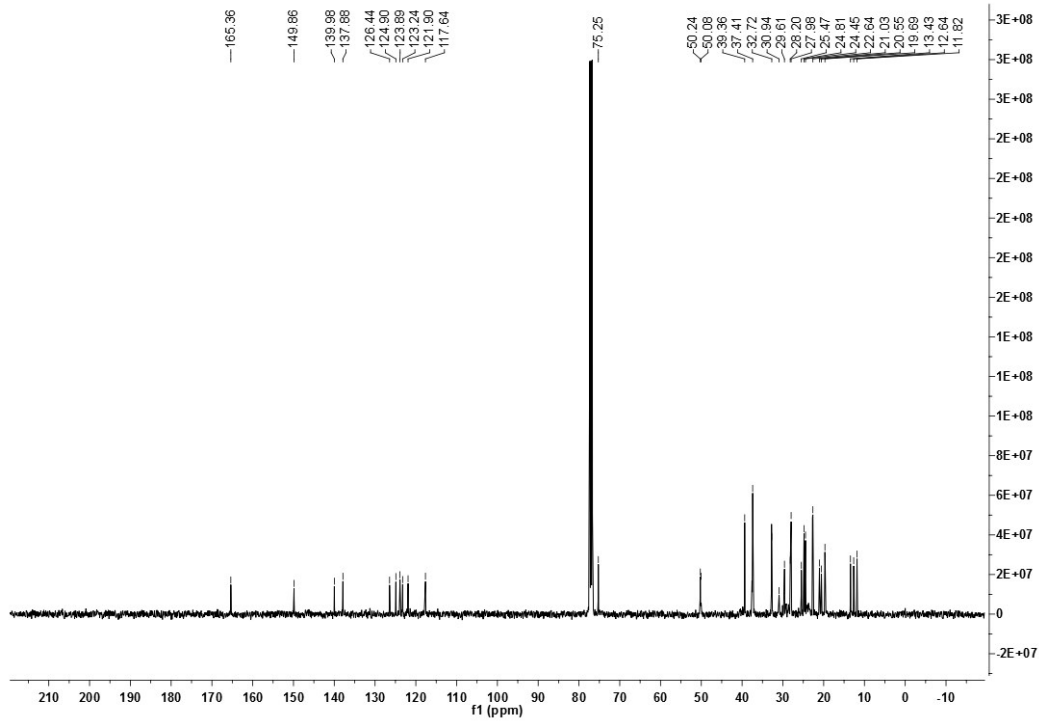


Fig. S6:  $^{13}\text{C}$ -NMR (400 MHz,  $\text{CDCl}_3$ ) spectrum of TIM 4

Sample Name	CRR-4	Position	Vial 36	Instrument Name	Instrument 1	User Name	
Inj Vol	-1	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	CRR-4.d	ACQ Method	SCS....m	Comment		Acquired Time	11/28/2016 10:30:04 AM

