Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supporting Information

Targeted delivery of siRNA using ternary complexes with branched cationic peptides and DOTMA/DOPE: the role of peptide sequence, peptide branching and lipids.

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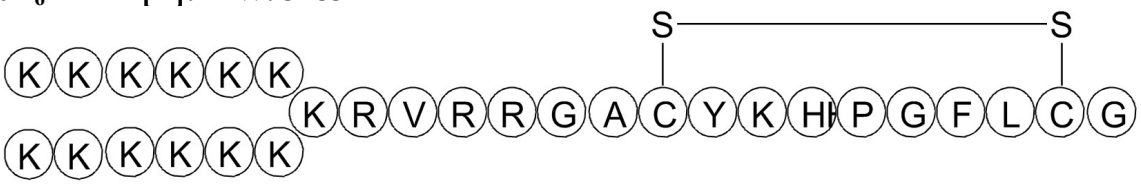
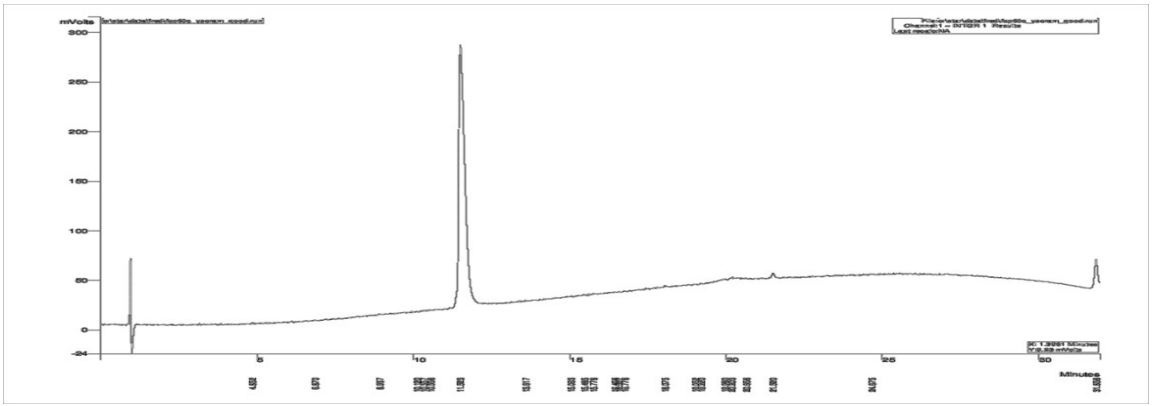
sK$_6$B1-L1-[Y]: MW. 3483

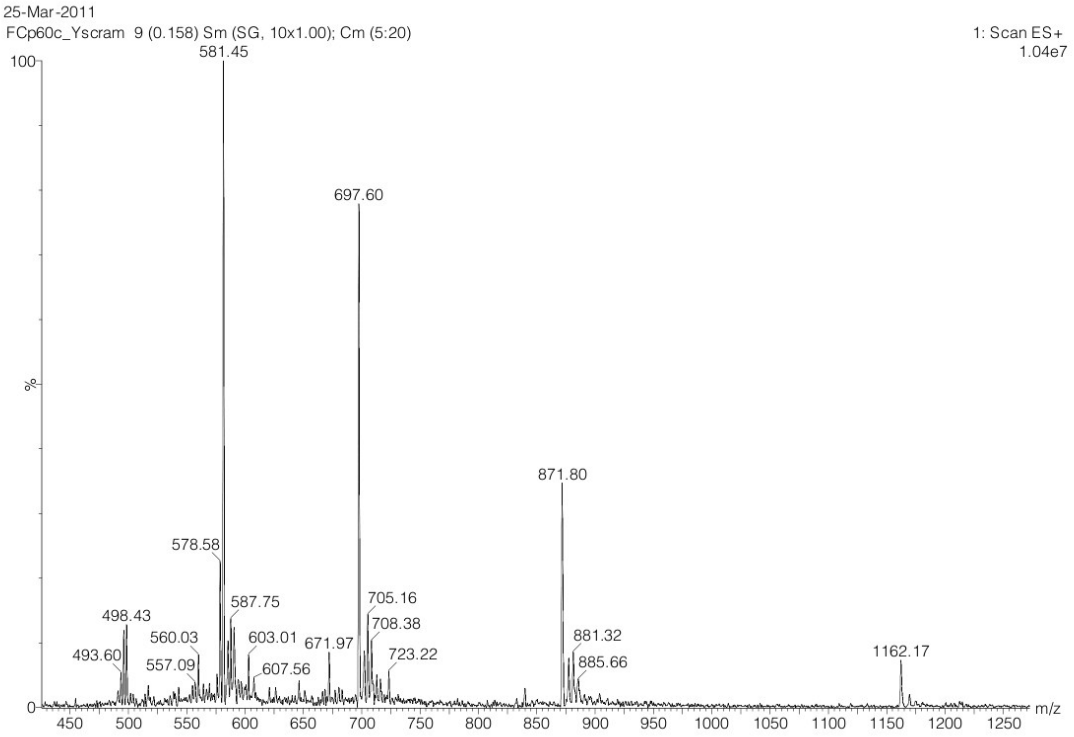


The disulfide bond was formed by aerial oxidation.

Purification of crude peptide was performed using a DiscoveryBIO Wide Pore C18 (Varian; 100 x 21.2 mm, 5 μm beads) flow rate of 10 mL/min, and UV detection at 215 and 254 nm. Linear gradient: 10-50% B over 25 min, A = H_2O, 0.1% TFA, B = CH_3CN, 0.1% TFA).



Analysis of the purified peptide was performed using an Onyx monolithic C18 column (Phenomenex; 100 x 3.0 mm), flow rate 0.85 mL/min, UV detection at 215nm. Linear gradient: 5-90% B over 30 min, A = H_2O, 0.1% TFA, B = CH_3CN, 0.1% TFA). R_T 11.523, *m*/*z* (ES+) 1162.17 ($[M + 3H]^{3+}$), 871.80 ($[M + 4H]^{4+}$), 697.60 ($[M + 5H]^{5+}$), 581.45 ($[M + 6H]^{6+}$).



FITC-labeling of branched peptides

To a stirred solution of peptide (1 mg/mL) in bicarbonate buffer (pH 8.99 @ 22.2ºC) was added fluorescein isothiocyanate (FITC, 10 eq. per peptide). The reaction mixture was stirred at room temperature for 4 h. Excess free FITC was removed by repeated dilution with water and separation by size exclusion spin filtration (VivaSpin; MWCO 2000 Da). The supernatant was then freeze-dried.

Exact FTIC-labelling conditions for each peptide:

$(H_3K)_4$B1-L1-Y (Mw 6012) – 1 mL of a 1 mg/mL solution in bicarbonate buffer – 0.166 umol. 64.8 uL of FITC (@10 mg/mL) – 1.66 umol.

