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Supplementary Information

Co-liposomes of Redox-Active Alkyl-Ferrocene Modified Low MW Branched PEI and DOPE for Efficacious Gene Delivery in Serum

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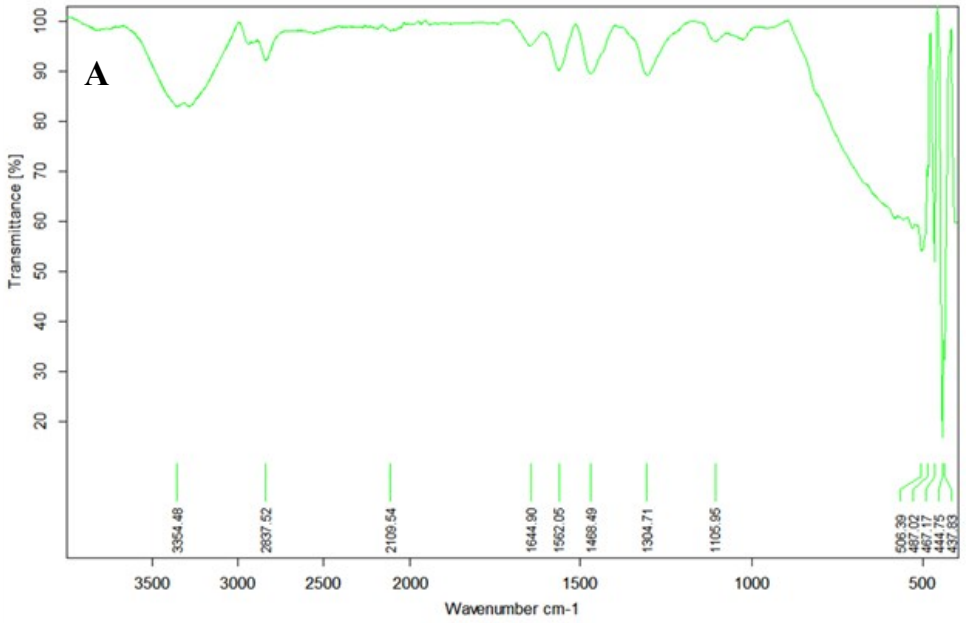
Table S1. Average particle size and Zeta potential of each suspension of various redox lipopolymers alone in water in their reduced and oxidized states.[a]

Lipopolymer	% Ferrocene grafting	Reduced		Oxidized		Average Zeta potential (mV)	
		Hydrodynamic diameter (nm)	PDI	Hydrodynamic diameter (nm)	PDI	Reduced	Oxidized
P8-C6-F1	15	87 ± 8	0.16 ± 0.02	170 ± 5	0.19 ± 0.03	46 ± 0.5	57 ± 2
P8-C6-F2	23	100 ± 10	0.16 ± 0.02	175 ± 5	0.21 ± 0.02	40 ± 1	48.5 ± 1.5
P8-C6-F3	48	132 ± 10	0.24 ± 0.03	205 ± 15	0.27 ± 0.05	37 ± 0.8	43 ± 2.5
P8-C11-F1	14	140 ± 8	0.11 ± 0.01	215 ± 5	0.13 ± 0.02	41 ± 1.2	48 ± 1.8
P8-C11-F2	24	165 ± 5	0.19 ± 0.02	270 ± 20	0.22 ± 0.03	37 ± 0.5	52 ± 2.2
P8-C11-F3	55	220 ± 5	0.23 ± 0.05	290 ± 20	0.26 ± 0.03	36 ± 1.5	57 ± 2.9

[a] Hydrodynamic diameters (average) and Poly Dispersity Index (PDI) obtained from the DLS measurements. Each measurement represents the mean ± SD of five independent measurements.