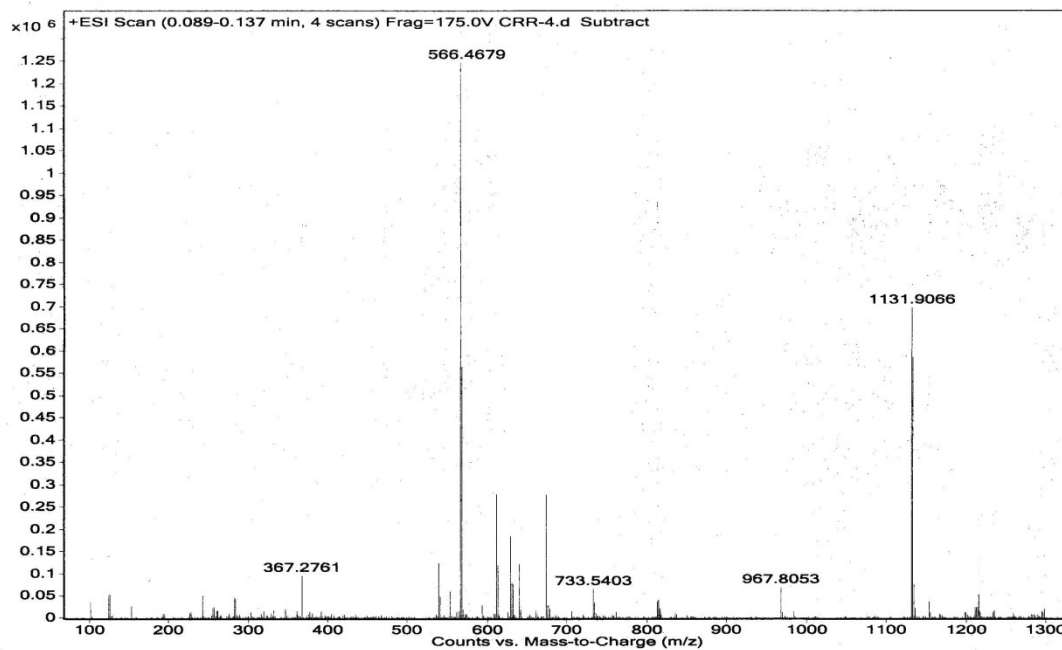
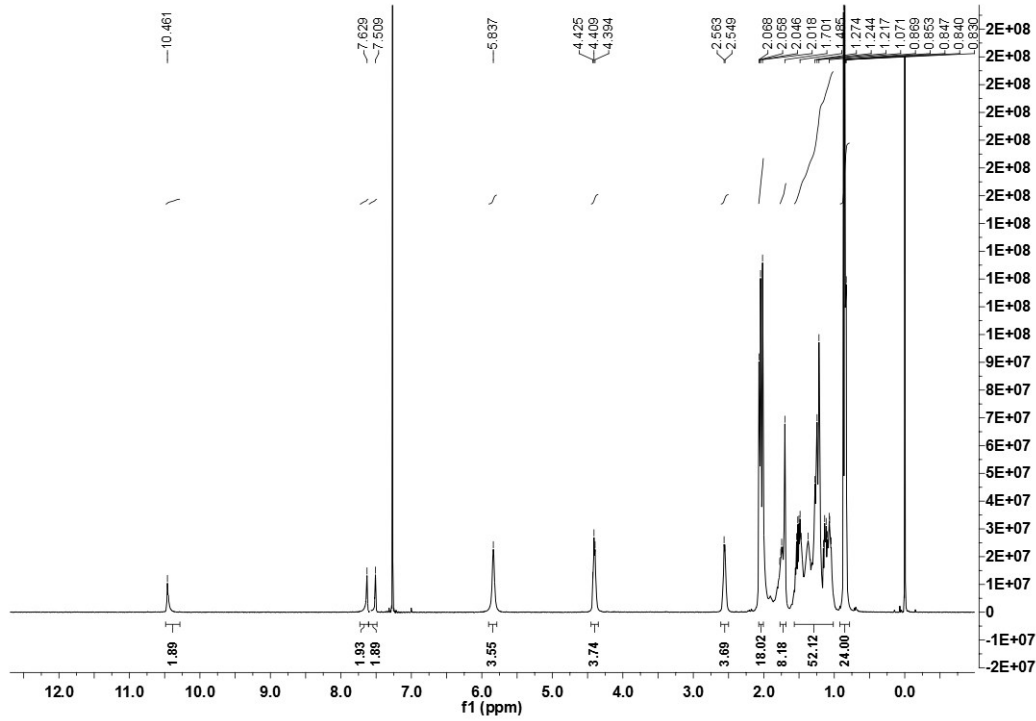
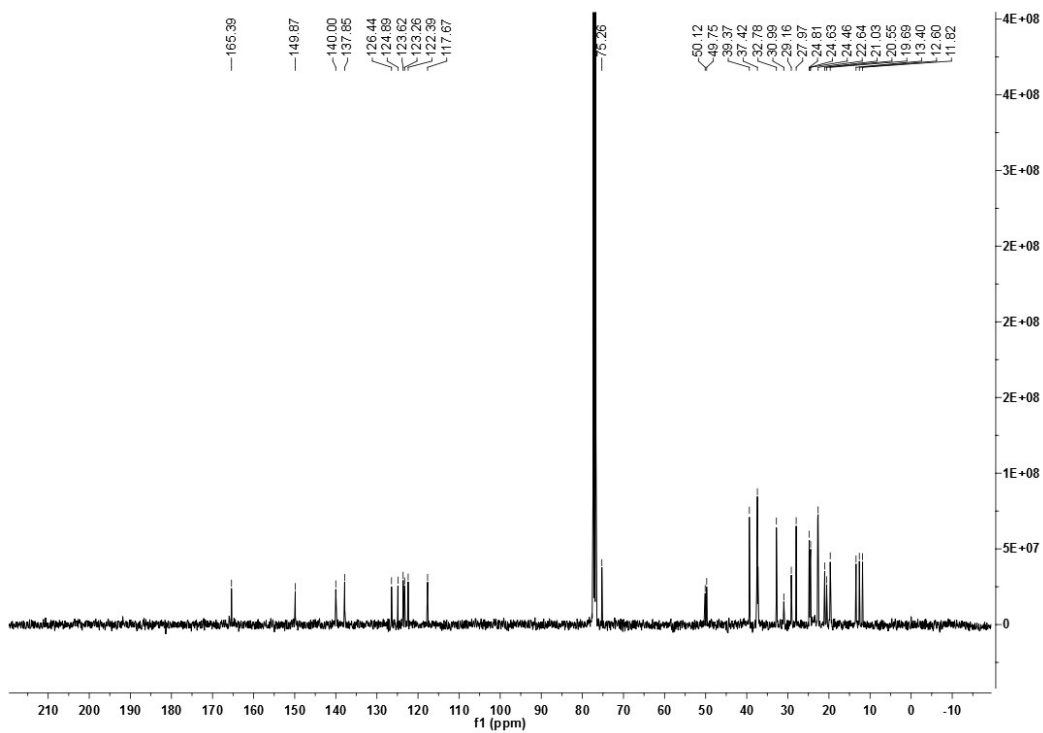


