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Supplemental information

Antisense oligonucleotide gapmers containing phosphoryl guanidine groups reverse MDR1-mediated multiple drug resistance of tumor cells

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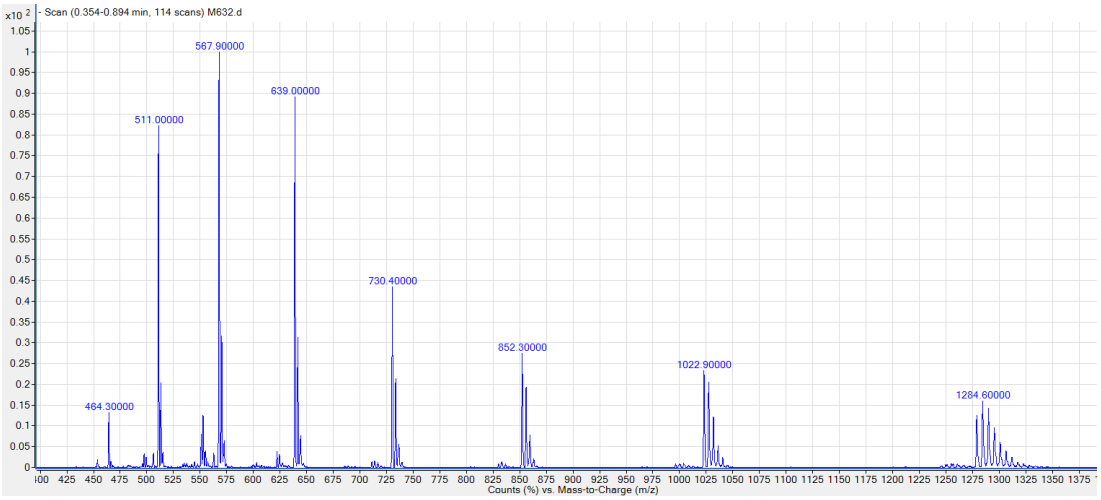

Figure S1. ESI spectrum (negative mode) for oligonucleotide R1.

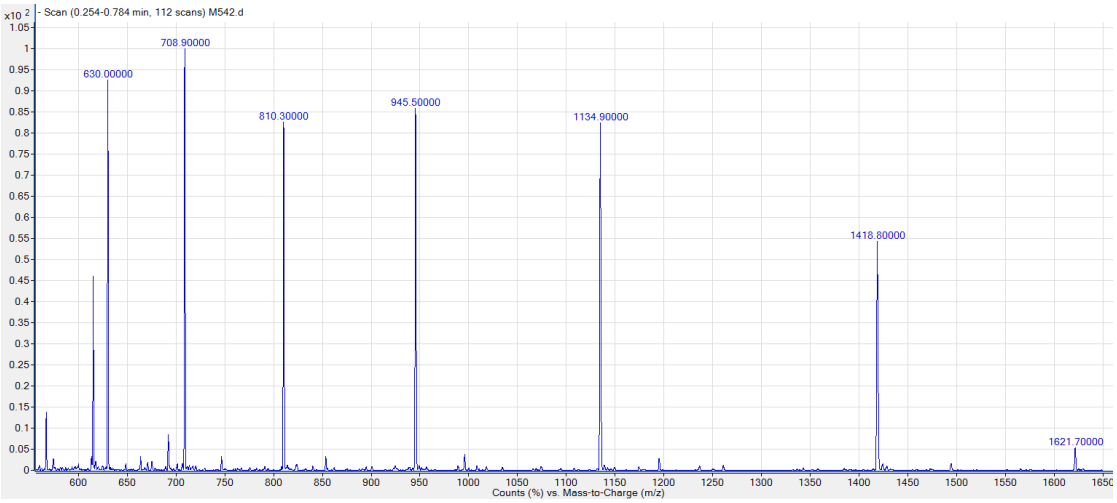

Figure S2. ESI spectrum (negative mode) for oligonucleotide R2.

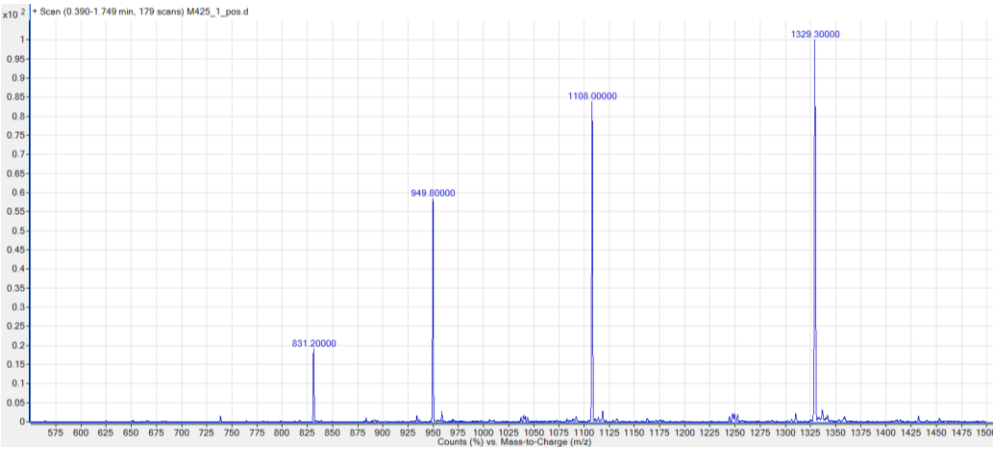

Figure S3. ESI spectrum (positive mode) for oligonucleotide R3.

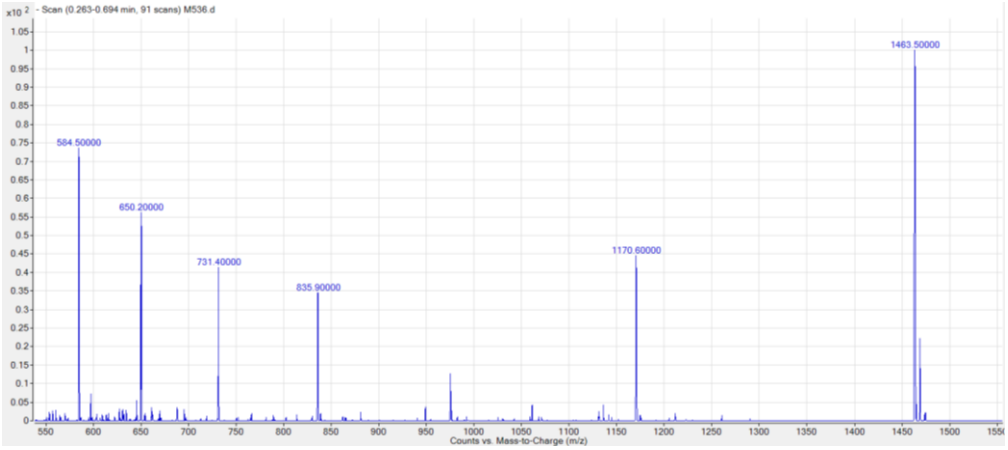

Figure S4. ESI spectrum (negative mode) for oligonucleotide R4.