K_6B2L1-Y (Mw 6817) – 1 mL of a 1 mg/mL solution in bicarbonate buffer – 0.147 umol. 64.8 uL of FITC (@10 mg/mL) – 1.47 umol.

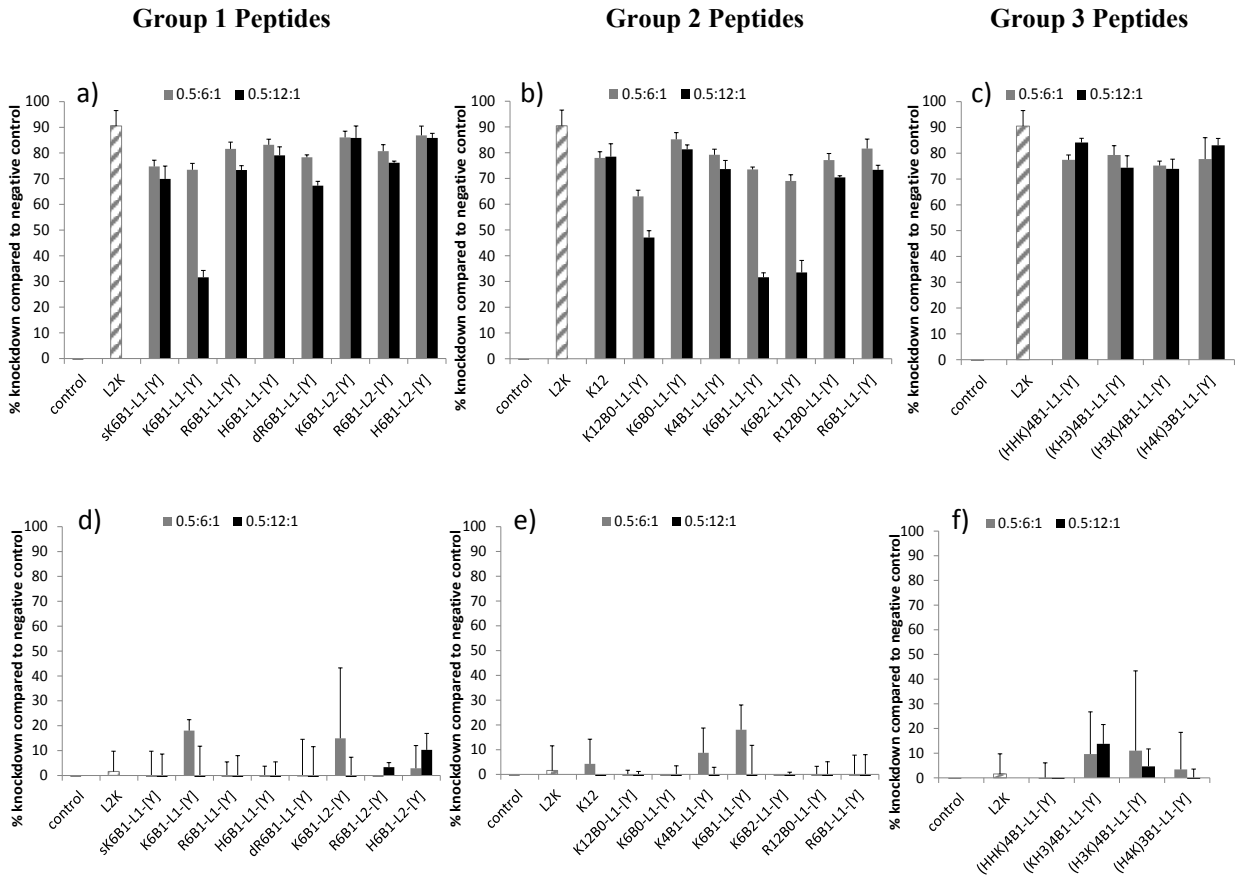


Figure S1: % Knockdown efficiency (calculated as a % of luminescence produced by each sample formulated with negative control siRNA, and normalised according to the protein content) of LPRs at 0.5:6:1 (grey bars) or 0.5:12:1 (black bars) charge ratios, prepared using (a, d) group 1, (b, e) group 2, and (c, f) group 3 peptides. LPRs were prepared either fully in OptiMEM (a, b, c); or in 12.5% water then diluted in serum-containing growth media (d, e, f). siRNA alone and L2K:siRNA at 5:1 weight ratio were used as negative and positive controls, respectively.

