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Supplemental information

DNA nuclear targeting sequences for enhanced non-viral gene transfer: An *in vitro* and *in vivo* study

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Supplemental figures

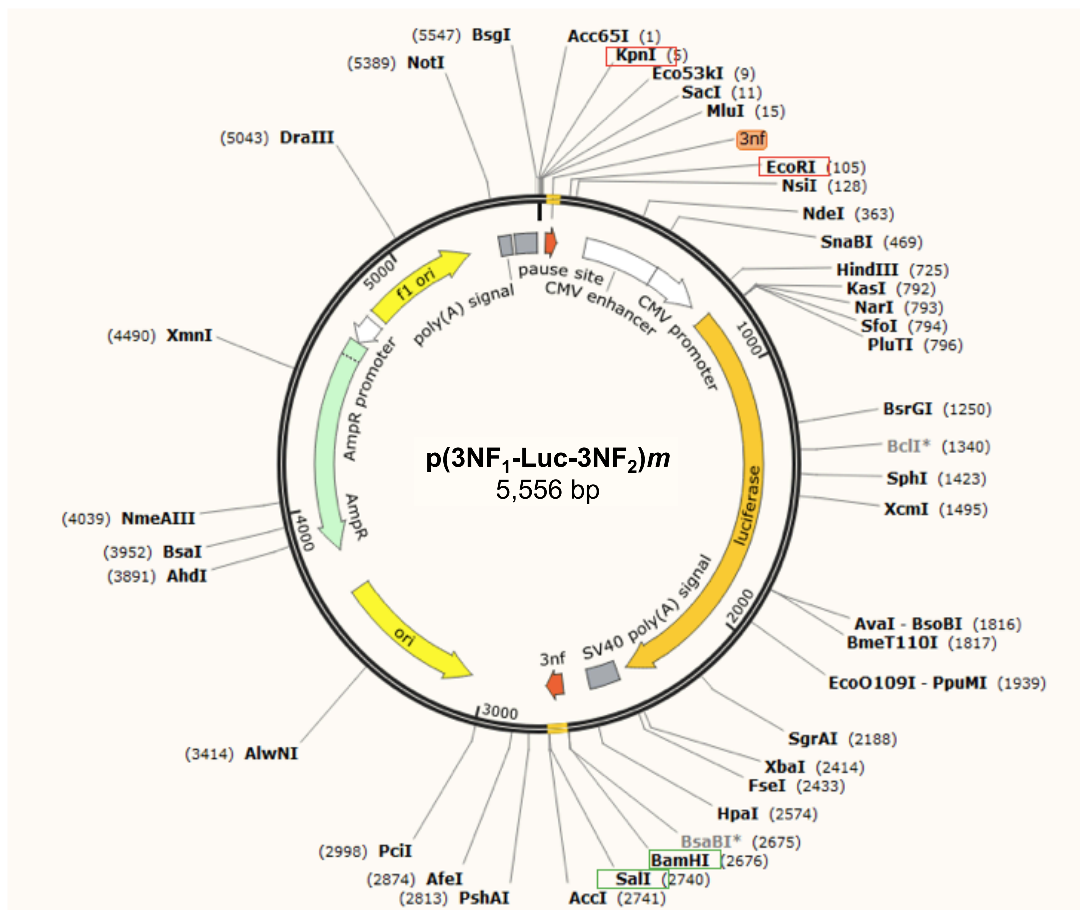


Figure S1. Detailed map of p(3NF$_1$-Luc-3NF$_2$)*m*. It includes a firefly luciferase gene under control of the strong CMV promoter. The optimized NFκB DNA nuclear Targeting Sequences (DTS), 3NF$_1$ and 3NF$_2$, are respectively upstream and downstream of the reporter gene. The restriction enzyme couples used to remove and replace 3NFs are framed in red and green (plasmid map realized with SnapGene viewer®).