A
Transmittance [%]
100
90
80
70
60
50
40
30
20
3354.48
2837.52
2109.54
1644.90
1562.05
1468.49
1304.71
1106.95
506.39
497.02
467.17
444.75
437.63
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1

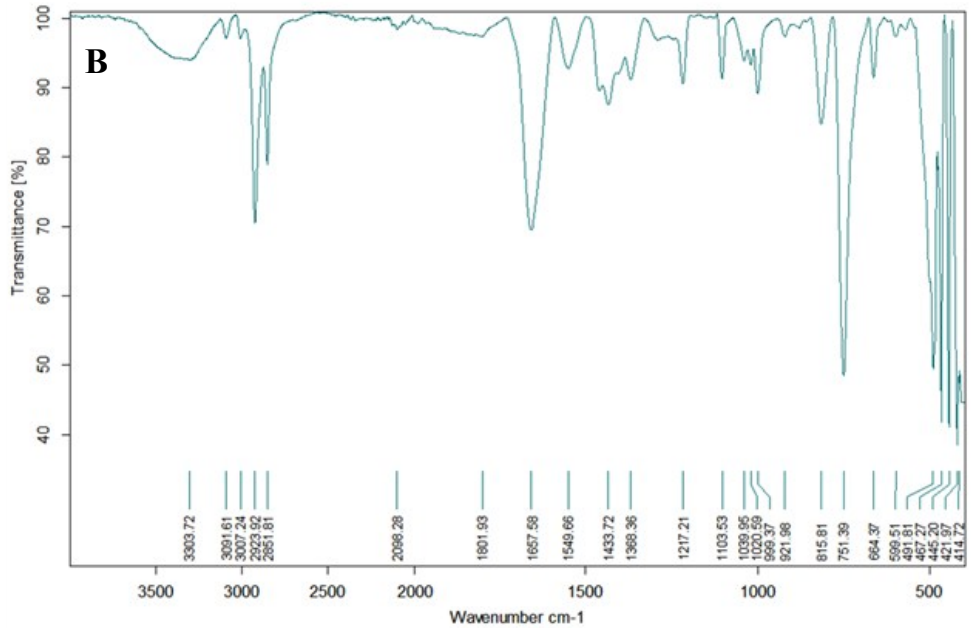

B
Transmittance [%]
100
90
80
70
60
50
40
3303.72
3091.61
3007.24
2923.92
2851.81
2098.28
1801.93
1657.58
1549.66
1433.72
1368.36
1217.21
1103.53
1039.95
1020.59
999.37
921.98
815.81
751.39
664.37
599.51
491.81
467.27
445.20
421.97
414.72
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1

Fig. S1 IR spectra of native BPEI (800 Da) (A) and an alkyl-ferrocene modified BPEI (800 Da), P8-C6-F1 (B).

The peak at 1560 cm^{-1} corresponds to the bending vibration of the NH_2 groups, while the peaks at 1045 and 1105 cm^{-1} are attributed to the symmetric and asymmetric stretching bands of imine groups respectively. The peaks at 1468, 2837 and 2940 cm^{-1} correspond to the symmetric and asymmetric stretching bands of CH_2 respectively. After the alkyl-ferrocene modification of the BPEI, the characteristic peaks of ferrocene were observed at 815, 1433 and 3091 cm^{-1}.