Fig. S7: ESI-HRMS Spectrum of TIM 4



**Fig. S8:**  $^1\text{H}$ -NMR (400 MHz,  $\text{CDCl}_3$ ) spectrum of **TIM 6**



**Fig. S9:**  $^{13}\text{C}$ -NMR (400 MHz,  $\text{CDCl}_3$ ) spectrum of **TIM 6**

Sample Name	CRR-5	Position	Vial 31	Instrument Name	Instrument 1	User Name	
Inj Vol	-1	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	CRR-5.d	ACQ Method	SCS...m	Comment		Acquired Time	11/28/2016 10:09:55 AM

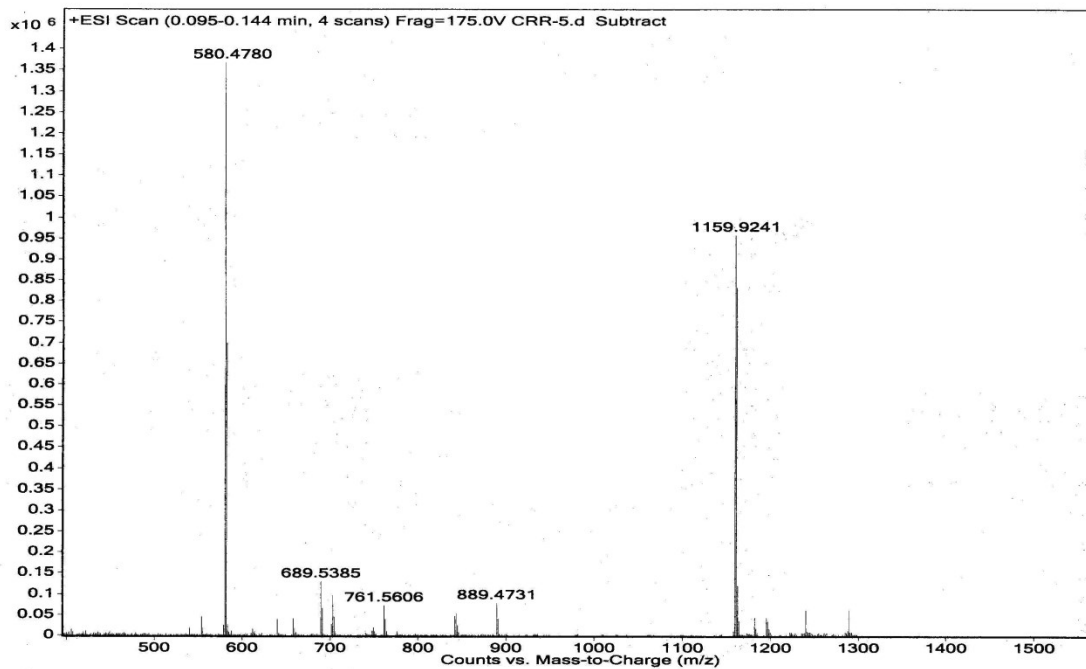