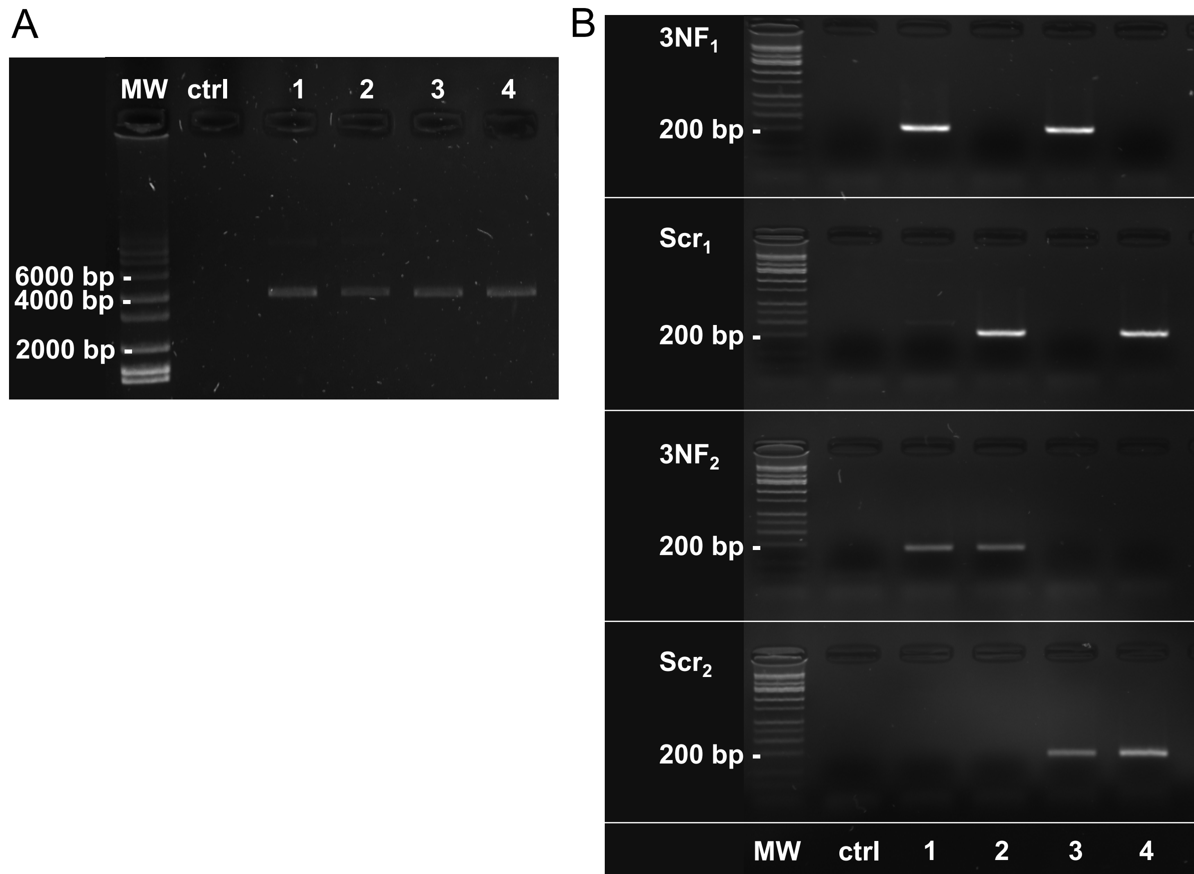


Figure S2. Characterization of p($3NF_1$-Luc-$3NF_2$)*m* and its monomer derivatives by agarose gel electrophoresis and PCR. (A) Electrophoretic profiles of p($3NF_1$-Luc-$3NF_2$)*m* and its derivatives. MW corresponds to the molecular weight marker; the weight of some bands is indicated on the left in bp. "Ctrl" corresponds to the control made of water used to dilute the different nucleic acid constructs. Numbers upon each lane indicates the plasmid ran with 1, p($3NF_1$-Luc-$3NF_2$)*m*; 2, p(Scr_1-Luc-$3NF_2$)*m*; 3, p($3NF_1$-Luc-Scr_2)*m* and 4, p(Scr_1-Luc-Scr_2)*m*. (B) PCR signatures of p($3NF_1$-Luc-$3NF_2$)*m* and its derivatives. Each plasmid can be identified thanks to specific couples of primers able to recognize each particular motif of interest known as $3NF_1$, $3NF_2$, Scr_1 and Scr_2. The lane "Ctrl" corresponds to the water used to dilute PCR matrix. Lane numbers below the PCR gel are 1, p($3NF_1$-Luc-$3NF_2$)*m*; 2, p(Scr_1-Luc-$3NF_2$)*m*; 3, p($3NF_1$-Luc-Scr_2)*m* and 4, p(Scr_1-Luc-Scr_2)*m*.