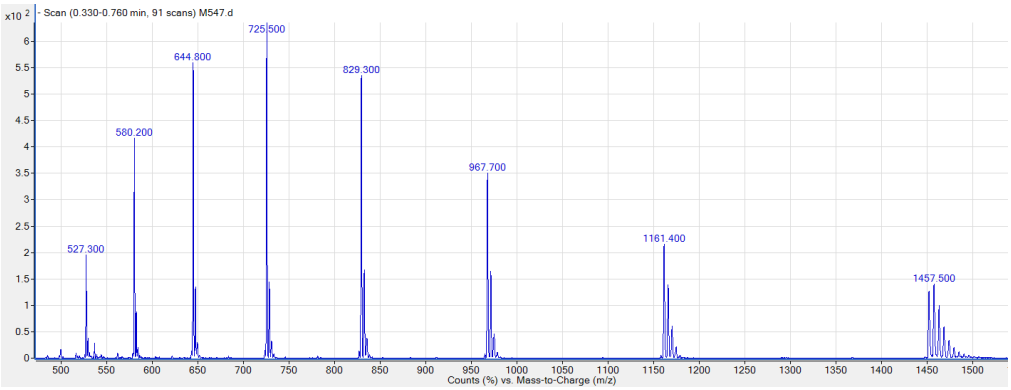

Figure S5. ESI spectrum (negative mode) for oligonucleotide R5.

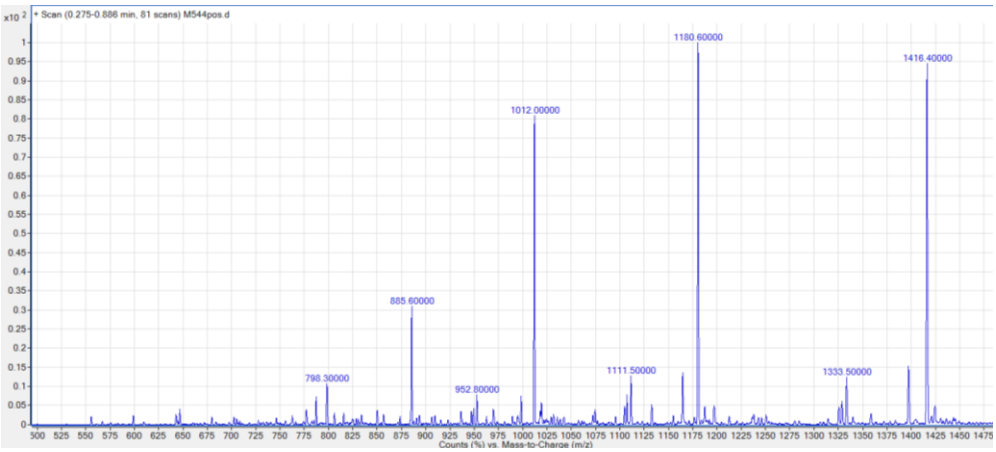

Figure S6. ESI spectrum (positive mode) for oligonucleotide R6.

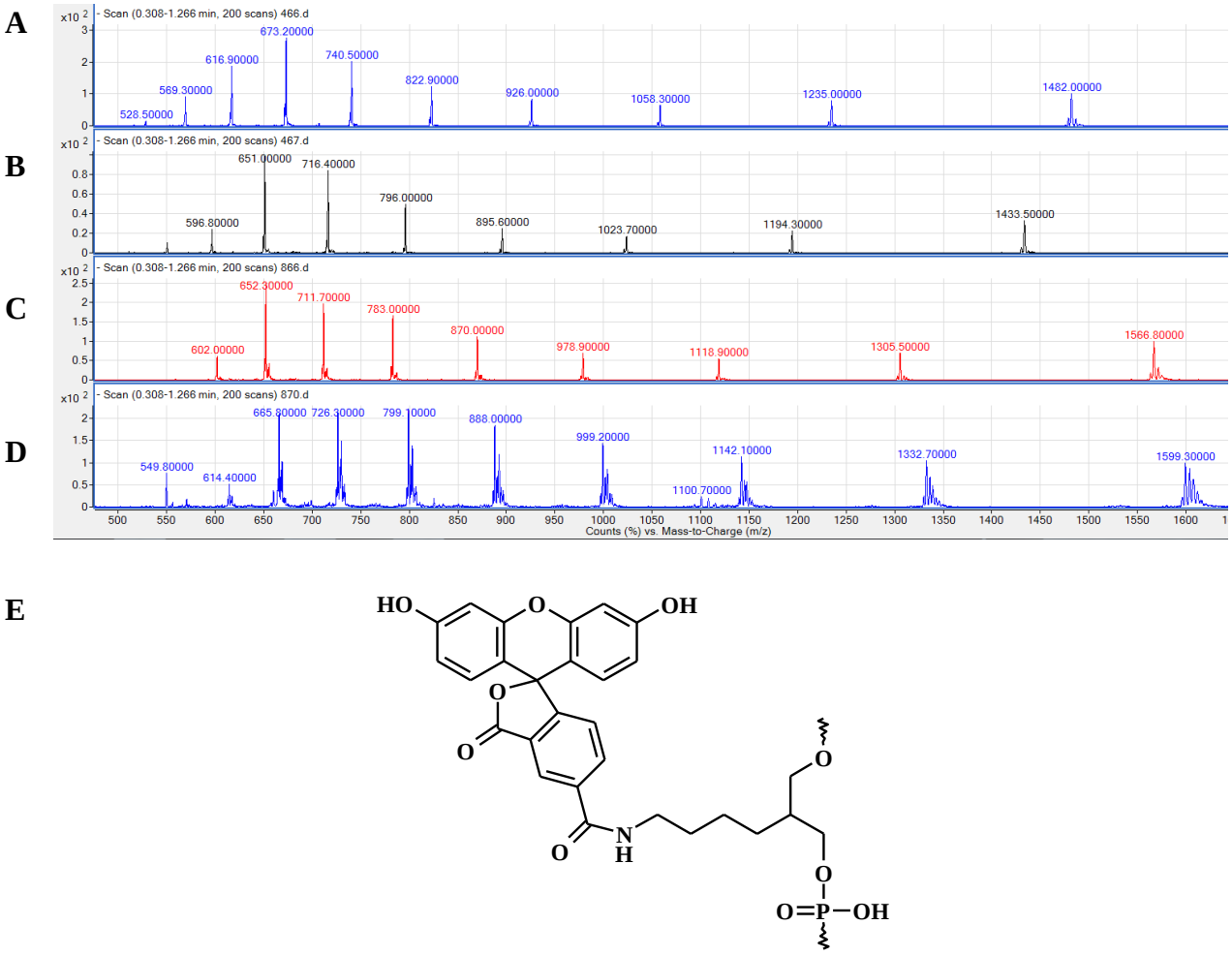

Figure S7. ESI spectrum (negative mode) for oligonucleotides of D series**.** (A) D1. (B) D3. (C) D2. (D) D4. (E) FAM structure.

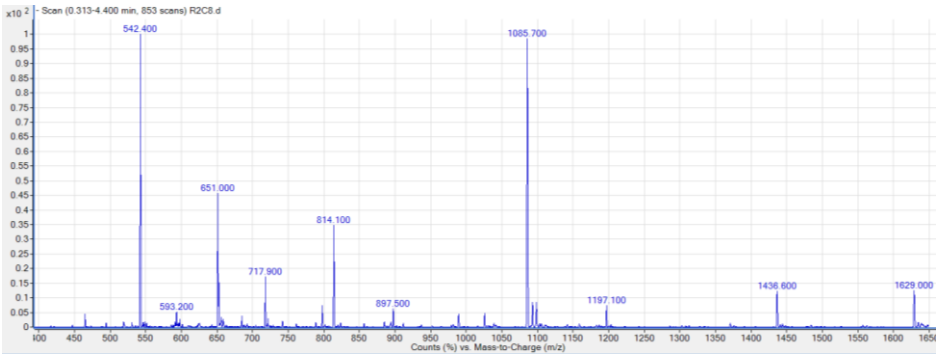

Figure S8. ESI spectrum (negative mode) for oligonucleotide D8.