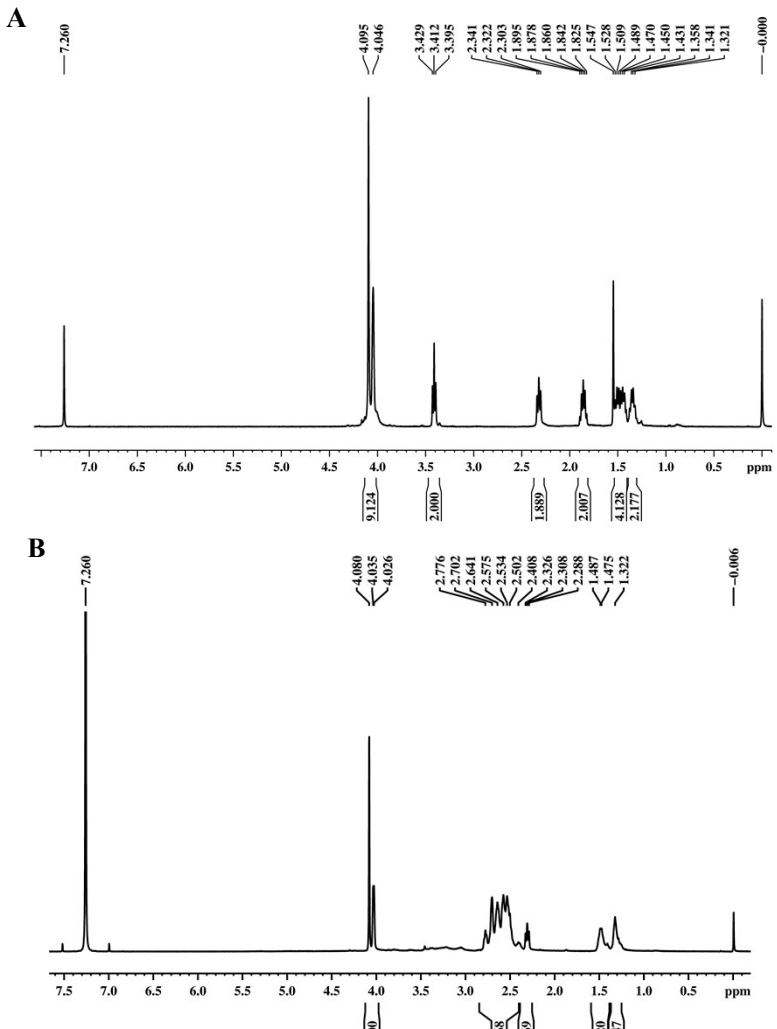


Fig. S2. ^{1}H NMR spectra of the (6-bromohexyl) ferrocene (A) and P8-C6-F1 (B) in $CDCl_3$.

In the ^{1}H-NMR of the reactant 6-bromohexyl ferrocene (Fig. S2A), the triplet at δ = 3.412 ppm corresponds to -CH_2-*CH_2*-Br which disappeared upon reaction with PEI. The ^{1}H-NMR of P8-C6-F1 (Fig. S2B) showed the peaks at δ = 4.03-4.08 ppm (9 Hs) which correspond to ferrocene moiety.

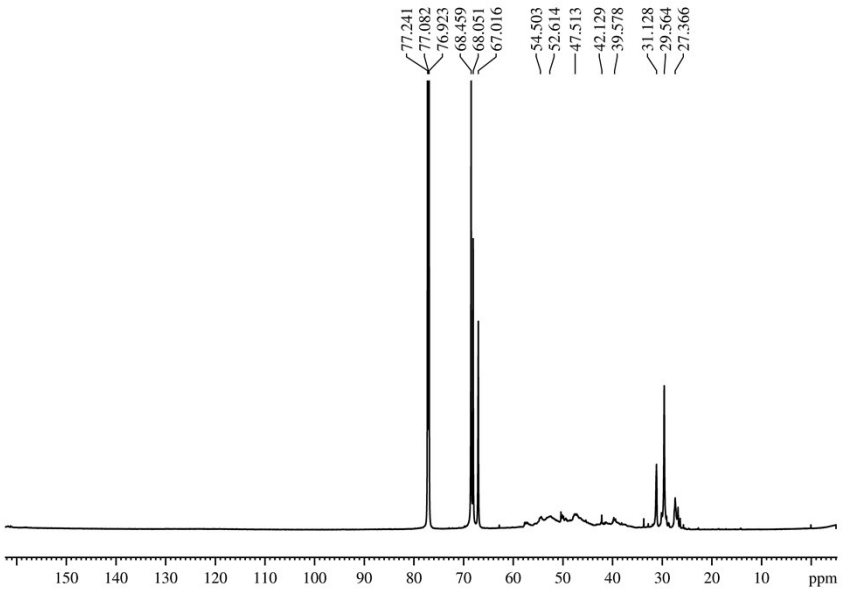


Fig. S3 ^{13}C NMR (200 MHz) of P8-C6-F1 in $CDCl_3$.

^{13}C NMR (Fig. S3) further confirmed the covalent grafting of alkyl ferrocene on the BPEI backbone. The signals between 39-55 ppm correspond to -*C*H_2*C*H_2N- and –N*C*H_2-$(CH_2)_5$Fc to PEI and the signals 67.0, 68.0 and 68.5 ppm correspond to ferrocene ring.