Figure S3. Gel retardation assay using p(3NF$_1$-Luc-3NF$_2$)*m* complexed with bPEI or His-lPEI at different cationic polymers/pDNA mass ratio. For each lane, the MR assayed is indicated upon the well. pDNA alone was used as control. (A) Migration profile obtained with bPEI. (B) Migration profile obtained for DNA complexation assay with His-lPEI.

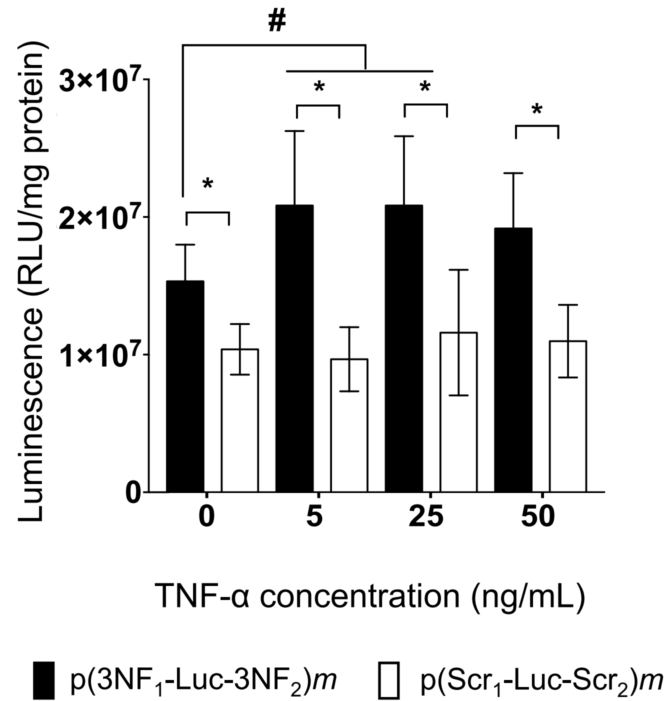
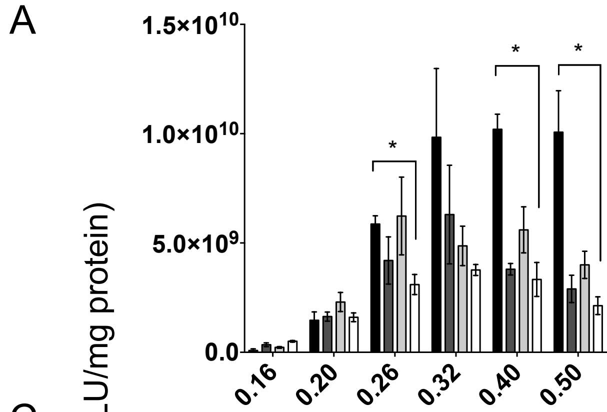
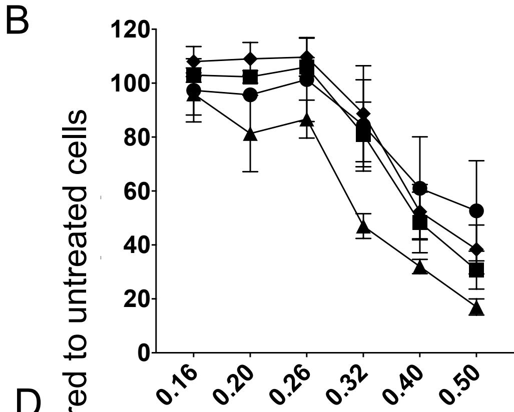
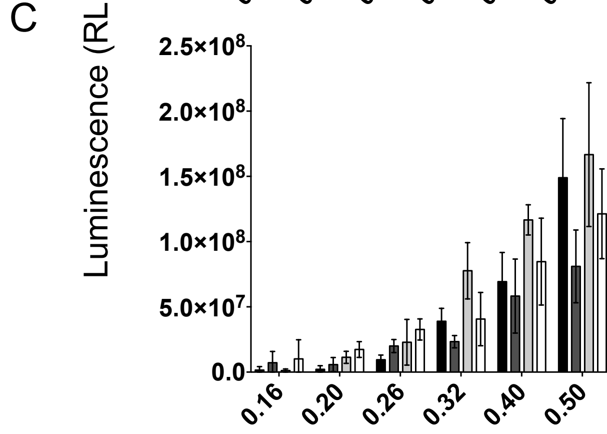
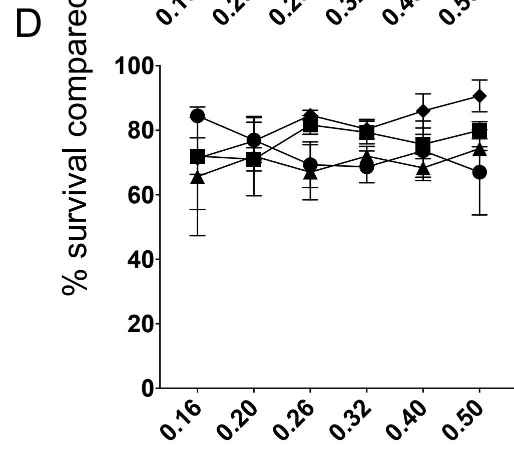