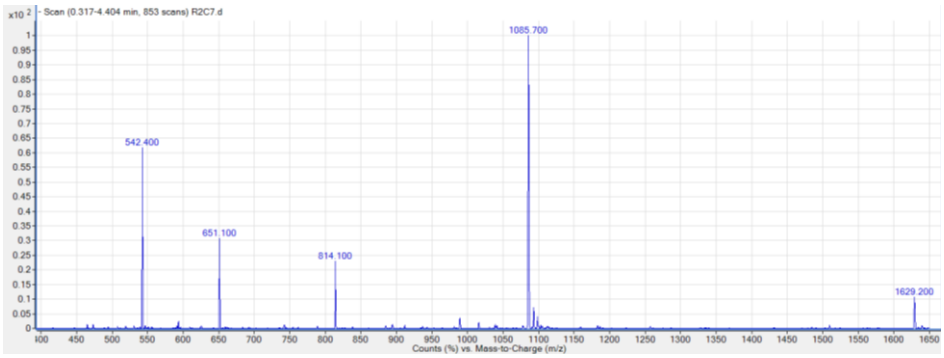

Figure S9. ESI spectrum (negative mode) for oligonucleotide D9.

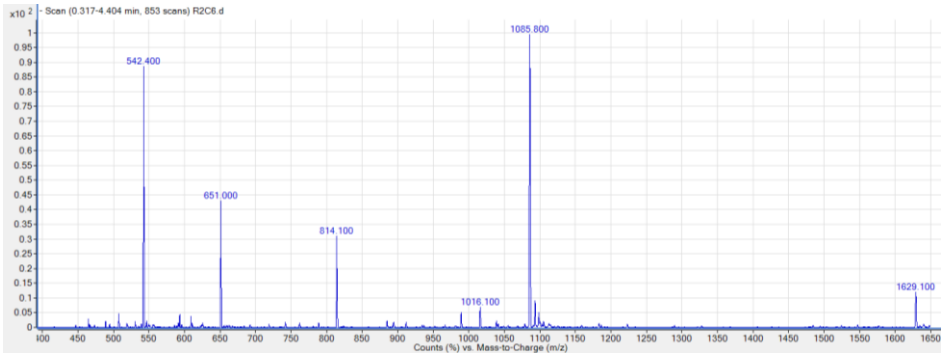

Figure S10. ESI spectrum (negative mode) for oligonucleotide D10.

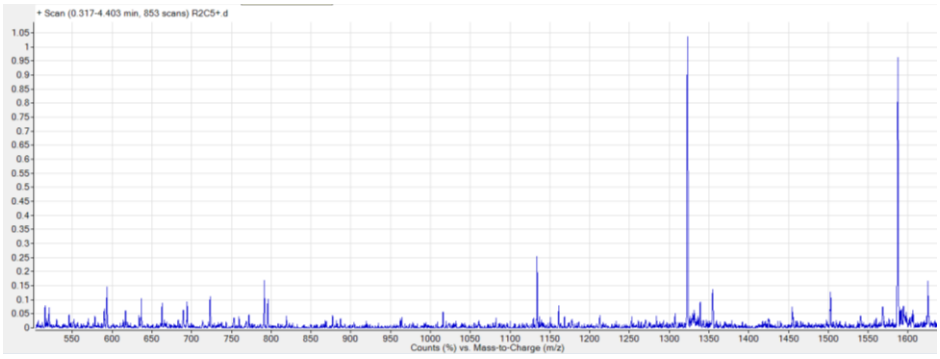

Figure S11. ESI spectrum (positive mode) for oligonucleotide D11.

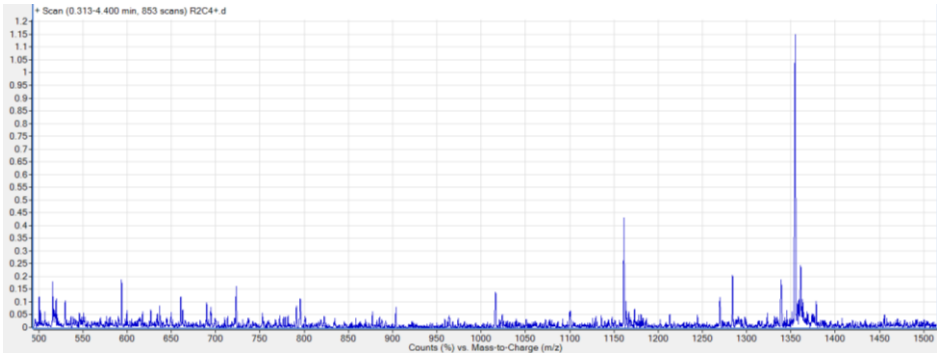

Figure S12. ESI spectrum (positive mode) for oligonucleotide D12.

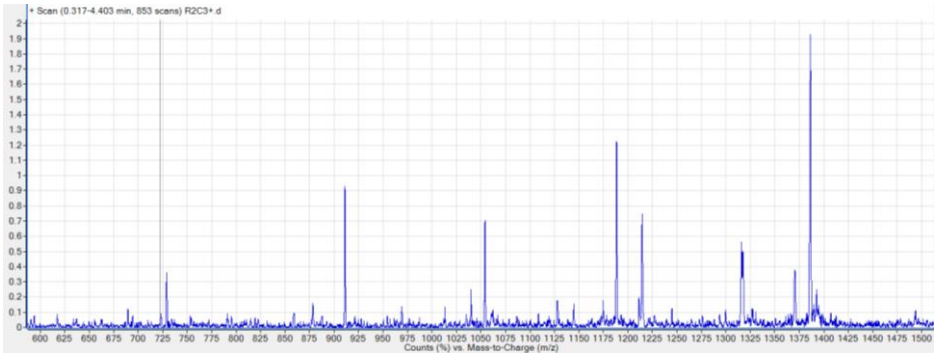

Figure S13. ESI spectrum (positive mode) for oligonucleotide D13.

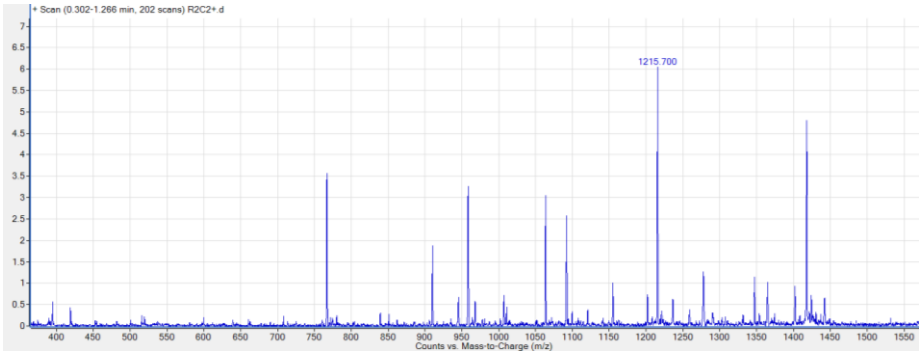

Figure S14. ESI spectrum (positive mode) for oligonucleotide D14.