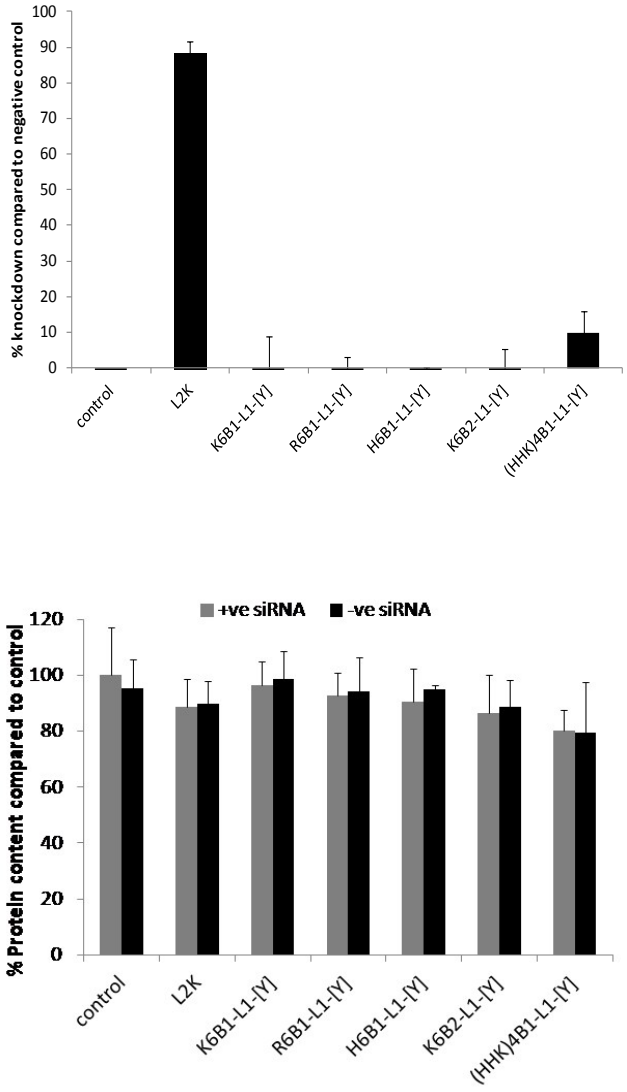
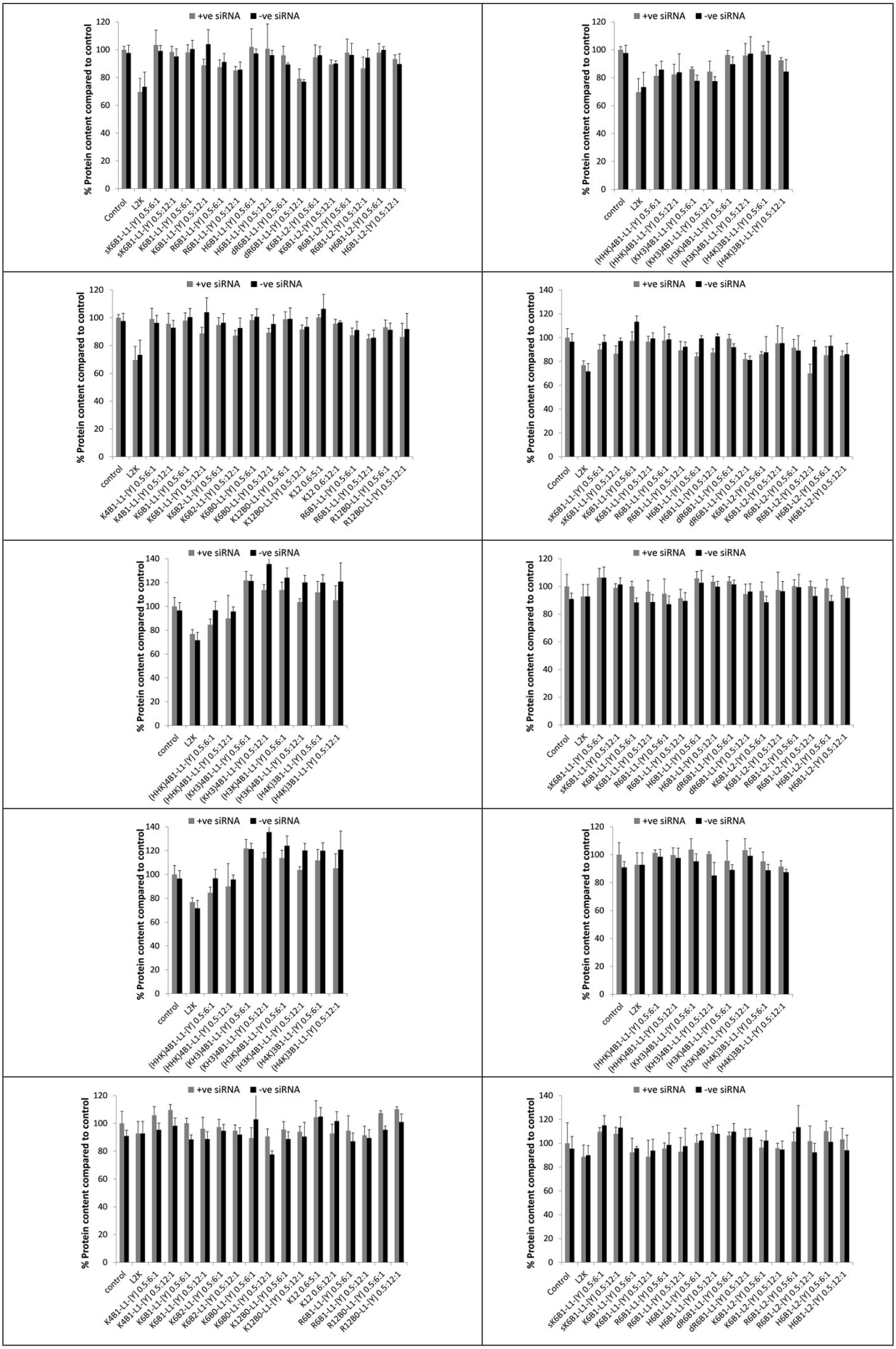


Figure S2 (a) **%** Knockdown efficiency (calculated as a % of luminescence produced by each sample formulated with negative control siRNA, and normalised according to the protein content) of selected PRs at 6:1 charge ratio, prepared fully in OptiMEM. siRNA alone and L2K:siRNA at 5:1 weight ratio were used as controls respectively. (b) % protein content of the PRs in figure (a) showing formulations prepared using Luc-siRNA (+ve siRNA) and negative control siRNA (-ve siRNA) in grey and black, respectively.


+ve siRNA
-ve siRNA
% Protein content compared to control
control
L2K
(HHK)4B1-L1-[V] 0.5:6:1
(HHK)4B1-L1-[V] 0.5:12:1
(KH3)4B1-L1-[V] 0.5:6:1
(KH3)4B1-L1-[V] 0.5:12:1
(H3K)4B1-L1-[V] 0.5:6:1
(H3K)4B1-L1-[V] 0.5:12:1
(H4K)3B1-L1-[V] 0.5:6:1
(H4K)3B1-L1-[V] 0.5:12:1
K4B1-L1-[V] 0.5:6:1
K4B1-L1-[V] 0.5:12:1
K6B1-L1-[V] 0.5:6:1
K6B1-L1-[V] 0.5:12:1
K6B2-L1-[V] 0.5:6:1
K6B2-L1-[V] 0.5:12:1
K6B0-L1-[V] 0.5:6:1
K6B0-L1-[V] 0.5:12:1
K12B0-L1-[V] 0.5:6:1
K12B0-L1-[V] 0.5:12:1
K12 0.6:5:1
K12 0.6:12:1
R6B1-L1-[V] 0.5:6:1
R6B1-L1-[V] 0.5:12:1
R12B0-L1-[V] 0.5:6:1
R12B0-L1-[V] 0.5:12:1
sK6B1-L1-[V] 0.5:6:1
sK6B1-L1-[V] 0.5:12:1
K6B1-L1-[V] 0.5:6:1
K6B1-L1-[V] 0.5:12:1
R6B1-L1-[V] 0.5:6:1
R6B1-L1-[V] 0.5:12:1
H6B1-L1-[V] 0.5:6:1
dR6B1-L1-[V] 0.5:6:1
dR6B1-L1-[V] 0.5:12:1
K6B1-L2-[V] 0.5:6:1
K6B1-L2-[V] 0.5:12:1
R6B1-L2-[V] 0.5:6:1
R6B1-L2-[V] 0.5:12:1
H6B1-L2-[V] 0.5:6:1
H6B1-L2-[V] 0.5:12:1