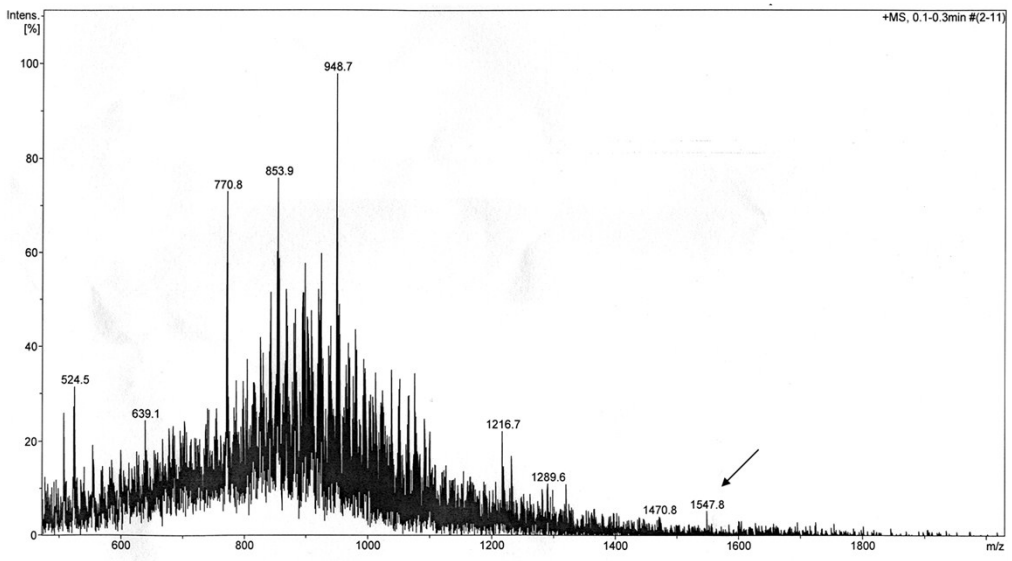
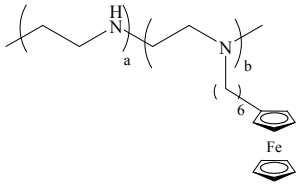


Fig. S4 ESI-MS spectra of P8-C6-F1.



Molecular weight of each repeating unit in native BPEI 800 Da is 43. Theoretical number of repeating units in unmodified BPEI 800 Da is 800/43 = 18.6

Mass of P8-C6-F1 (15% grafting) based on ^{1}H NMR is

[(43 x 0.8) + (312 x 0.151)] x 18.6 = 1550.3

In the mass spectrum of P8-C6-F1, a peak at m/z =1547.8 was observed which indicates the molecular ion peak of P8-C6-F1.

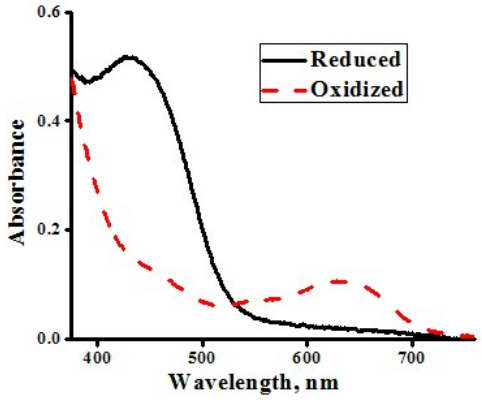


Fig. S5 UV-Vis spectra of lipopolymer P8-C6-F1 (A) before and (B) after oxidation at a concentration of 1 mg/mL.

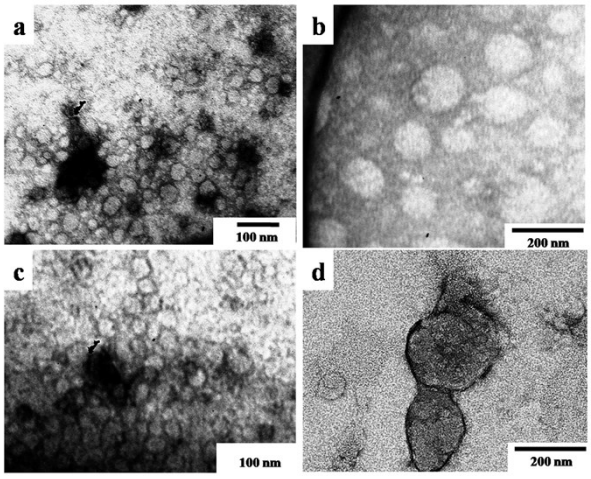


Fig. S6 Representative TEM images of redox lipopolymers P8-C6-F1 (a, b) and P8-C11-F1 (c d) before (a, c) and after oxidation (b, d).