Fig. S10: ESI-HRMS Spectrum of TIM 6

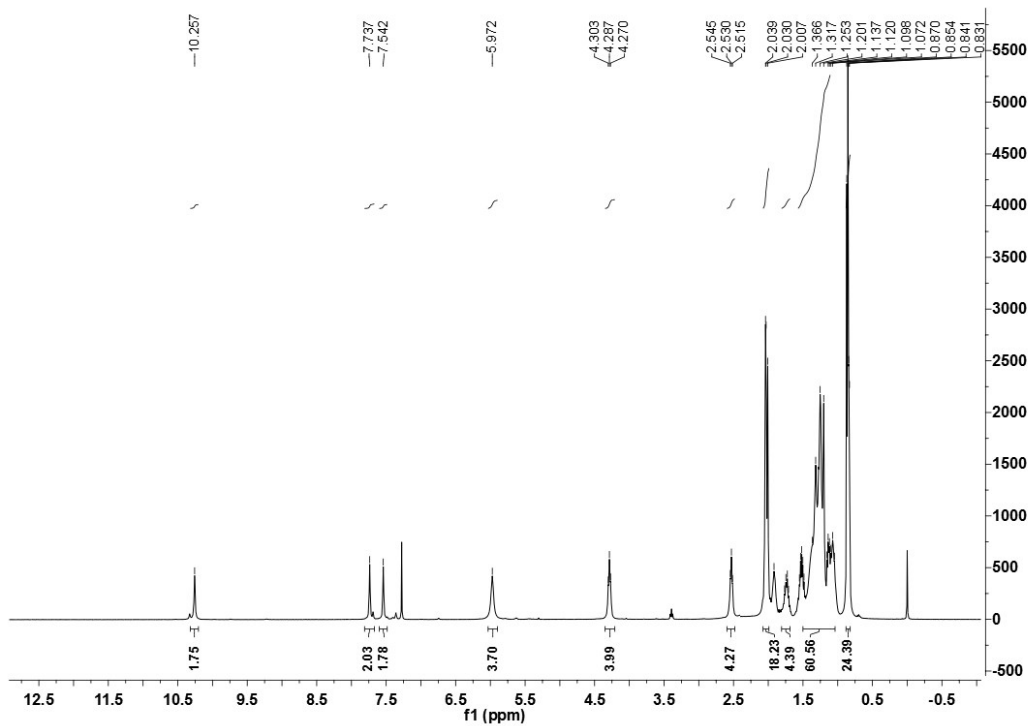


Fig. S11: <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) spectrum of TIM 10

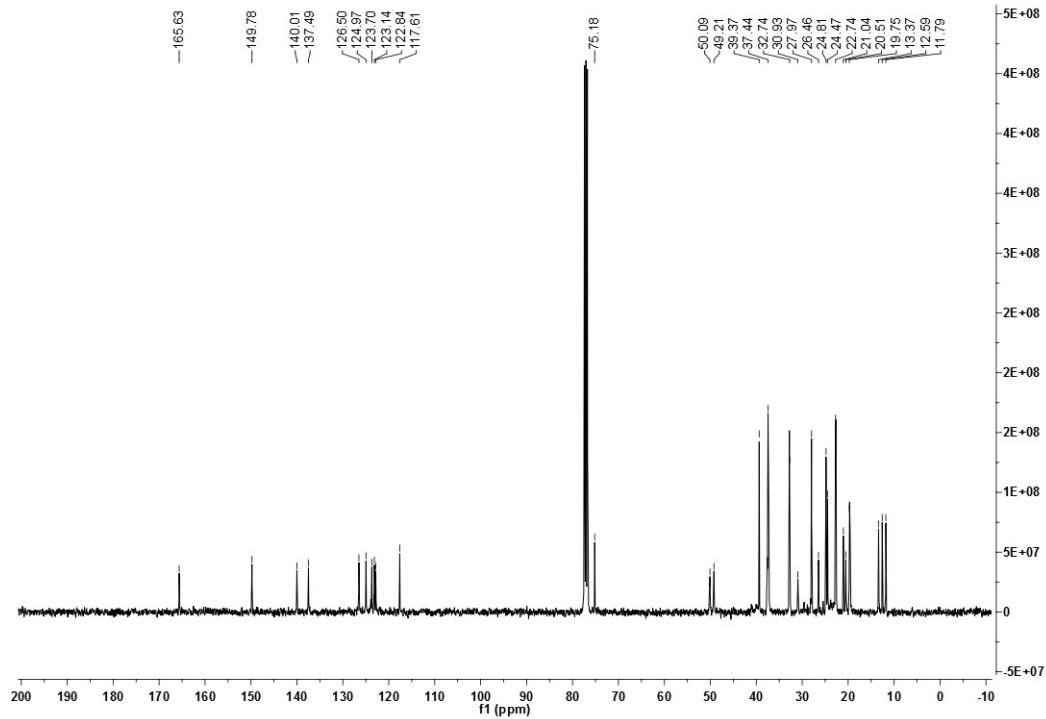


Fig. S12:  $^{13}\text{C}$ -NMR (400 MHz,  $\text{CDCl}_3$ ) spectrum of TIM 10

Sample Name	CRR-6	Position	Vial 63	Instrument Name	Instrument 1	User Name	
Inj Vol	-1	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	CRR-6.d	ACQ Method	SCS...m	Comment		Acquired Time	11/16/2016 3:05:47 PM