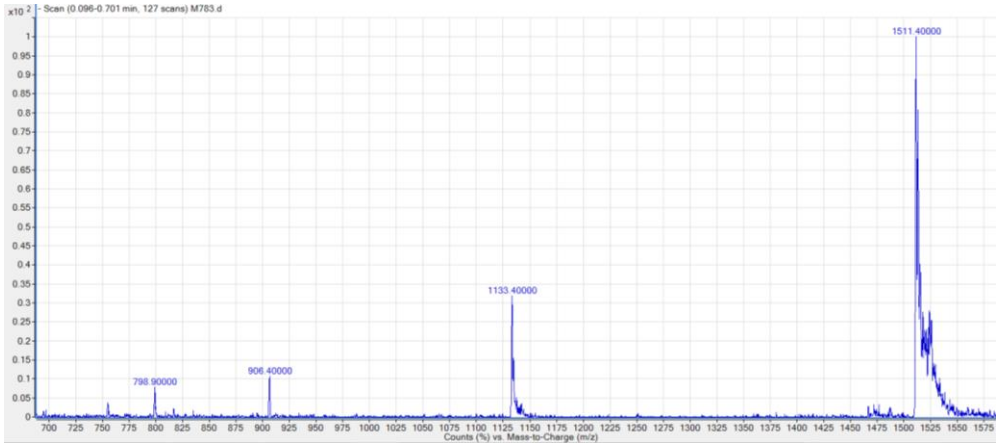

Figure S15. ESI spectrum (negative mode) for oligonucleotide M1.

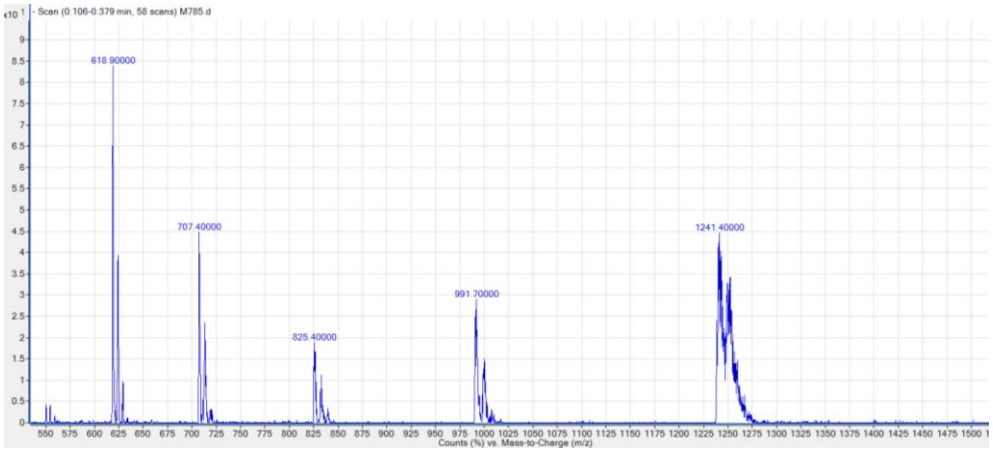

Figure S16. ESI spectrum (negative mode) for oligonucleotide M2.

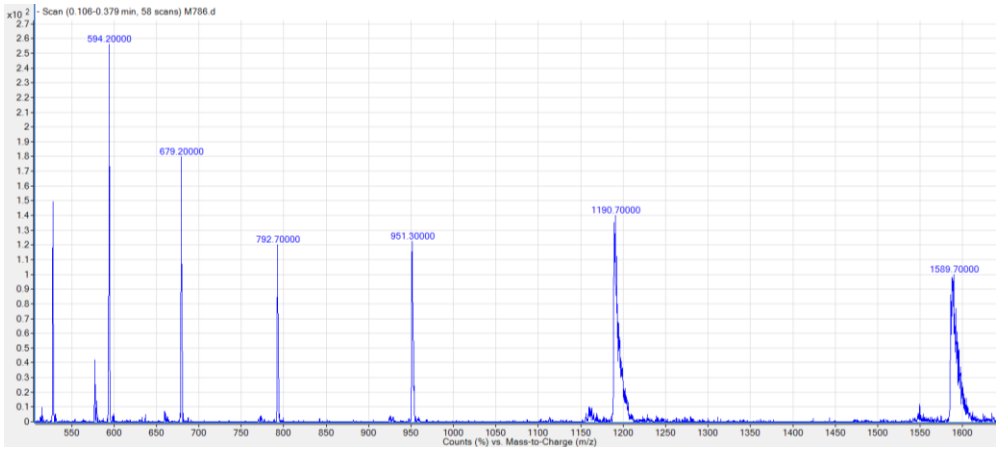

Figure S17. ESI spectrum (negative mode) for oligonucleotide M3.

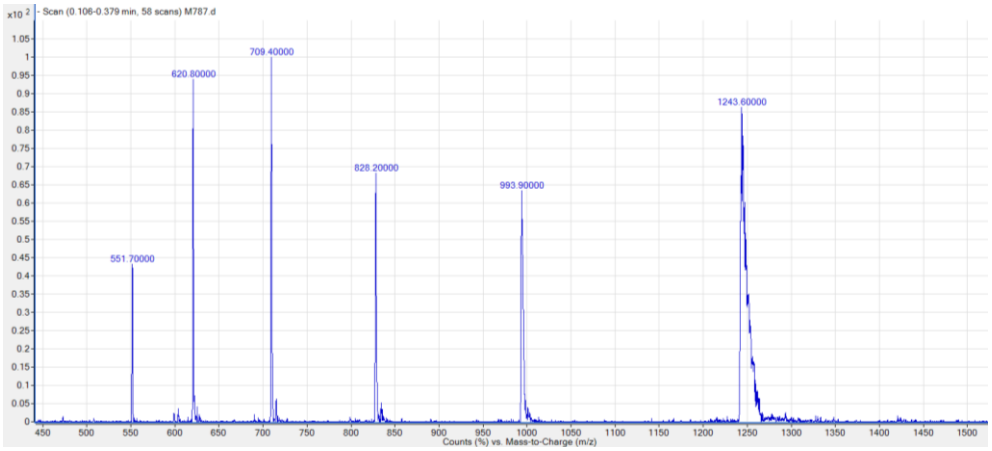

Figure S18. ESI spectrum (negative mode) for oligonucleotide G1.

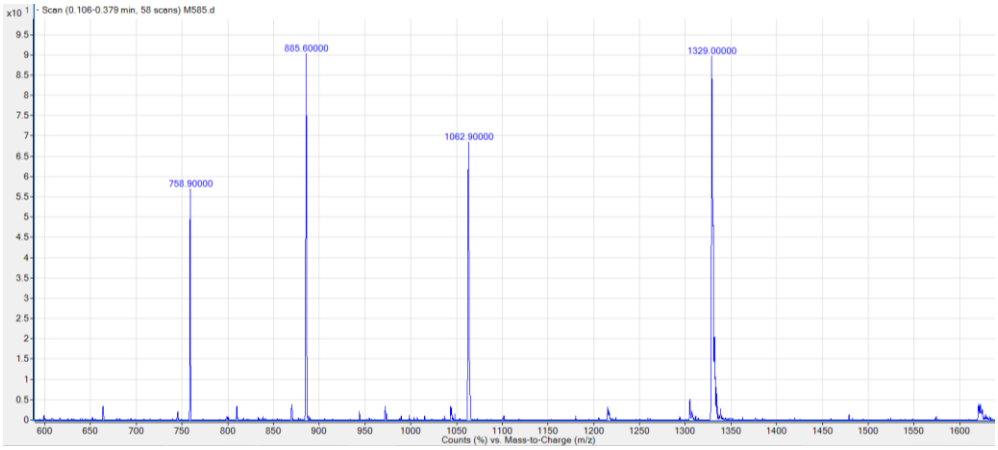

Figure S19. ESI spectrum (negative mode) for oligonucleotide G2.

