Supplementary information

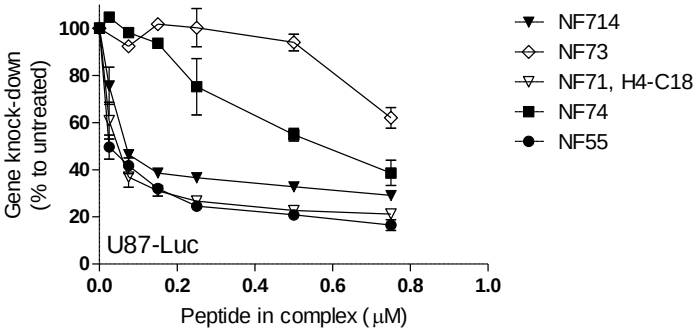
Supplementary table 1. Size of CPP/siRNA complexes measured by DLS and TEM. Sizes of CPP/siRNA particles formed at MR30. Sizes expressed by intensity.

CPP			MR30		MR40		Size in TEM (nm) MR30
			Size in DLS (nm)	pdI	Size in DLS (nm)	pdI	
NF55			58 ± 1 (9%) 209 ± 75 (48%) 589 ± 87 (43%)	0.45 ± 0.05	213 (99%) 4370 (1%)	0.27	10 – 50 nm ø particles, and elongated conglomerates
PF6			44 ±15 (12%) 150 ±6 (88%)	0.02 ±0.02	nd	nd	30 – 40 nm ø spherical nanoparticles
H4	**C18**	**NF71**	**44 ± 12 (15%) 195 ± 14 (85%)**	**0.39 ± 0.02**	**173 (94%) 3917 (5%)**	**0.19**	**20 – 60 nm ø diameter particles**
H6	C0	NF700	151 ± 17 (100%) 1 h later 517 ± 25 (100%)	0.18 ± 0.02 1.00 ± 0.06	1227 (99%)	0.19	>500 nm , only a few particles
	C10	NF701	198 ±28 (15%) 599 ± 24 (85%)	0.25 ± 0.01	2012 (100%)	0.19	200 – 400 nm x 15 – 20 nm ø worm-like particles
	C18	NF702	70 ± 9 (28%) 285 ± 56 (72%)	0.43 ± 0.03	141 (13%) 838 (87%)	0.46	
	C20	**NF70**	**54 ± 17 (20%) 240 ± 35 (79%)**	**0.39 ±0.03**	**210 (98%) 4888 (2%)**	**0.39**	**30 – 40 nm ø particles**
	C22	NF703	79 ± 25 (38%) 391 ± 219 (62%)	0.44 ± 0.05	122 (99%) 1545 (1%)	0.23	10- 20 nm ø particles
H8	**C20**	**NF72**	**70 ± 34 (24%) 289 ± 78 (76%)**	**0.42 ± 0.05**	**175 (76%) 1494 (24%)**	**0.39**	**10- 20 nm ø particles**

Supplementary table 2. Hemolytic concentration (HC50) of free peptides and CPP/siRNA complexes formed at MR30.

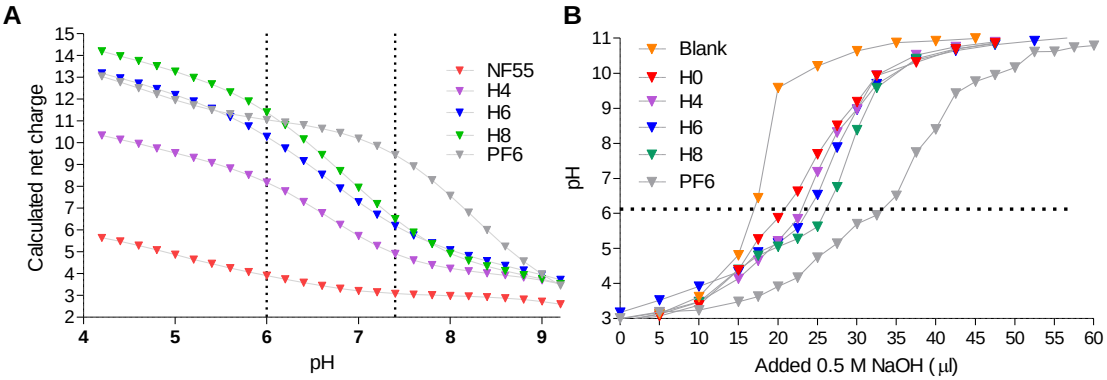
CPP	HC50 (μM)	
	Free peptide	CPP/siRNA complex (peptide concentration)
NF55	1.7	3.2
PF6	0.9	2.1
H4-C18	1.2	2.5
H6-C20	4.3	8.3
H8-C20	>10	nd