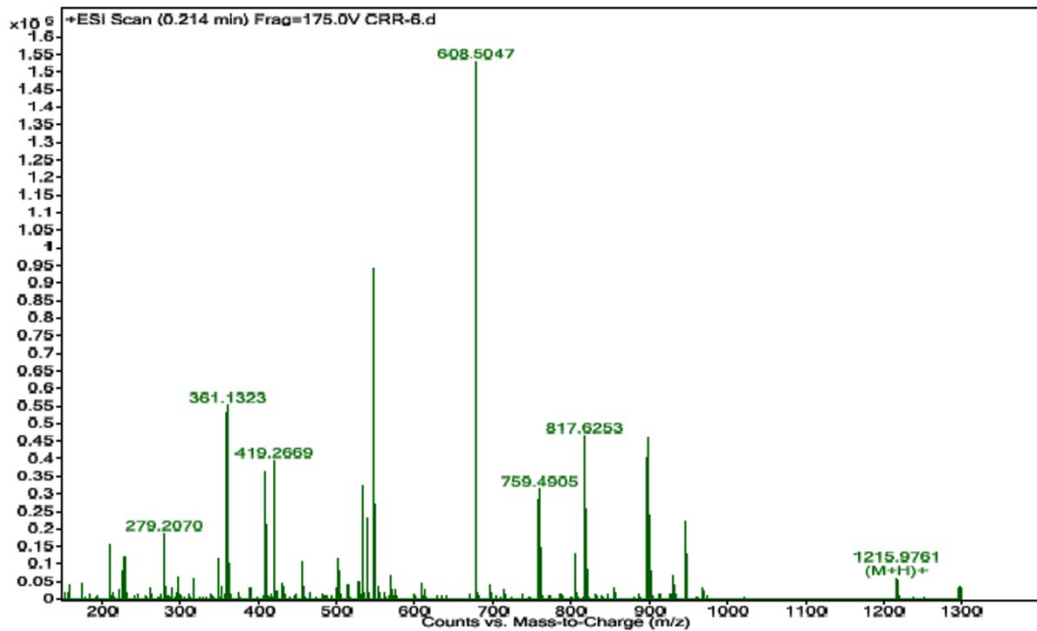


Fig. S13: ESI-HRMS Spectrum of TIM 10



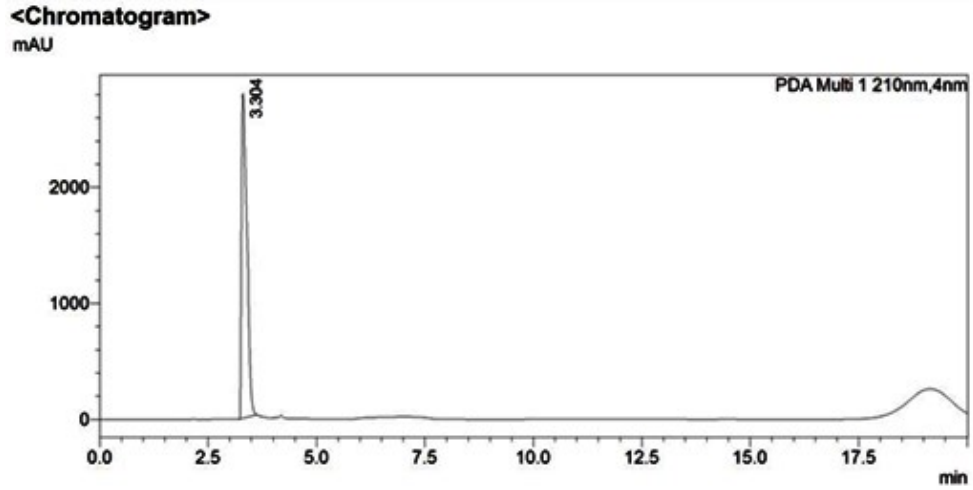


Fig. S14: HPLC chromatogram of TIM 2