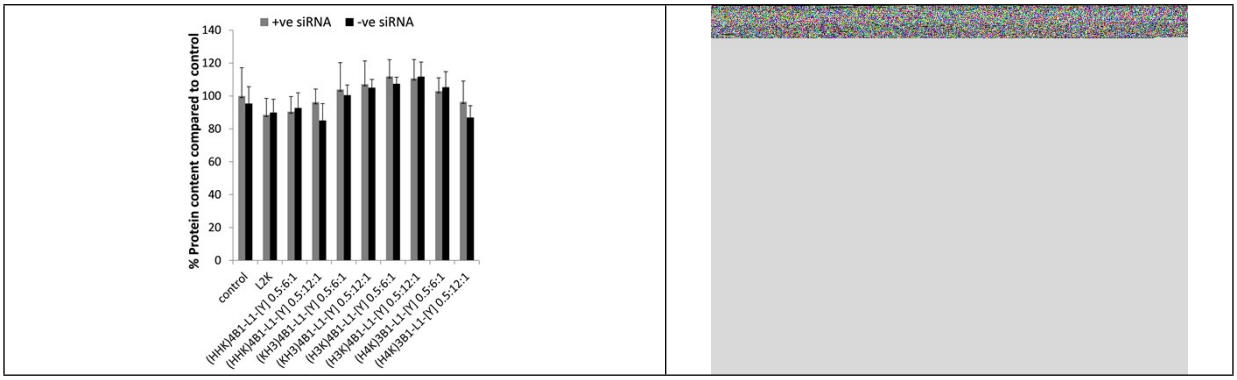


Figure S3: % Protein content compared to untreated control cells of LPRs prepared using (a, d, g, j) group 1, (b, e, h, k) group 2, and (c, f, i, l) group 3 peptides. LPRs were prepared either in (a, b, c) water then diluted in OptiMEM; in (d, e, f) OptiMEM fully; (g, h, i) water then diluted in serum-containing growth media, and (j, k, l) OptiMEM then diluted in serum-containing growth media. The protein content of formulations prepared using Luc-siRNA (+ve siRNA) and negative control siRNA (-ve siRNA) are shown in grey and black, respectively.

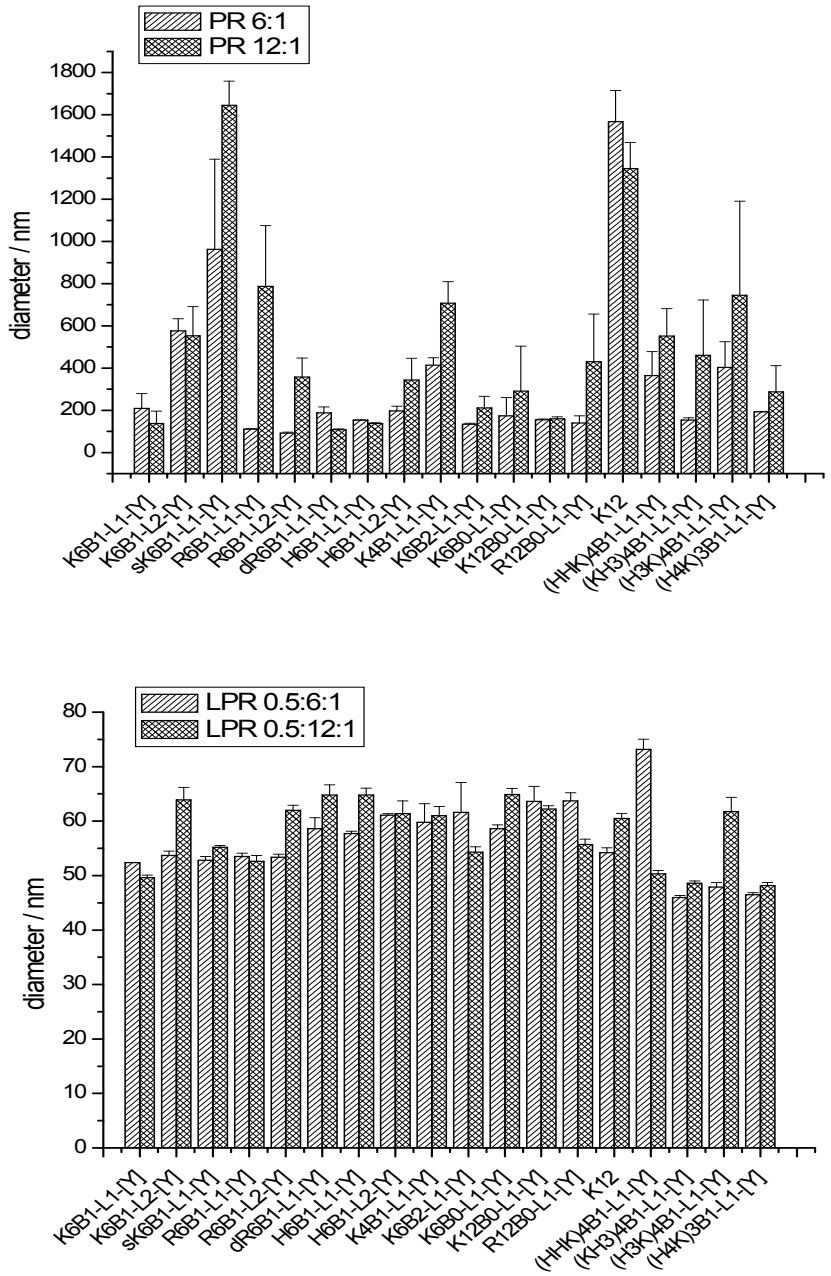


Figure S4: Apparent hydrodynamic size (nm) of (a) PR and (b) LPR complexes prepared at 6:1 and 12:1 or 0.5:6:1 and 0.5:12:1 charge ratios respectively using group 1, 2 and 3 peptides. The siRNA concentration in each sample was 26 ug mL^{-1}.