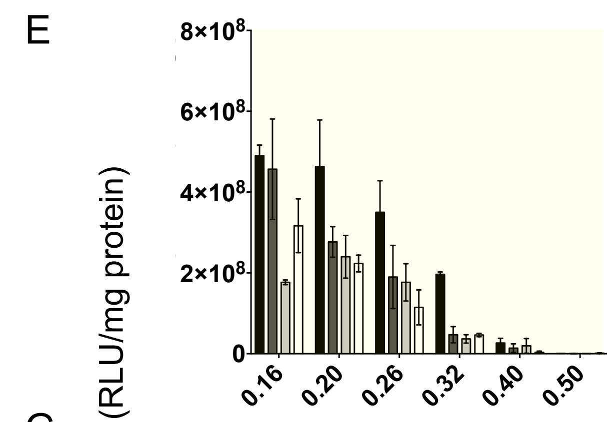
Figure S4. ***In vitro* transfection of 16HBE cells using His-lPEI polyplexes incorporating either p(3NF$_1$-Luc-3NF$_2$)*m* or p(Scr$_1$-Luc-Scr$_2$)*m* in presence of increasing concentrations of TNF-α.** Transfections were carried out in 16HBE cells using His-lPEI polyplexes. Luminescence signals are expressed in the unit of Relative Light Unit per milligram of protein (RLU/mg protein) as mean values of 6 wells +/- SD. The character * denotes p-value ≤ 0.05 for the two-tailed unpaired t-test used to compare p(3NF$_1$-Luc-3NF$_2$)*m* with p(Scr$_1$-Luc-Scr$_2$)*m* at a given TNF-α dose. The character # denotes p-value ≤ 0.05 for the two-tailed unpaired t-test used to compare p(3NF$_1$-Luc-3NF$_2$)*m* without *versus* with TNF-α at indicated doses.