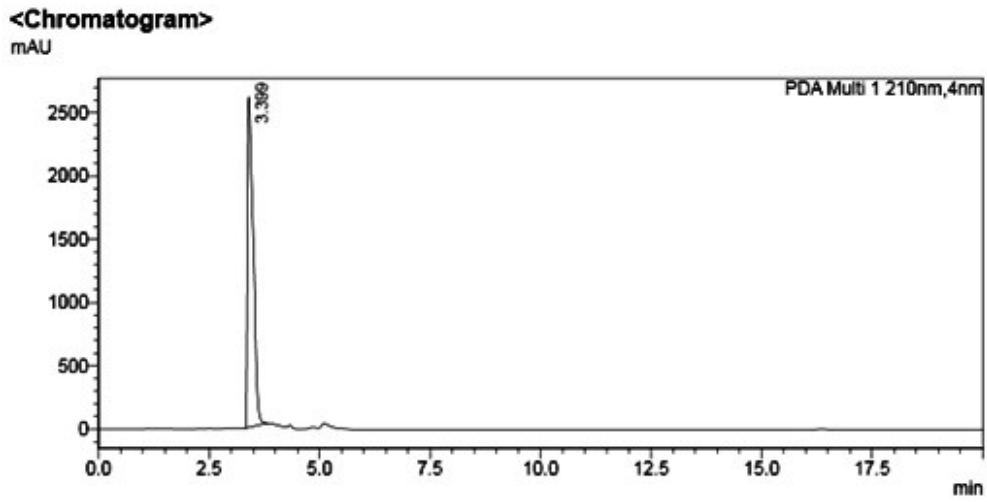


Fig. S15: HPLC chromatogram of TIM 4

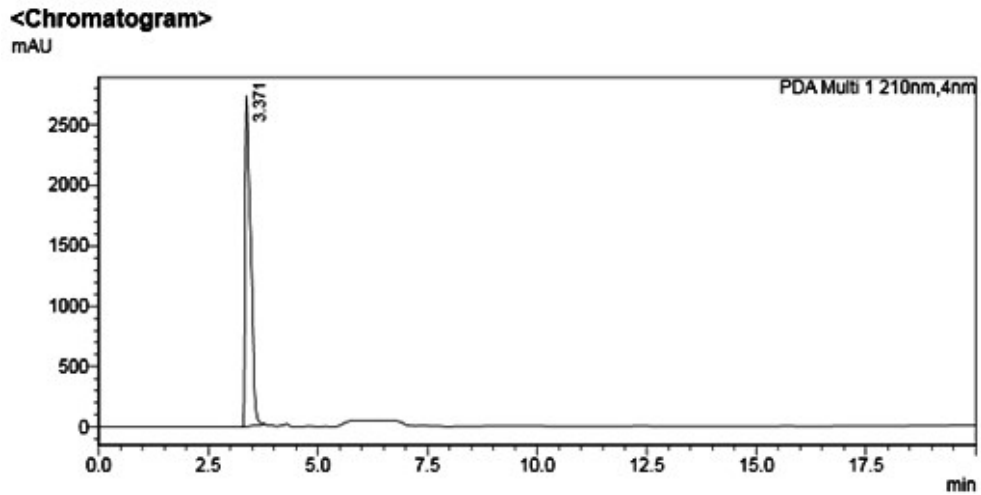
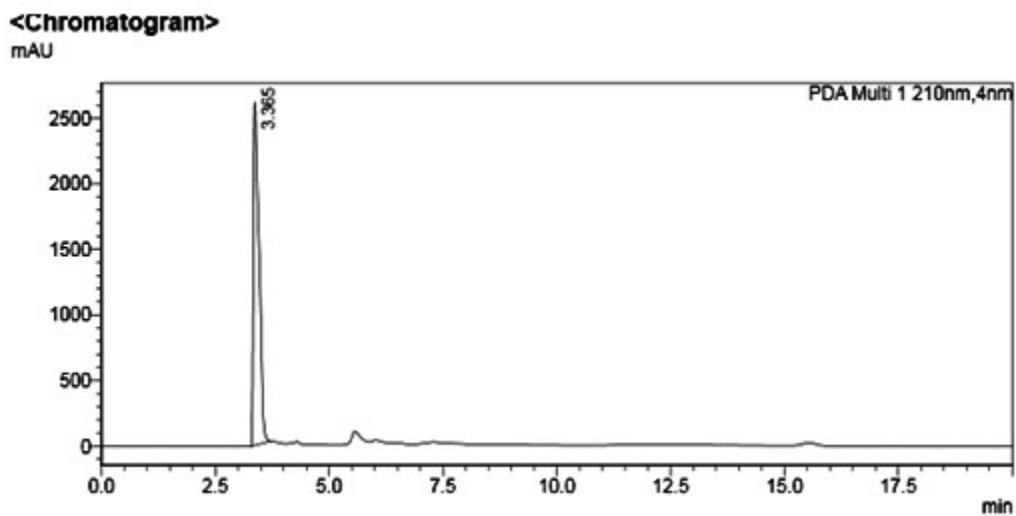
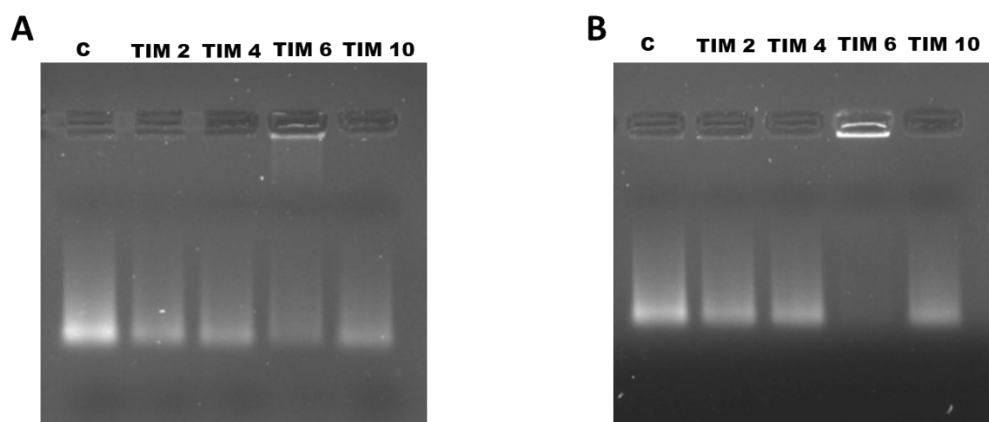