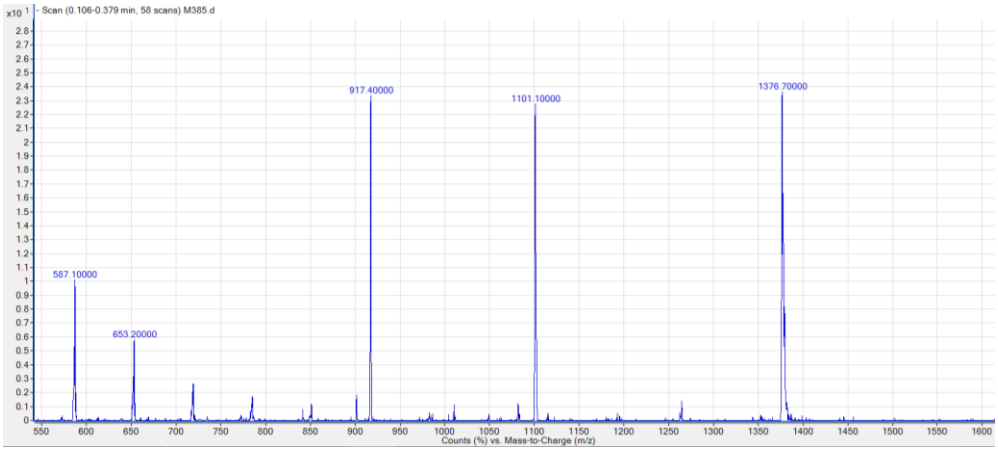

Figure S20. ESI spectrum (negative mode) for oligonucleotide G3.

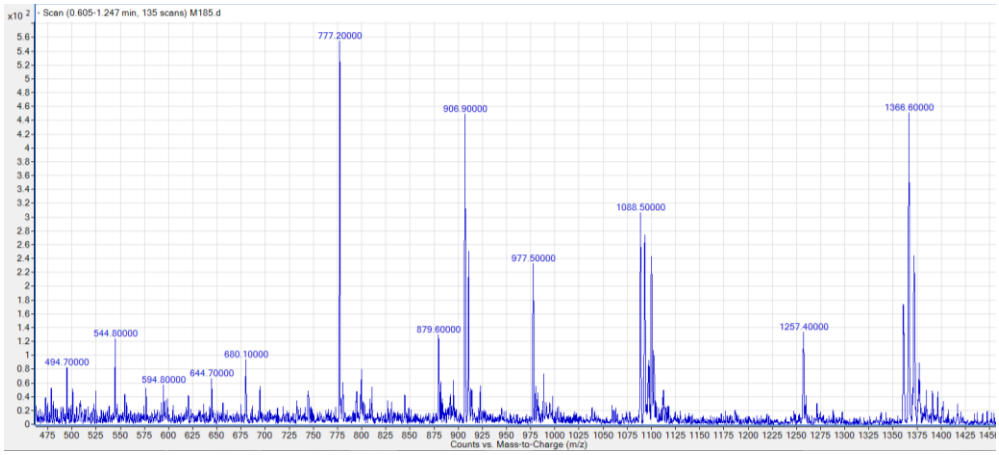

Figure S21. ESI spectrum (negative mode) for oligonucleotide G4.

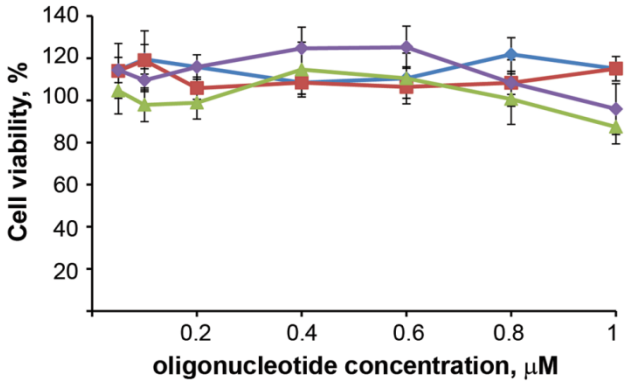

Figure S22. The effect of modified oligonucleotides on the viability of A549 cells. Cell viability relative to control (intact A549 cells). Circles – oligonucleotide D1, squares – oligonucleotide D2, triangles – oligonucleotide D3, and diamonds – oligonucleotide D4. Cells were incubated in the presence of oligonucleotides (0.1 – 1 μM) for 24 hours. The percentage of living cells was determined using MTT test. The data were statistically processed using the Student's t-test (two-tailed, unpaired); a *p* value of ≤ 0.05 was considered to indicate a significant difference; ** - data were statistically insignificant. All experimental points were run in triplicate for statistical analysis. Data are presented as mean±SEM.

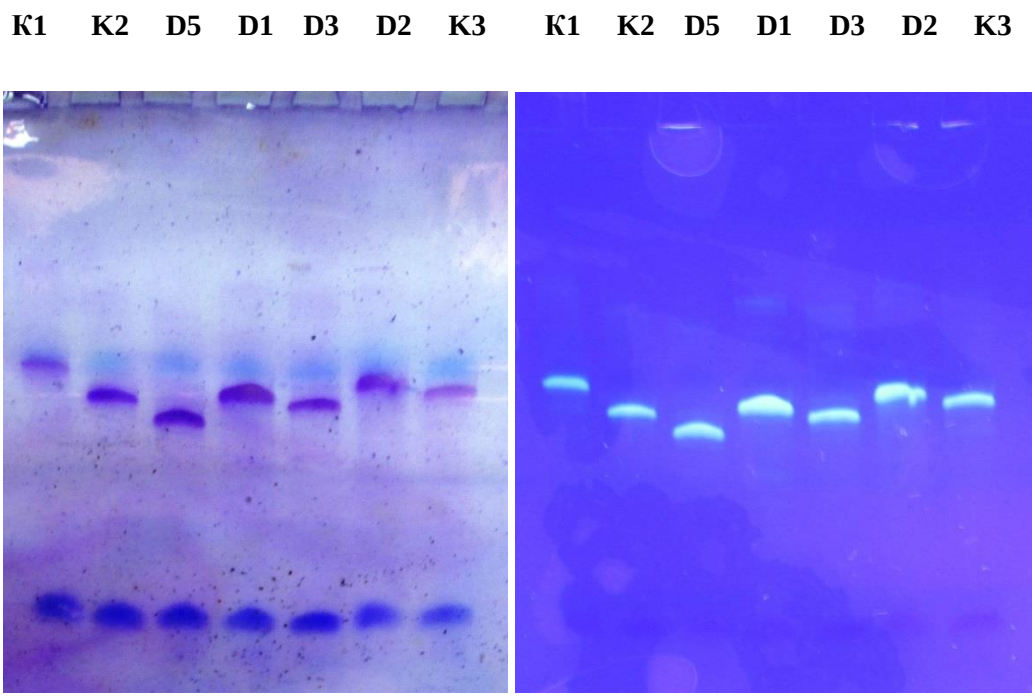

Figure S23. Typical 20% polyacrylamide/8 M urea gel colored by Stains-all dye showing the homogeneity of oligonucleotides. 2′-OMe ribonucleotides are in capital letters; s – phosphorothioate group.