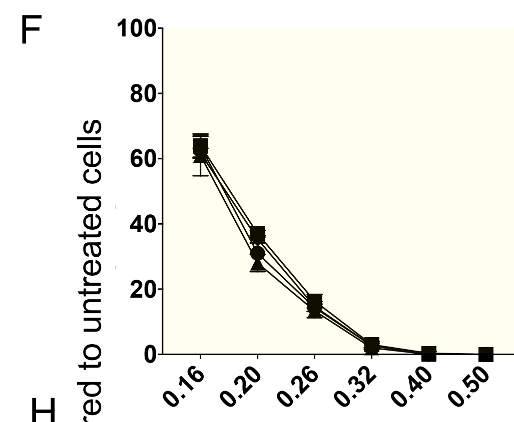


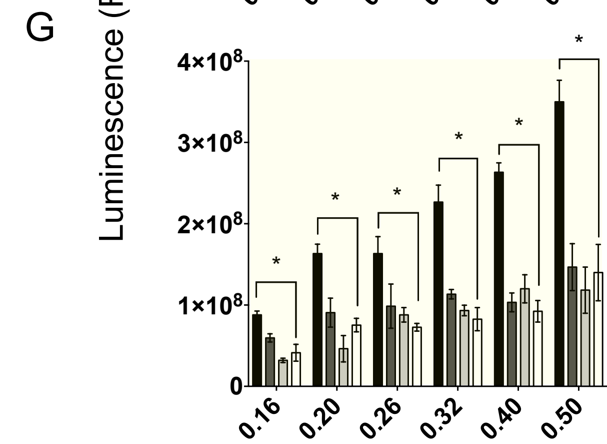


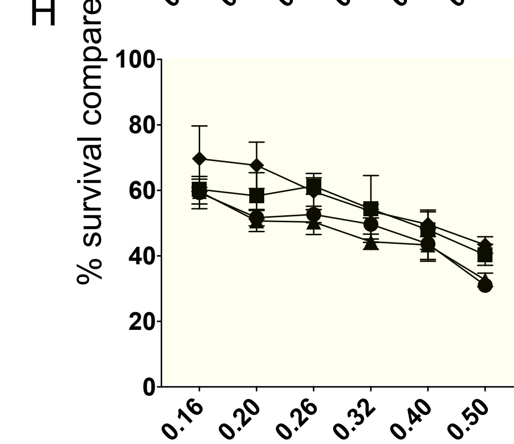












p($3NF_1$-Luc-$3NF_2$)m p($3NF_1$-Luc-Scr_2)m
p(Scr_1-Luc-$3NF_2$)m p(Scr_1-Luc-Scr_2)m

p($3NF_1$-Luc-$3NF_2$)m p($3NF_1$-Luc-Scr_2)m
p(Scr_1-Luc-$3NF_2$)m p(Scr_1-Luc-Scr_2)m

Figure S5. *In vitro* transfection using ascending doses of His-lPEI or bPEI polyplexes incorporating either p($3NF_1$-Luc-$3NF_2$)*m*, p($3NF_1$-Luc-Scr_2)*m*, p(Scr_1-Luc-$3NF_2$)*m* or p(Scr_1-Luc-Scr_2)*m*. Experiments were carried out in (A, B, E, and F) SK-Mel-28 and (C, D, G, and H) C2C12 cells using (A-D) His-lPEI or (E-H) bPEI polyplexes. Results are mean values of 3 wells +/- SD. The sign * denotes statistically significant differences (p-value ≤ 0.05, multiple t-test Holm-Sidak method) between p($3NF_1$-Luc-$3NF_2$)*m* and p(Scr_1-Luc-Scr_2)*m*. Cell-survival is given as percentage compared to untreated cells.