Supplementary Figure 1. Downregulation of reporter gene in U87 cells with CPP/siLuc nanoparticles. Experiment done in serum containing (10% FBS) media and cells lysed 24 h post-treatment. [a] – the length of the saturated fatty acid added to the N-terminus of the peptide sequence (18 – stearoyl). [b] – synthesis continued from the sidechain amino group instead of α-amino group.

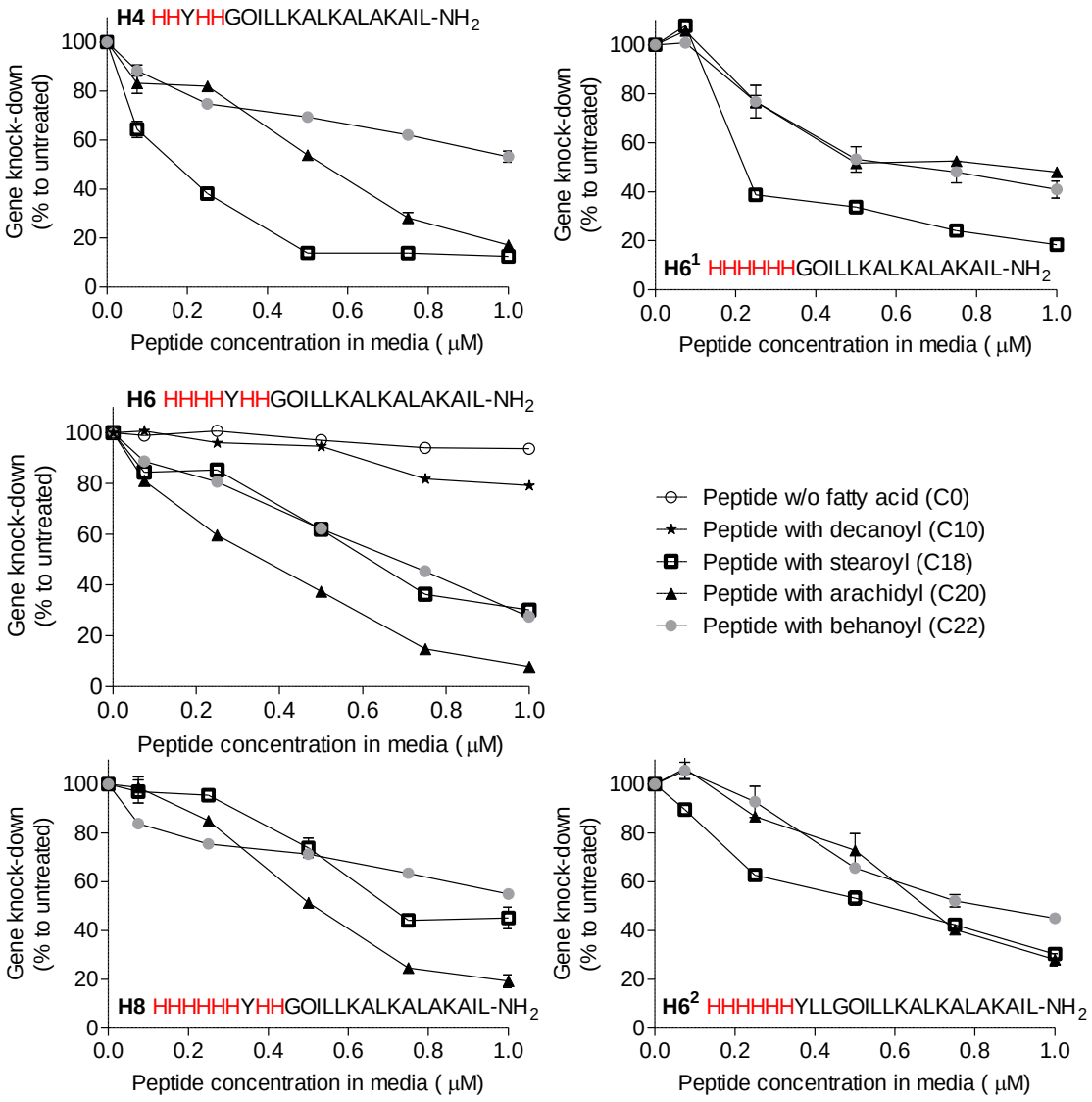


CPP	Fatty acid[a]	Peptide sequence (C-terminally amidated)
NF71	C18	HHYHHGO[b]ILLKALKALAKAIL
NF714	C18	HHYHHGO[b]INLKALKALAKAIL
NF73	C18	AGYLLGO[b]IHLKAHKAHAKAHL
NF74	C18	AGYLLGO[b]ILHKAHHKLHKAIL