K1 – FAM-GUGUUAUCAGACAUGAAACGGC;

K2 - FAM-CCAGUUCAAACAUUUCCCCU;

D5 – FAMs-AsGsUsCsUsCsGsAsCsUsUsGsCsUsAsCsC;

D1 - FAMs-AsGsUsCsUsCsGsAsCsUsUsGsCsUsAsCsCsUsCsA;

D3 - FAMs-GsAsCsAsUsCsCsAsUsUsCsAsAsAsUsGsGsUsUsUsG;

D2 - FAMs-CsUsCsCsGsAsAsGsAsAsAsUsAsAsGsAsUsCsC;

K4 – FAM-GGACUAUCGCUCAUGGUUUC.

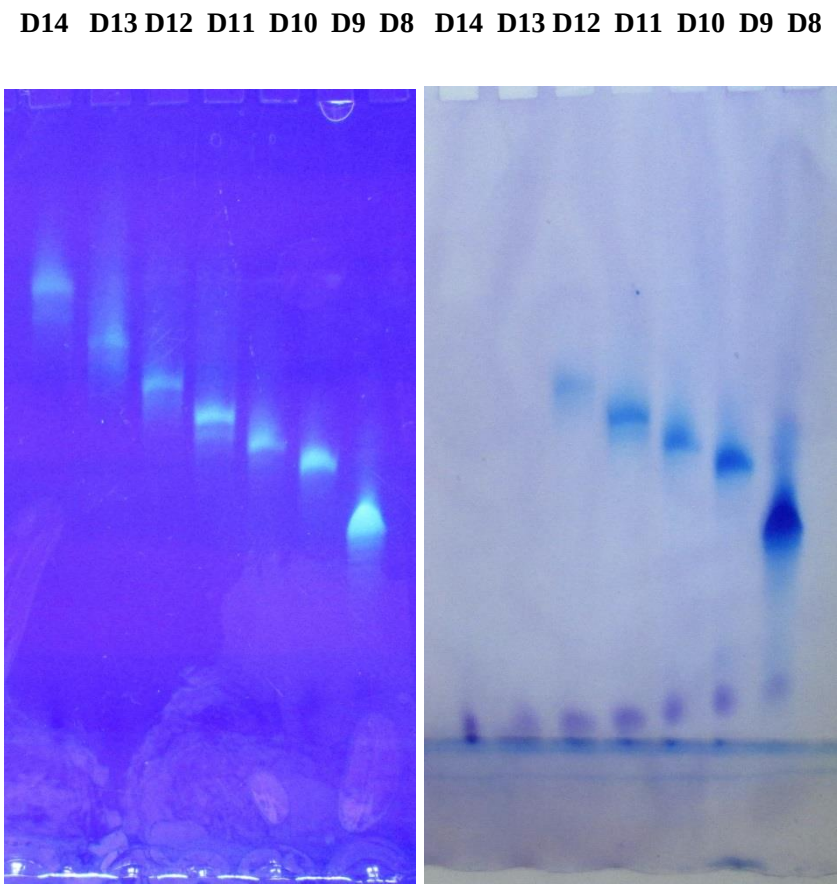

Figure S24. Typical 20% polyacrylamide/8 M urea gel colored by Stains-all dye showing the homogeneity of oligonucleotides. Deoxyribonucleotides are in lowercase letters; * – 1,3-dimethylimidazolidin-2-imine (Dmi) group.

D14 - FAM*-c*a*c*t*c*g*c*a*a*g*c*a*c*c*c*t*atcag;

D13 - FAM*-c*a*c*t*c*g*c*a*a*g*c*a*c*c*ctatcag;

D12 - FAM*-c*a*c*t*c*g*c*a*a*g*c*a*ccctatcag;

D11 - FAM*-c*a*c*t*c*g*c*a*a*g*caccctatcag;

D10 - FAM*-c*a*c*t*c*g*c*a*agcaccctatcag;

D9 - FAM*-c*a*c*t*c*g*caagcaccctatcag;

D8 - FAM-cactcgcaagcaccctatcag.