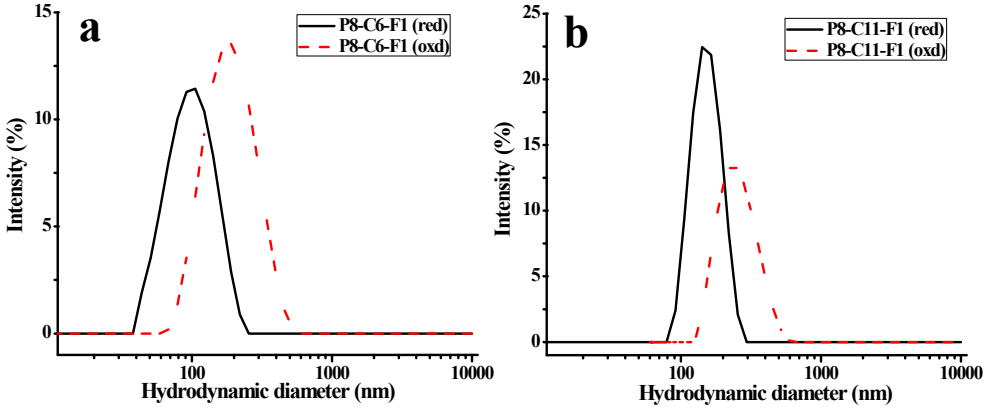


Fig. S7 Hydrodynamic diameter distribution of the neat redox lipopolymeric aggregates in water (a) P8-C6-F1 and (b) P8-C11-F1 before (solid line) and after (dashed line) oxidation by $FeCl_3$.

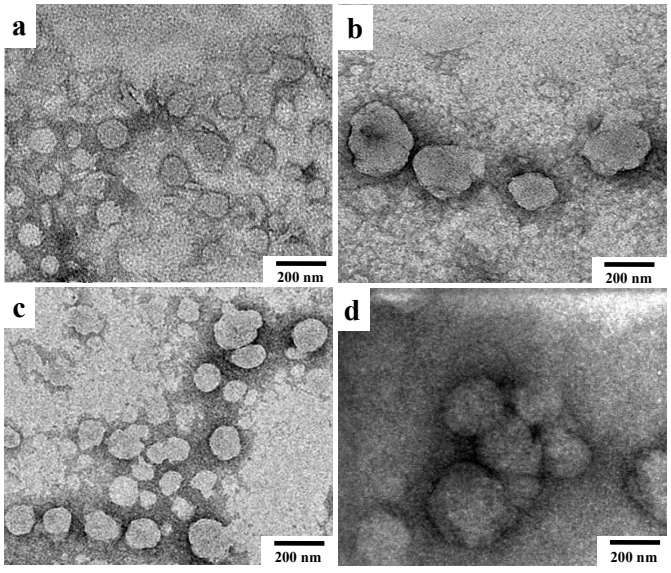


Fig. S8 Representative TEM images of co-liposomes containing DOPE and redox lipopolymers P8-C6-F1 (a, b) and P8-C11-F1 (c, d) before (a, c) and after oxidation (b, d).

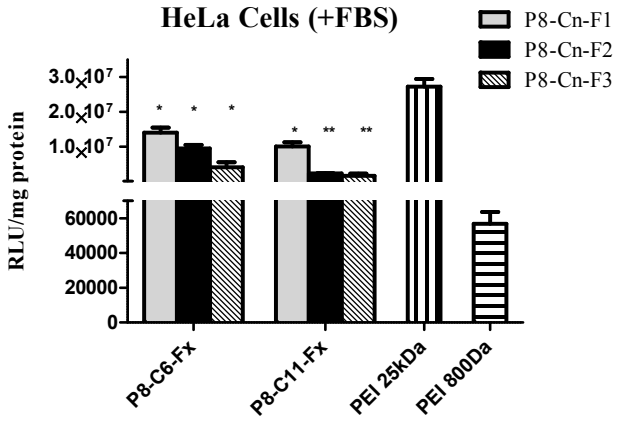


Fig. S9 Gene transfection capability (luciferase activity) of the ferrocene modified polymers alone and native BPEI 800Da using BPEI (25kDa) as a positive control in HeLa cells in the presence of 10% serum. The luciferase activities were examined for statistical significance in comparison with BPEI (25 KDa) transfections (*$P < 0.05$ and **$P < 0.01$, Two-tailed Student's t-test).