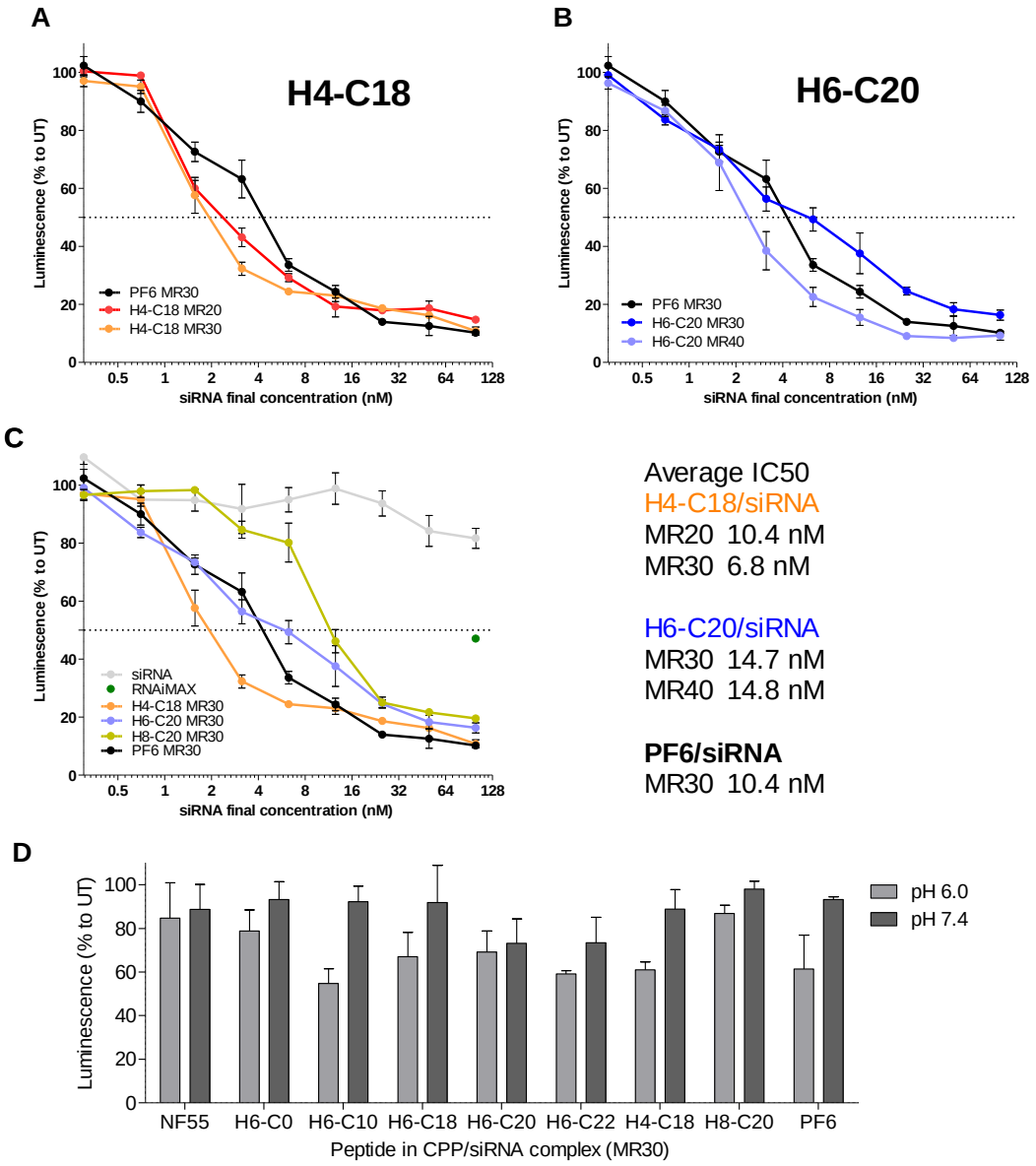
Supplementary Figure 2. **Inclusion of histidines in the sequence increased the net charge of the peptide at lower pH and increased its buffering efficiency** A) The calculated net charge of histidine-rich NickFect peptides and reference peptides NF55 and PF6 at different pH. Dotted line marks pH 7.4 to pH 6.0. For calculations MarvinSketch 15.9.14, ChemAxon Ltd was used. B) Titration of 2 ml 200 µM peptide solution with 0.5 M NaOH. Measured pH and the increase of NaOH amount needed to achieve same pH compared to control (H0). Dotted line marks pH 6.0.