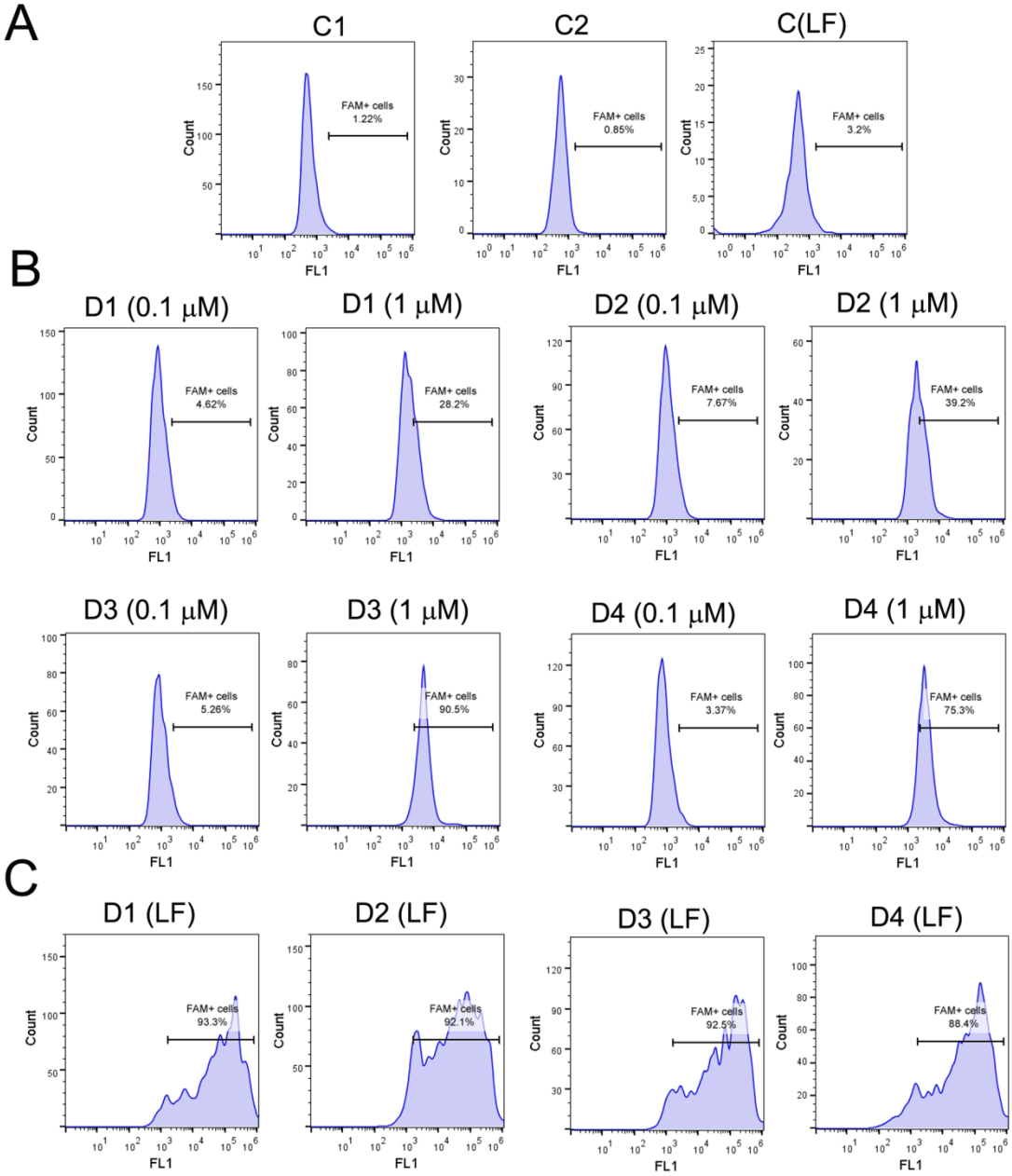

Figure S25. Intracellular accumulation of fluorescein-labelled oligonucleotides D1 – D4 in A549 cells mediated by Lipofectamine 2000 or under carrier-free conditions. (A) Controls. C1 – control, cells incubated without oligonucleotides; C2 – control, cells incubated without Lipofectamine 2000; C(LF) – cells incubated with Lipofectamine 2000. (B) Accumulation of D1–D4 in A549 cells in the absence of transfection agents. (C) Lipofectamine 2000–mediated delivery of D1–D4 in A549 cells. Under carrier-free conditions A549 cells were incubated in the presence of oligonucleotides D1–D4 at concentrations of 0.1 and 1 μM for 4 h. In the case of liposome-mediated delivery A549 cells were incubated with complexes formed by oligonucleotides D1–D4 (1 μM) with Lipofectamine 2000 for 4 h. The percentage of fluorescent cells was measured by flow cytometry 4 h post-transfection. Data are presented as histogram plots of event count versus fluorescence (FL1).

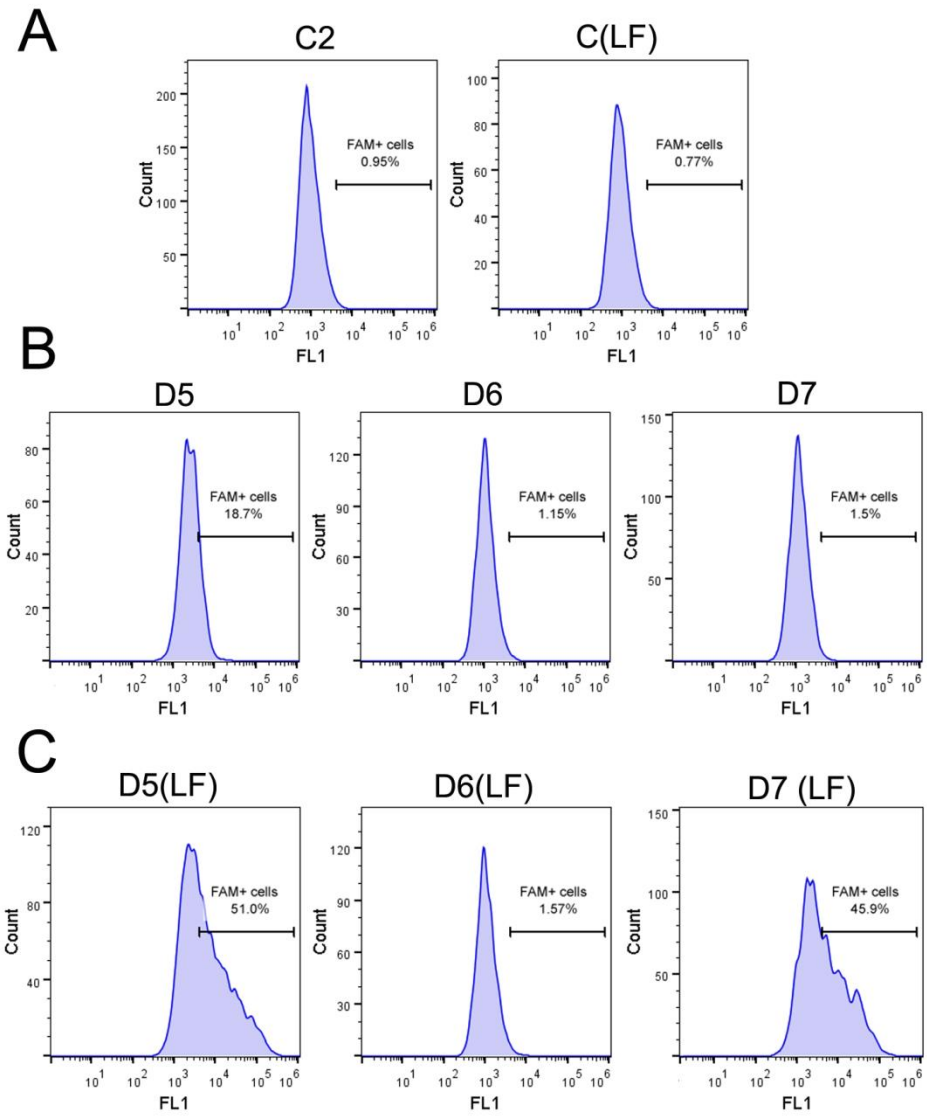

Figure S26. Intracellular accumulation of fluorescein-labelled oligonucleotides D5 – D7 in A549 cells mediated by Lipofectamine 2000 or under carrier-free conditions. (A) Controls. C2 – control, cells incubated without Lipofectamine 2000; C(LF) – cells incubated with Lipofectamine 2000. (B) Intracellular accumulation of D5–D7 in A549 cells under carrier free conditions. (C) Lipofectamine 2000–mediated delivery of D5–D7 in A549 cells. Under carrier-free conditions A549 cells were incubated in the presence of oligonucleotides D5 – D7 at concentrations of 5 μM for 4 h. In the case of liposome-mediated delivery A549 cells were incubated with complexes formed by oligonucleotides D5–D7 (1 μM) with Lipofectamine 2000 for 4 h. The percentage of fluorescent cells was measured by flow cytometry 4 h post-transfection. Data are presented as histogram plots of event count versus fluorescence (FL1).