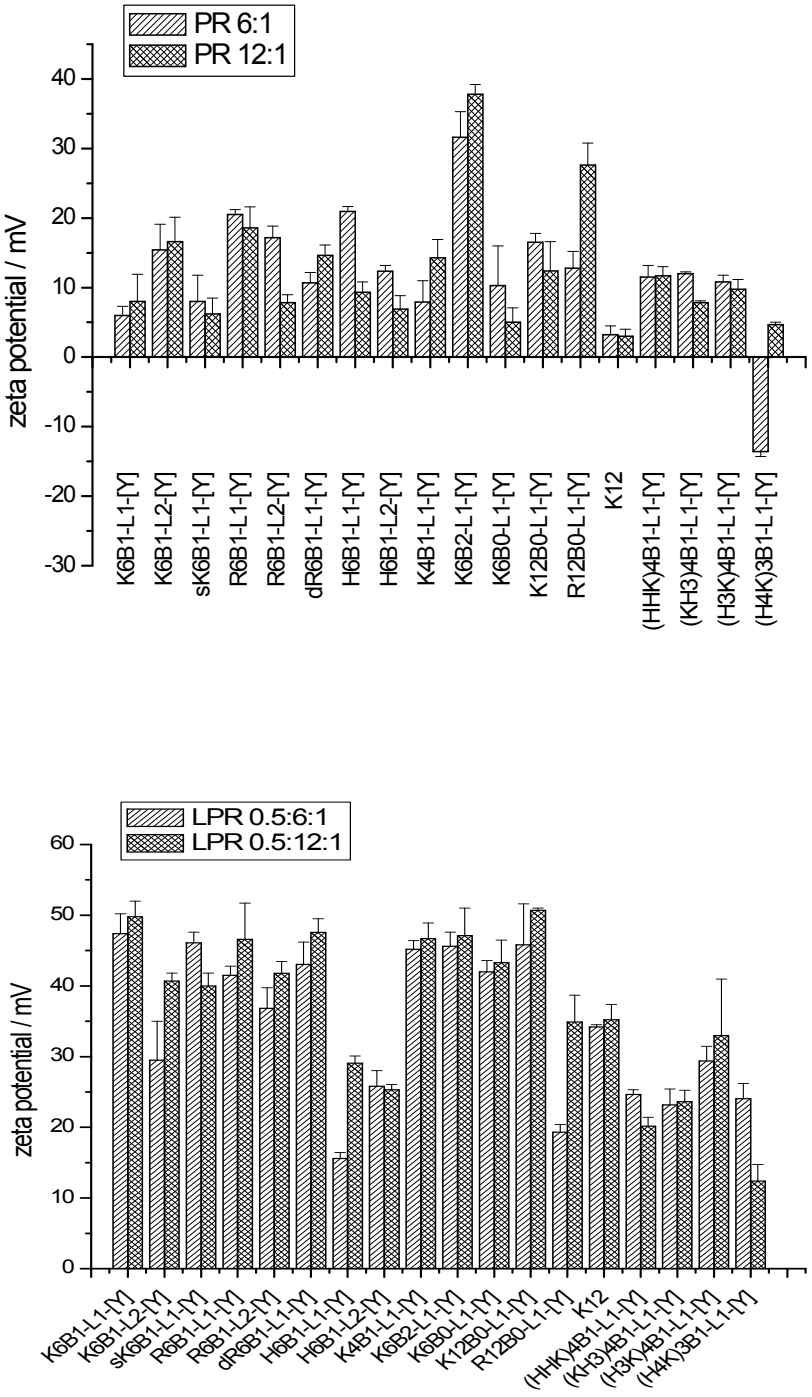


Figure S5: Zeta potential (mV) of (a) PR and (b) LPR complexes prepared at 6:1 and 12:1 or 0.5:6:1 and 0.5:12:1 charge ratios respectively using group 1, 2 and 3 peptides. The siRNA concentration in each sample was 2.9 ug mL^{-1}.

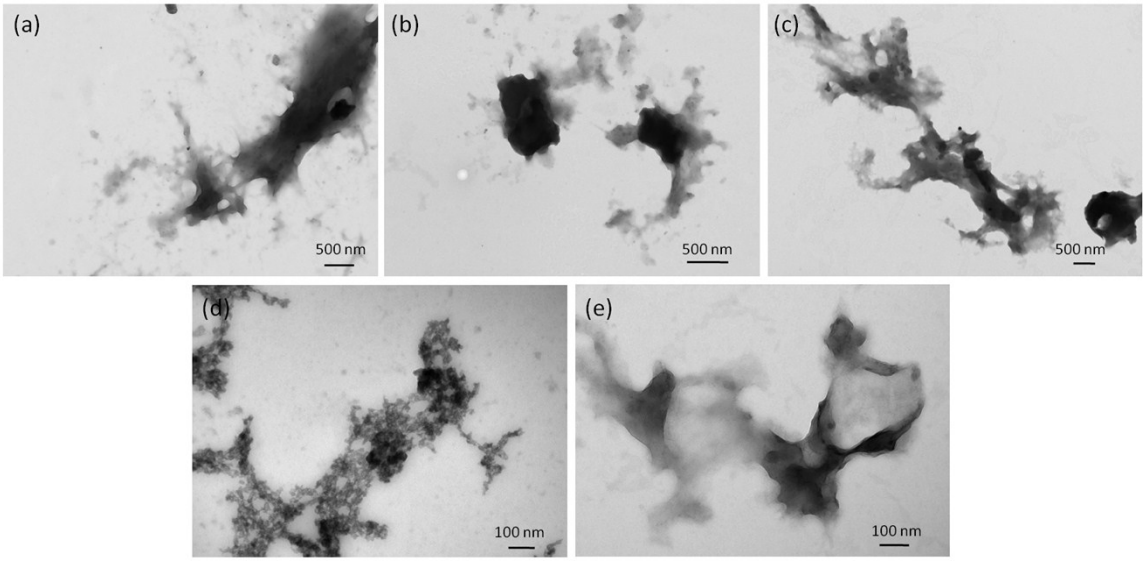


Figure S6: Transmission electron microscopy of PR complexes prepared with the peptides (a) **K_6B1-L1-[Y]**, (b) **R_6B1-L1-[Y]**, (c) **H_6B1-L1-[Y]**, (d) **K_{12}B0-L1-[Y]** and (e) **K_6B2-L1-[Y]** at 6:1 PR charge ratio and a final Sigma siRNA concentration of 0.05 mg mL^{-1}.