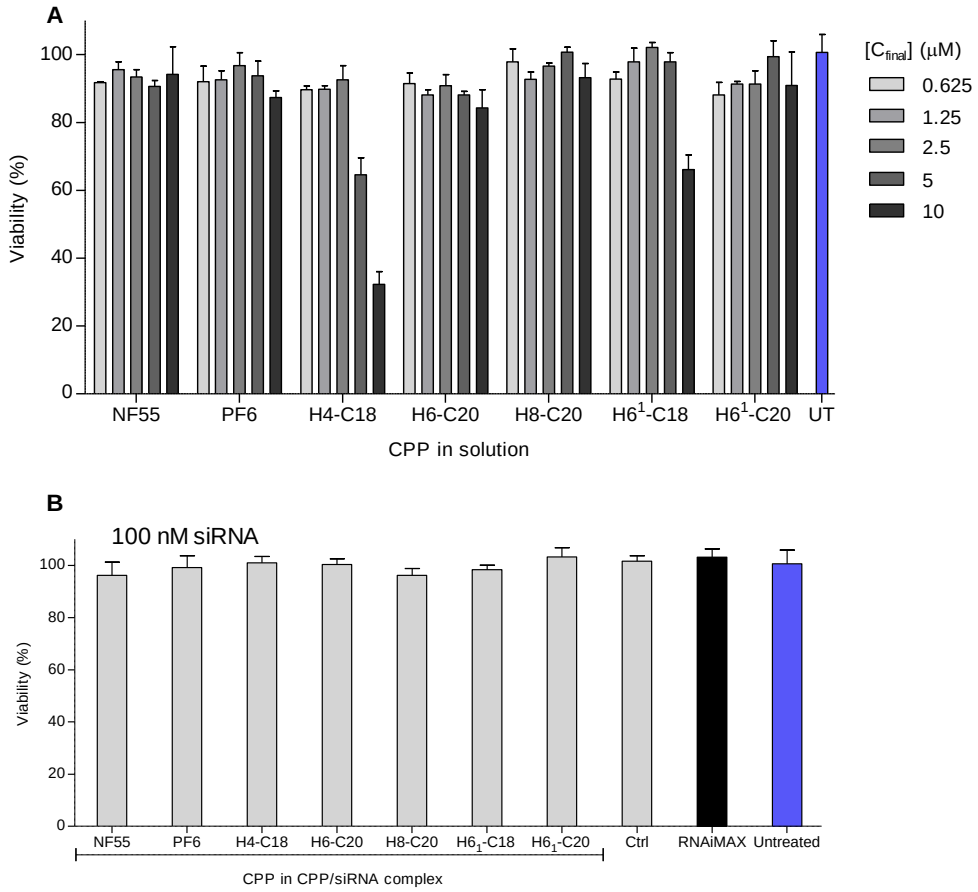
Supplementary Figure 3. Downregulation of luciferase activity with NF/siLuc complexes. From each peptide series three saturated fatty acid analogues with N-terminal stearoyl, arachidyl or behanoyl were tested on U87 sells stably expressing luciferase activity. For H6 series, additional decanoyl (C10) and peptide w/o fatty acid (C0) were tested. All experiments were performed in serum containing (10% FBS) media. Molar ratios of peptide to siRNA 0-40 were used for non-covalent complex formation and added to the cells with 25 nM siRNA final concentration in media, resulting in peptide final concentration 0.075 to 1 μM. For each sample, luminescence was measured 2-h post-treatment and results normalized to total protein content in cell lysates. Results are shown as percentage of remaining luciferase activity normalized to untreated cells.