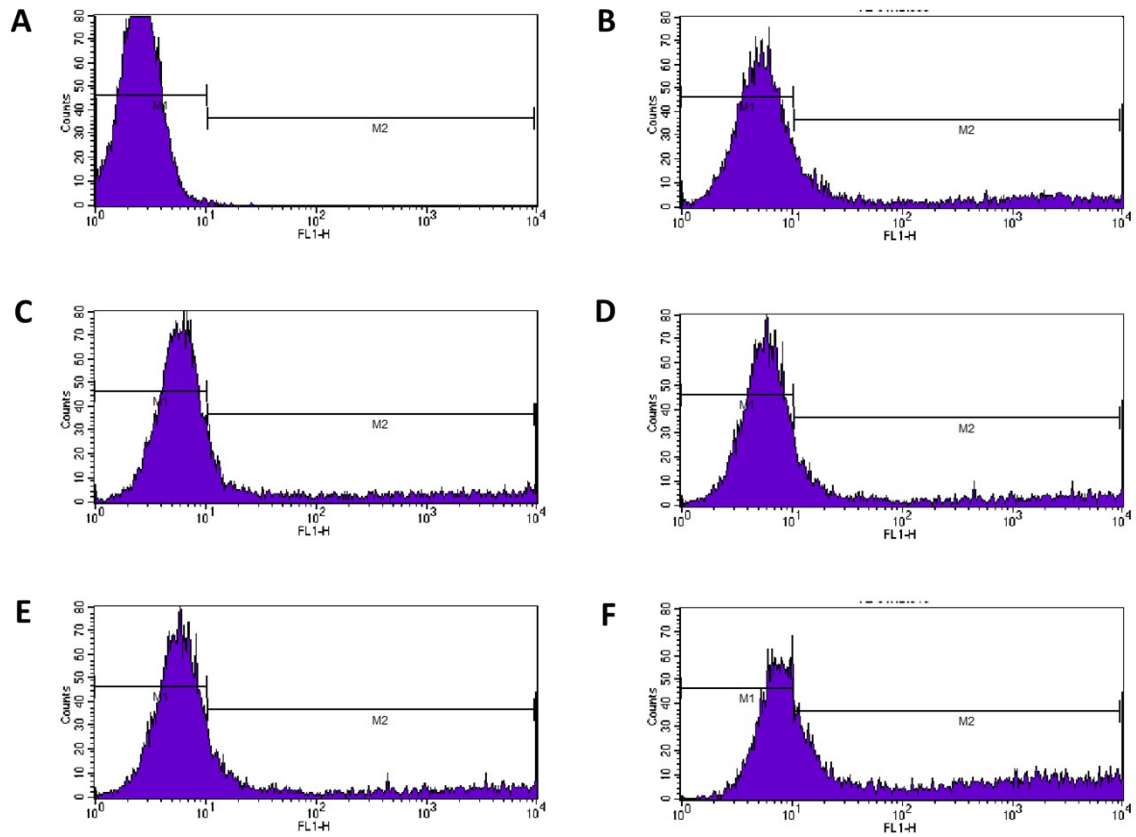
Fig. S16: HPLC chromatogram of TIM 6



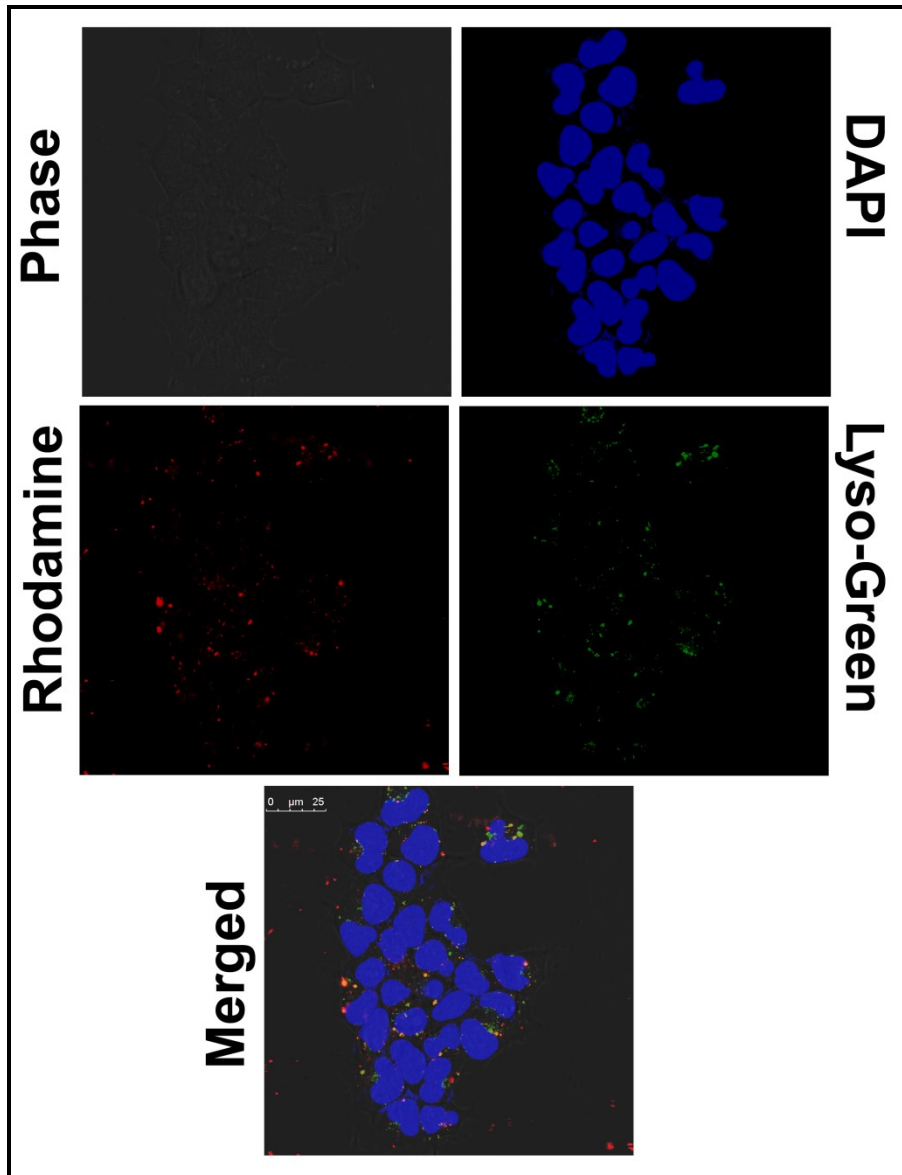
**Fig. S17:** HPLC chromatogram of TIM 10



**Fig. S18:** Electrophoretic gel patterns of DNA complexes incubated with 0.3  $\mu\text{g}$  of heparin sodium salt for 30 minutes. DNA complexes are prepared with four dimeric lipid complexes at N/P ratios 2: 1 (A) and 4:1 (B). Naked DNA serves as control “C”.



**Fig. S19** Representative FACS histograms of HEK293 cells treated by A) pEGFP-N3 only, B) TIM 2-pEGFP-N3, C) TIM 4-pEGFP-N3, D) TIM 6-pEGFP-N3, E) TIM 10-pEGFP-N3 and F) L2K-pEGFP-N3 plasmid complexes.



**Fig. S20** Confocal laser scanning microscope images for endosomal co-localization of lipoplexes derived from rhodamine-PE labeled **TIM 6** formulation (lipid:DOPE:PE) and pCMV- $\beta$ gal DNA. Acidic compartments and nuclear regions were stained with lysotracker green and DAPI blue respectively in HEK 293 cells.