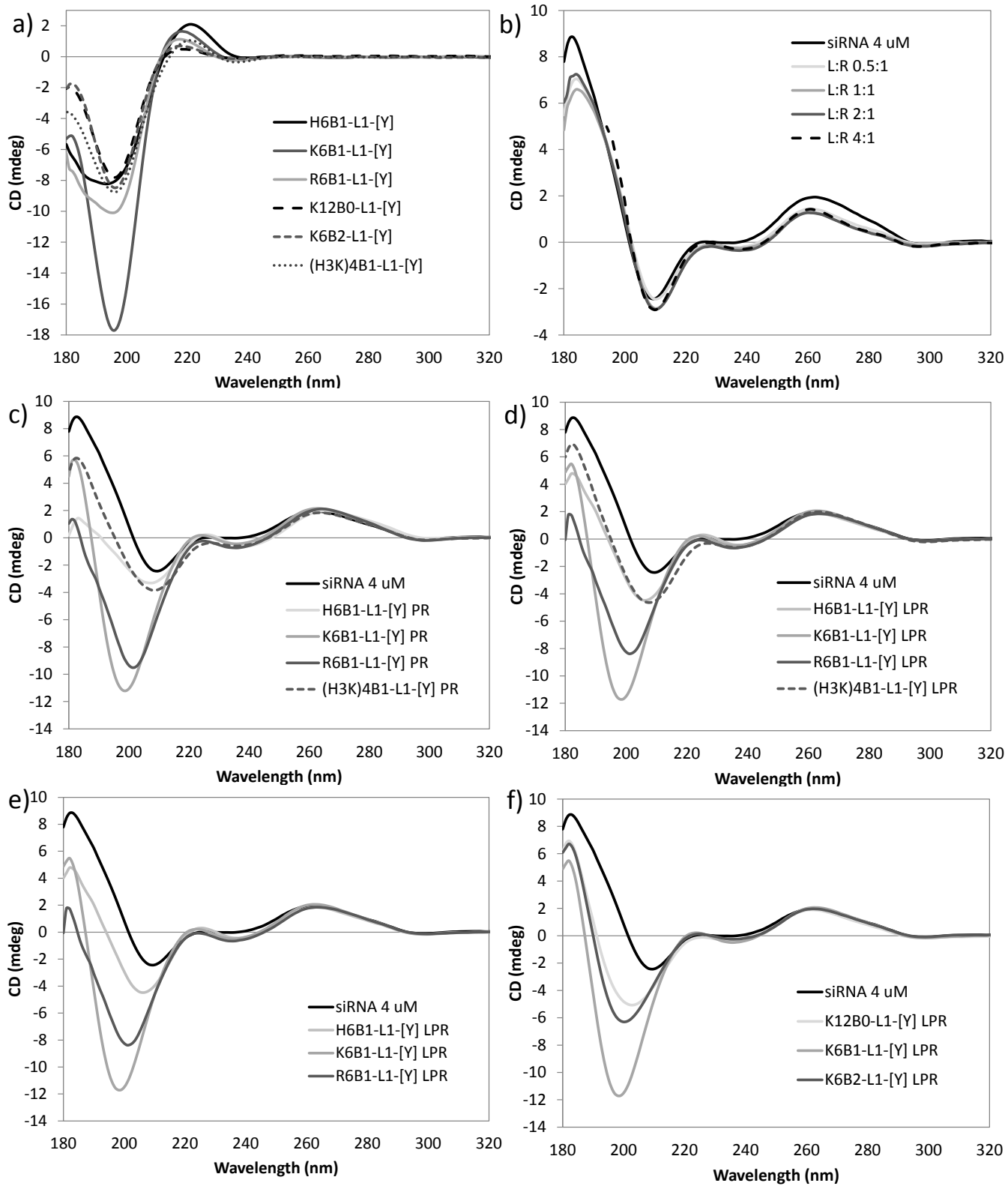


Figure S7: CD spectra of (a) peptides **H_6B1-L1-[Y]**, **K_6B1-L1-[Y]**, **R_6B1-L1-[Y]**, **K_{12}B0-L1-[Y]**, **K_6B2-L1-[Y]** and **$(H_3K)_4$B1-L1-[Y]** at concentrations equivalent to those used in PR and LPR complexes; (b) DOTMA:DOPE:siRNA (LR) complexes at 0.5:1, 1:1, 2:1 and 4:1 charge ratios; (c) PR complexes at 6:1 charge ratio prepared using the above peptides compared to free siRNA; (d-f) LPR complexes at 0.5:6:1 charge ratio prepared using the above peptides compared to free siRNA. The siRNA concentration in all samples was 4 M. Measurements were performed in a 1 mm path length cuvette.

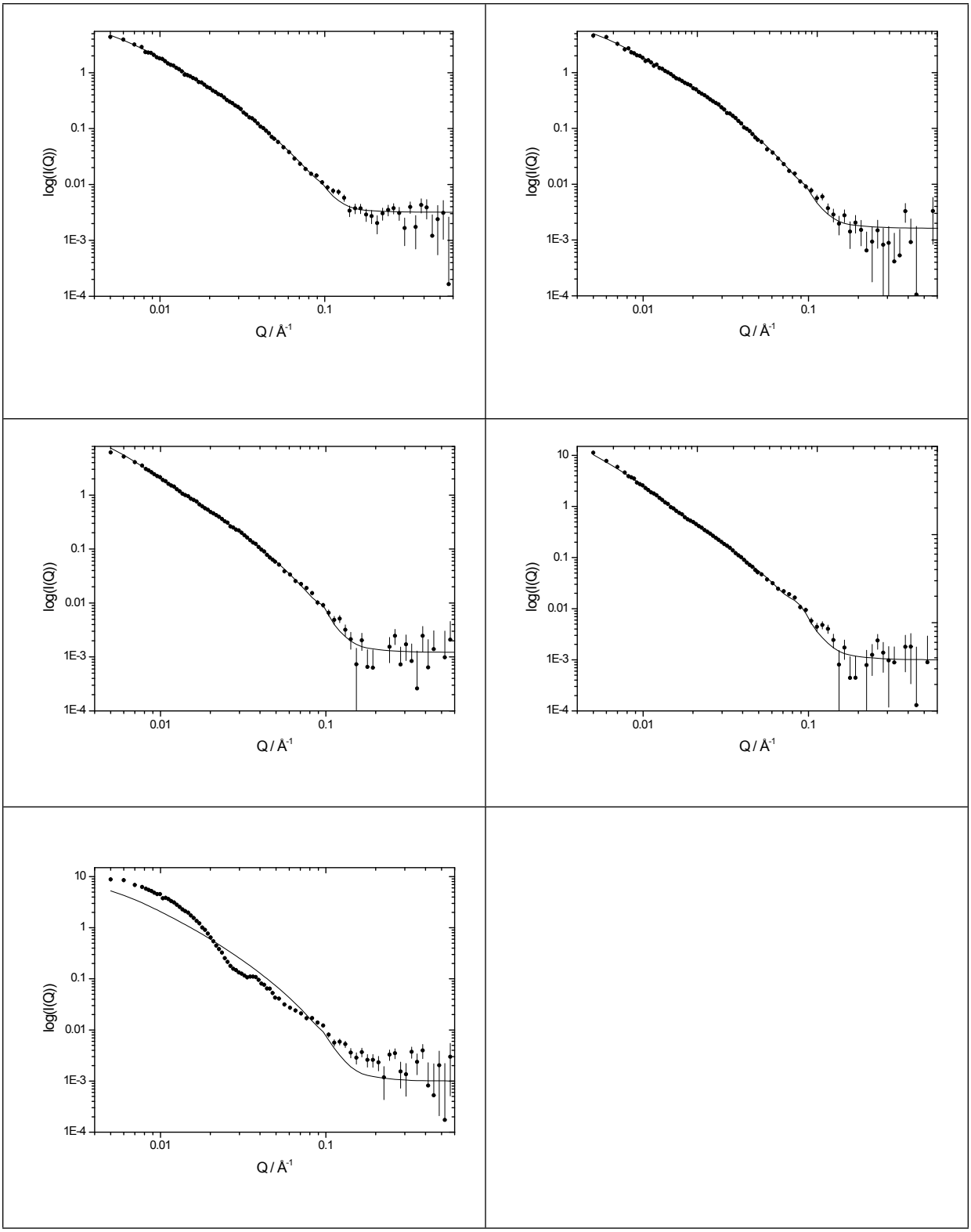


Figure S8. Small angle neutron scattering data (dots) at 298 K and the best fit to the data (solid line) obtained using the mixed sheet and stack model for LPRs prepared from a 1:1 molar ratio of DOTMA:DOPE and containing (a) **R_6B1-L1-[Y]** (b) **K_{12}B0-L1-[Y],** (c) **K_6B2-L1-[Y],** (d) **K_6B1-L1-[Y],** (e) **K_6B0-L1-[Y]** at a 0.5:6:1 charge ratio.