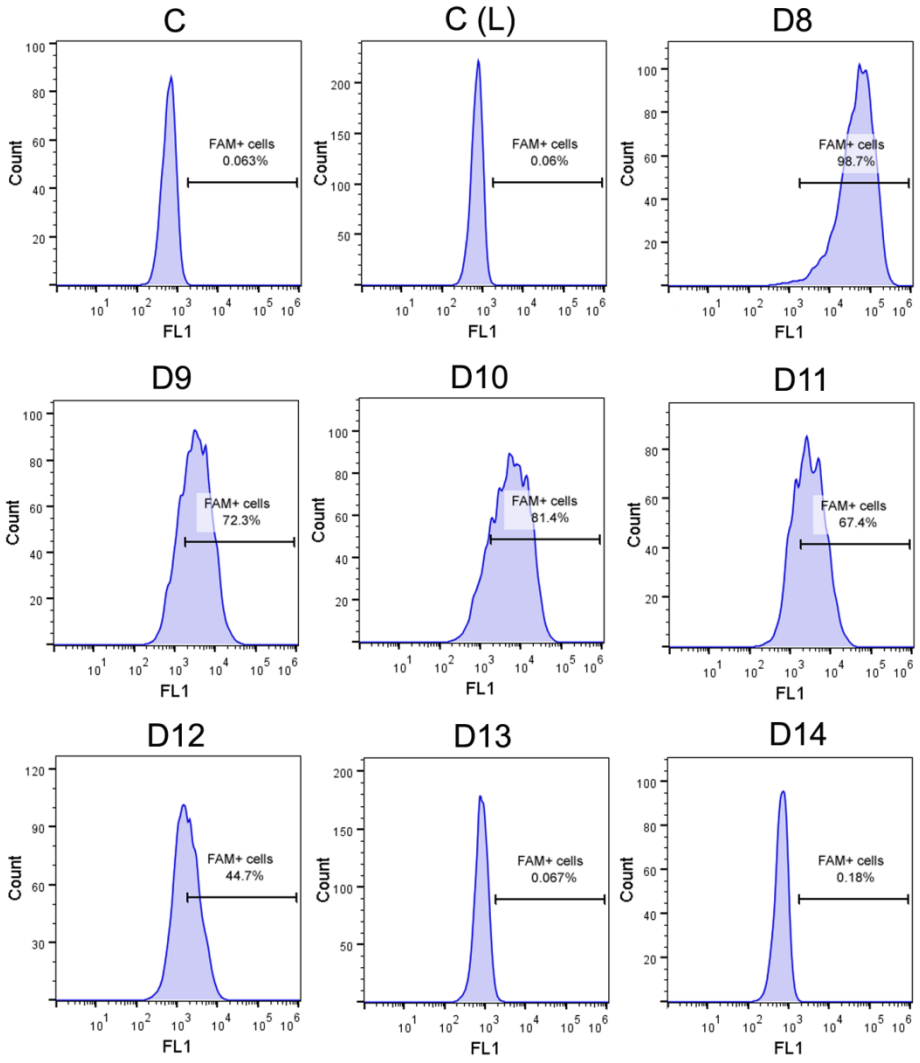

Figure S27. Intracellular accumulation of fluorescein-labelled oligonucleotides D8 – D14 in KB8-5 cells mediated by liposomes 2X3-DOPE. C – control, cells incubated without 2X3-DOPE; C(L) – cells incubated in the presence of 2X3-DOPE. KB-8-5 cells were incubated with complexes formed by oligonucleotides D8-D14 (1 μM) with 2X3-DOPE for 4 h. The percentage of fluorescent cells was measured by flow cytometry 4 h post-transfection. Data are presented as histogram plots of event count versus fluorescence (FL1).

Table S1. Half-life times of oligonucleotides in the presence of 50% foetal bovine serum.

Oligonucleotide	Modification	Half-life time ($\tau_{1/2}$)[a]
R1	deoxy/PO	10 min
R2	deoxy/PS	10 h
R3	deoxy/PG	> 21 days
R4	2′-O-Me/PO	70 min
R5	2′-OMe/PS	> 24 h
R6	2′-O-Me/PG	> 21 days

[a]Oligonucleotides R1–R6 (0.3 nmol) were incubated in DMEM supplemented with 50% FBS for 1 min – 24 h (oligonucleotides R1, R2, R4 and R5) and for 21 day (oligonucleotides R3 and R6) at 37°C followed by analyses of digestion products by 20% denaturing polyacrylamide gel electrophoresis (R1, R2, R4, and R5) or by liquid chromatography–electrospray ionisation–tandem mass spectrometry (LC-ESI-MS/MS) (R3 and R6). All experimental points were run in triplicate for statistical analysis.

Table S2. The effect of ASO modification on their accumulation in A549 cells.

ASO	Modification	Accumulation of ASO in A549 cells			
		Carrier-free mode		Liposome-mediated delivery	
		Fluorescence intensity, RFU	Fluorescent cells, %	Fluorescence intensity, RFU	Fluorescent cells, %
D1[a]	2′-OMe/PS	2.2±0.1	36.8±3.4	140.5±11.1	94.3±2.2
D2[a]	2′-OMe/PS	4±0.4	44±7	87.6±21	91.3±0.3
D3[a]	2′-OMe/PS	14.1±1.5	90.8±5	120±6.4	92.8±2.5
D4[a]	2′-OMe/19PS/2PG	12.23±2	84.6±6	117.5±17.5	89.3±7
D5[a]	2′-OMe/PS	3.1±0.4	31.5±2	42.6±4.3	53.3±4
D6[a]	deoxy/PG	1.3±0.5	1.9±0.5	0.7±0.3	0.7±0.1
D7[a]	deoxy/PO	1.4±1	2.7±0.6	40±1.2	70±2
D8[b]	deoxy/PO	1.2±0.6	2.6±0.8	59.5±3	98.9±1
D9[b]	deoxy/7PG	1.5±0.3	6±1.3	4.3±2	83.4±3.5
D10[b]	deoxy/9PG	1.7±0.3	8.1±0.9	7.7±2.2	88.9±4
D11[b]	deoxy/11PG	1.7±0.1	10.8±1	3.9±1.1	80.1±2.6
D12[b]	deoxy/13PG	1.9±0.8	19.4±2	2.1±0.9	65.2±5.2
D13[b]	deoxy/15PG	1.8±1	12.2±1.2	0.8±0.4	8.4±4.2
D14[b]	deoxy/17PG	1.3±0.9	2.7±2	0.7±0.5	1.0±0.5

A549 cells were incubated in the presence of oligonucleotides D1–D14 at concentration of 5 μM in the absence of transfectant or at concentration of 1 μM complexed with Lipofectamine 2000 ([a]) or liposomes 2X3-DOPE ([b]) for 4 h. The percentage of fluorescent cells was measured by flow cytometry 4 h post-transfection. Data are presented as mean ± SEM. The data were statistically processed using the Student's t-test (two-tailed, unpaired); a p value of ≤ 0.05 was considered to indicate a significant difference. All experimental points were run in triplicate for statistical analysis.

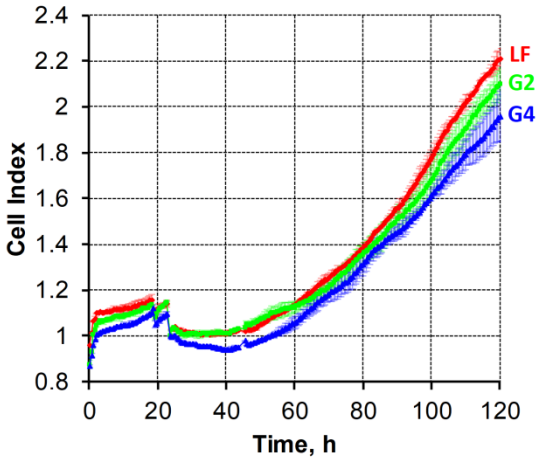

Figure S28. Real-time analysis of the effect of modified ASOs on the growth rate of KB-8-5 cells in the absence of vinblastine. Cells were transfected with 1 μM of oligonucleotide G2, or 0.1 μM of oligonucleotide G4 pre-complexed with Lipofectamine 2000 for 4 h; LF – cells treated with Lipofectamine 2000 only. Cell viability was recorded in real time using an xCELLigence instrument for 120 h. The results are shown as mean cell index ± standard error.