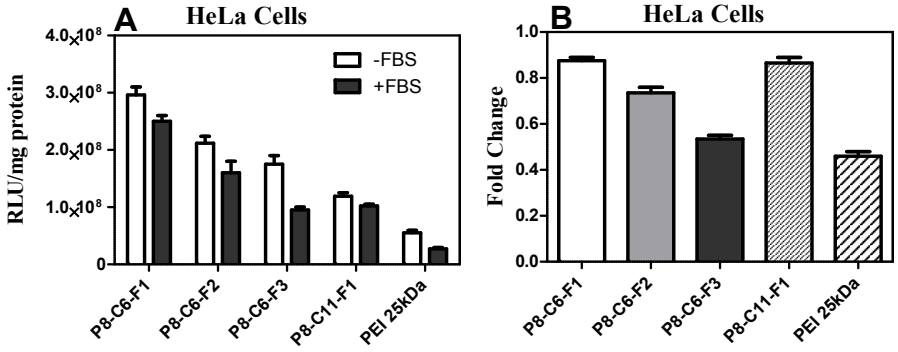


Fig. S10 (A) Luciferase activities of efficient lipopolymeric co-liposomal formulations in the absence of serum (-FBS) and presence of serum (+FBS). (B) The fold change in transfection activities in serum relative to those in absence of serum. The results are based on triplicates of three independent experiments.

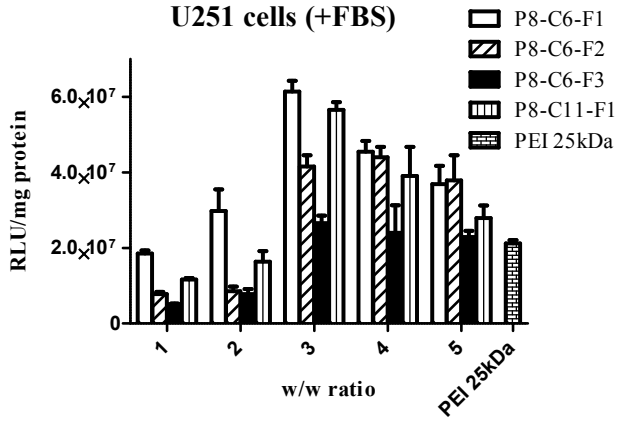


Fig. S11 Transfection efficacies (luciferase activity) of four efficient redox lipopolymer formulations in their reduced state in U251 cells in the presence of serum (10% FBS). The cells were transfected with 0.4 μg of pGL3 control plasmid DNA while using BPEI (25kDa) as a positive control.

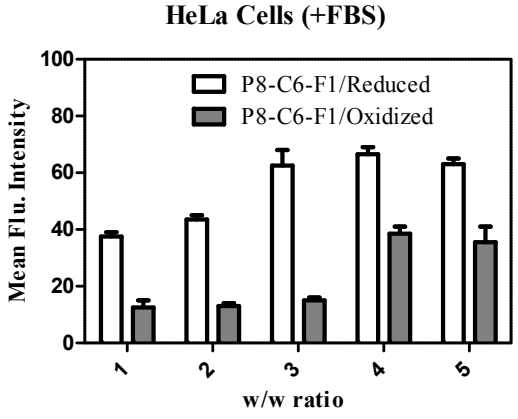


Fig. S12 GFP expression analysis of transfections obtained from flow cytometry for the most efficient co-liposomal formulation at different w/w ratios before and after the oxidation of ferrocene in HeLa cells in the presence of serum (10% FBS).

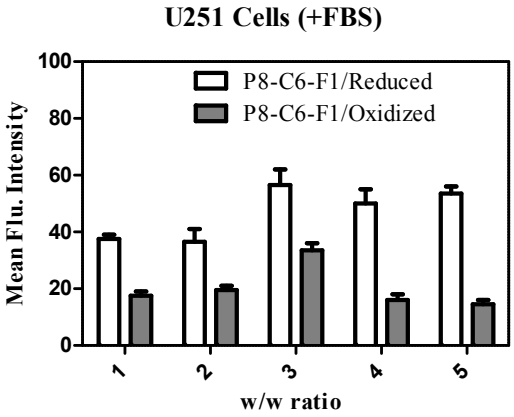


Fig. S13 GFP expression analysis of transfections obtained from flow cytometry for the most efficient co-liposomal formulation at different w/w ratios before and after the oxidation of ferrocene in U251 cells in the presence of serum (10% FBS).