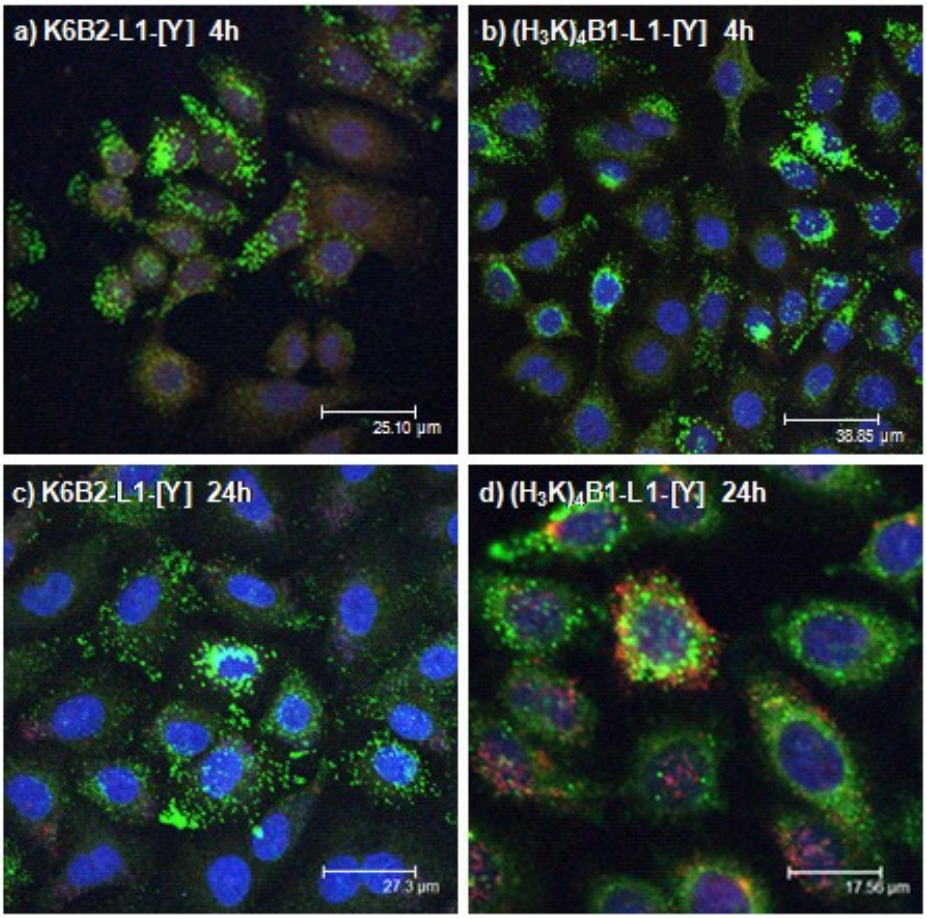


Figure S9: Confocal microscopy of A549-luc cells transfected with LPR complexes prepared fully in OptiMEM containing peptides (a) **K_6B2-L1-[Y]** and (b) **$(H_3K)_4$B1-L1-[Y]** after 4 hours of incubation or (c) **K_6B2-L1-[Y]** and (d) **$(H_3K)_4$B1-L1-[Y]** after 24 hours of incubation. LPR complexes were prepared with BODIPY-HPC incorporated into DOTMA/DOPE lipid (green) and rhodamine labelled Silencer® Negative Control siRNA (red) at a lipid:peptide:siRNA charge ratio of 0.5:6:1. The cell nucleus was stained with DAPI (blue). All peptides were unlabelled.