Supplementary Figure 4. SiRNA concentration and media pH dependent downregulation of luciferase expression by CPP/siRNA complexes formed between histidine-rich NickFects and siLuc. Complexes between siRNA and CPPs H4-C18 (MR20, MR30), H6-C20 (MR30, MR40), H8-C20 (MR30) and control peptides PF6 (MR30) and NF55 (MR30) were formed and downregulation of luciferase was assessed. Complexes were added to cells accordingly that the siRNA final concentration in wells was 100, 50, 25, 12.5, 6.25, 3.13, 1.6, 0.7 and 0.3 nM. Luminescence was normalized to protein content and RLU/mg of untreated cells were taken as 100% for normalization. A) Complexes formed with H4-C18, B) H6-C20 or C) comparison of the best peptides in the set. D) Luciferase expression downregulation after transfection of cells in HEPES buffer at pH 6.0 or 7.4. Buffer was supplemented with 10% FBS. After 4 h co-incubation with complexes buffer on cells was replaced with cell culture media and incubated for further 20 h. Luminescence from expressed reporter gene was measured and results are normalized to untreated cells (100%). SiRNA final concentration on cells was 10 nM.



Supplementary Figure 5. MTS based viability assay on U87 cells. A) Viability of cells after treatment with peptides at concentration range 0.625 µM – 10 µM and B) after treatment with peptide complexes at MR30, siRNA final concentration 100 nM. Controls RNAiMAX (RM), free siRNA at given concentration (Ctrl) and untreated cells (UT). All experiments were performed in serum containing conditions. MTS is added 24 h post-transfection and incubated for 2 h followed by the measurement of absorption.



Supplementary Figure 6. Negative staining TEM images of CPP/siRNA nanoparticles. The siRNA nanoparticles with H6-C0, H6-C10, H6-C18, H6-C22, NF55 or Jet-PEI were formed by mixing siRNA with CPP at molar ratio 30:1 (A-E), or with transfection reagent (F) in water. The formed complexes were stained with aqueous uranyl acetate solution, and imaged at 13 000× magnification. Scale bars: 200 nm.