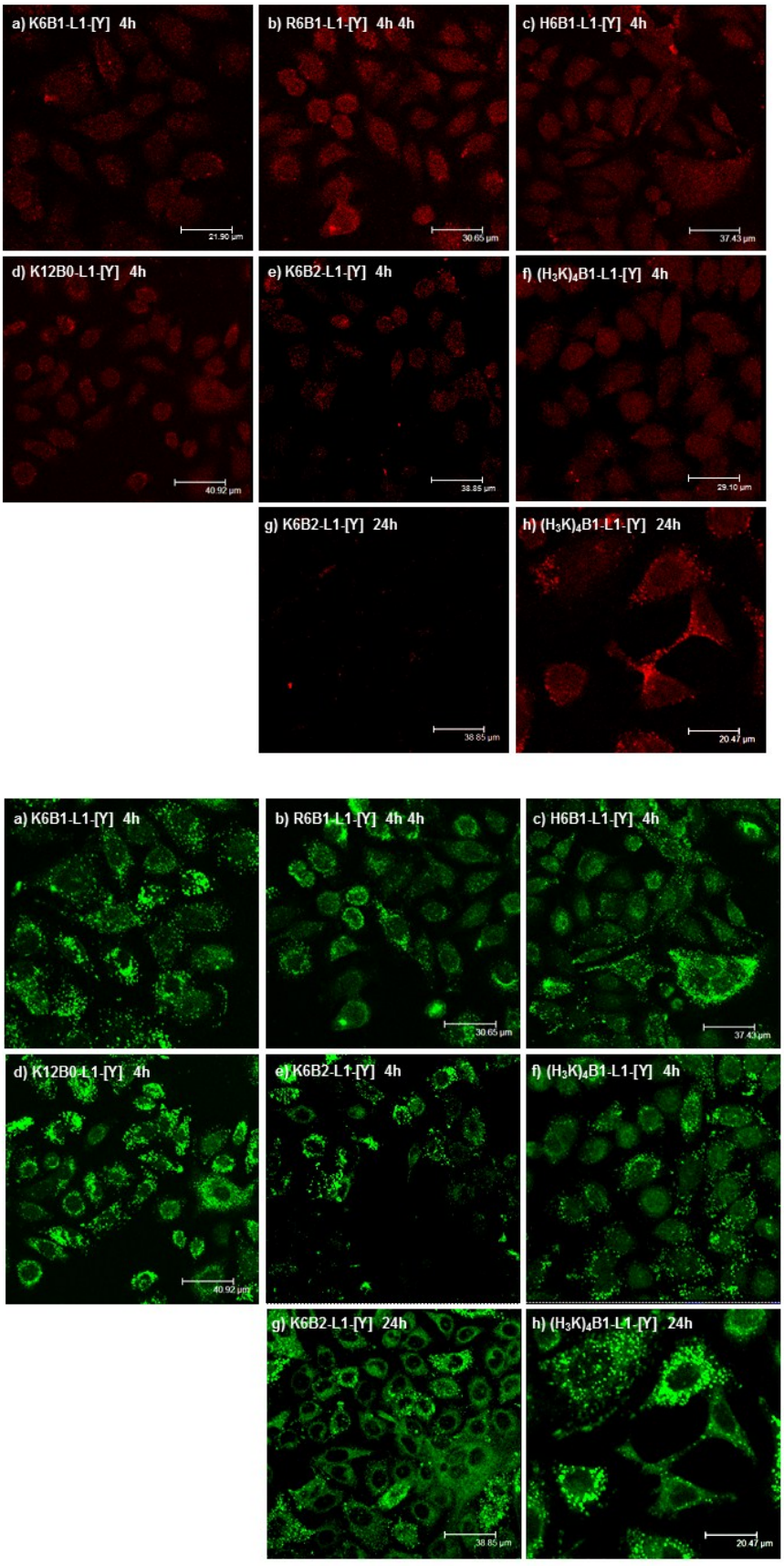


Figure S10: Red and green channel confocal microscopy images from Figure 9

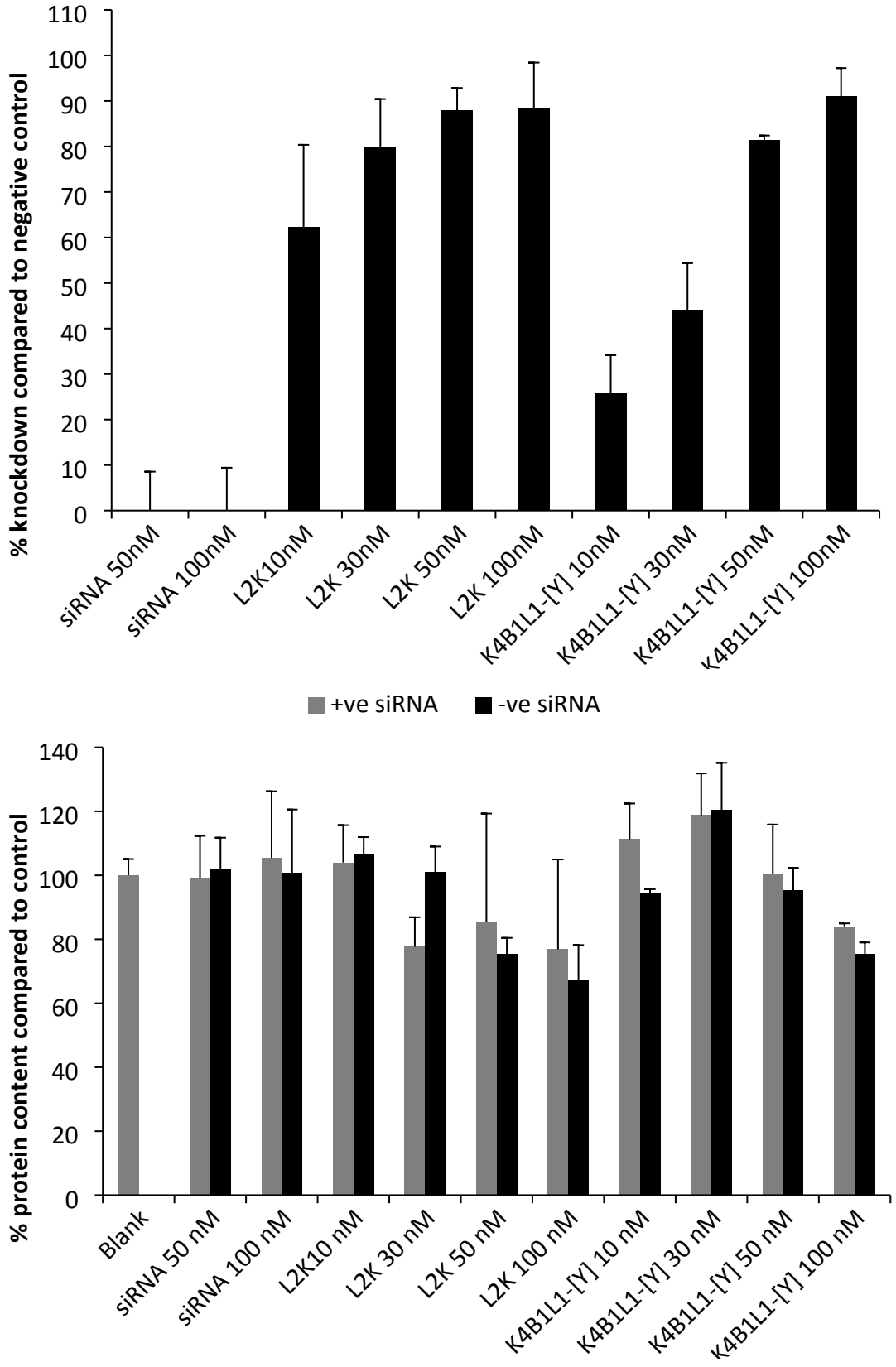


Figure S11: (a) **%** Knockdown efficiency (calculated as a % of luminescence produced by each sample formulated with negative control siRNA, and normalised according to the protein content) of L2K and K4B1L1-[Y] LPR at 0.5:12:1 prepared in OptiMEM using 10, 30, 50 and 100 nM siRNA per well. (b) % protein content of the formulations in figure (a) prepared using Luc-siRNA (+ve siRNA) and negative control siRNA (-ve siRNA) in grey and black, respectively.

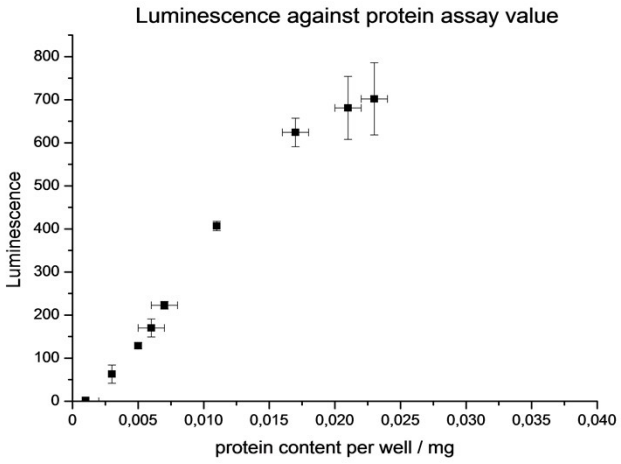


Figure S12: Correlation between luminescence and protein content per well in A549-Luc cells.