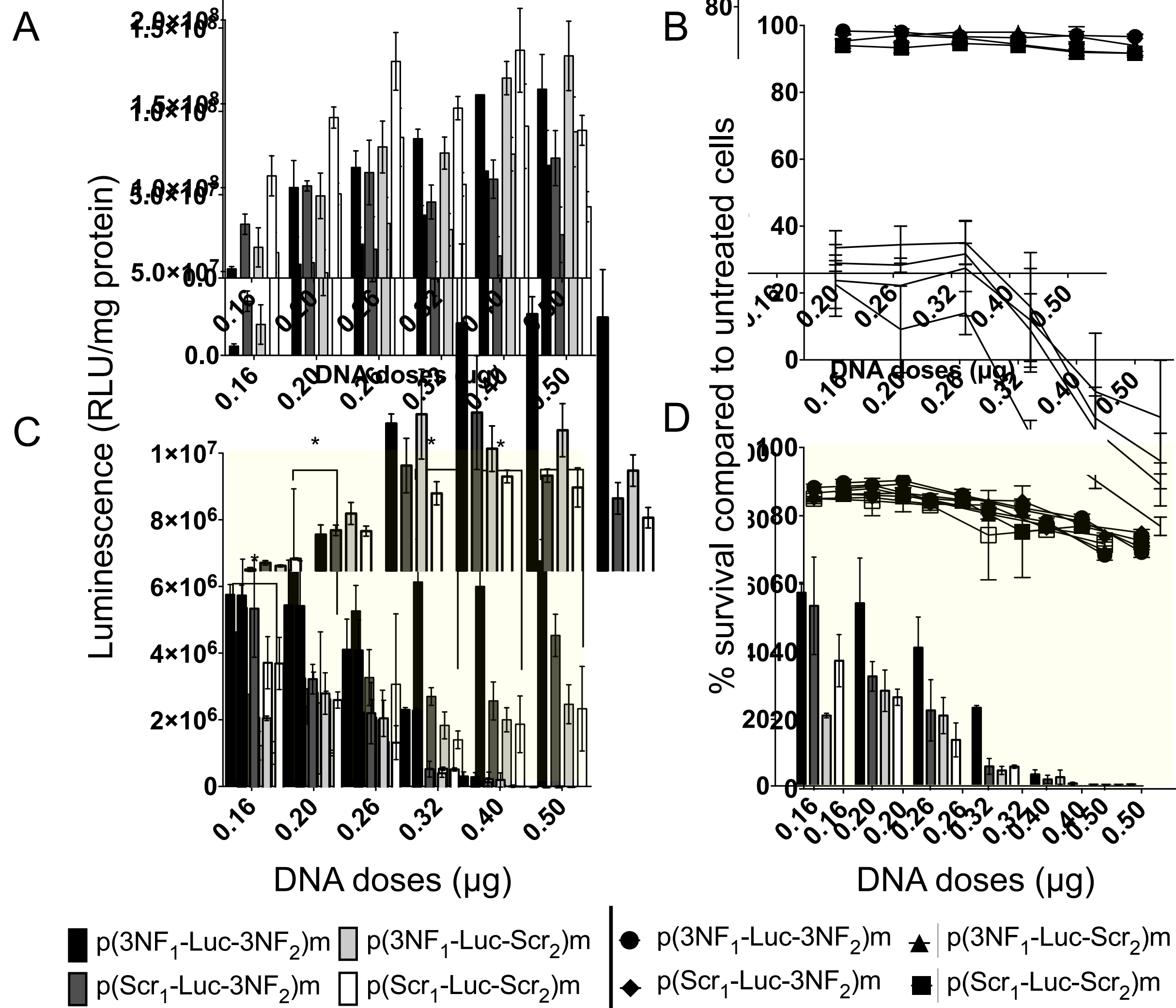
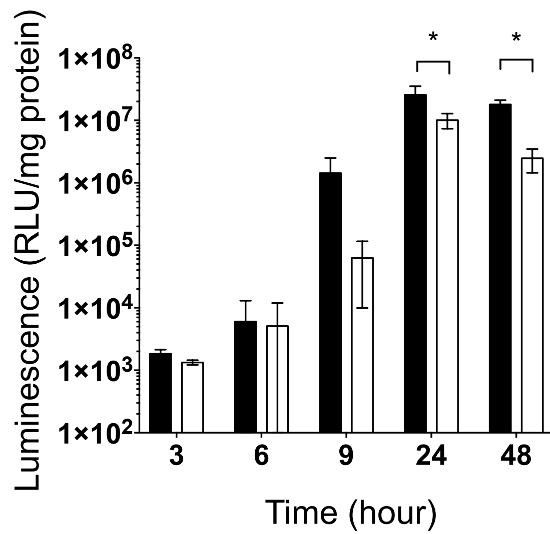
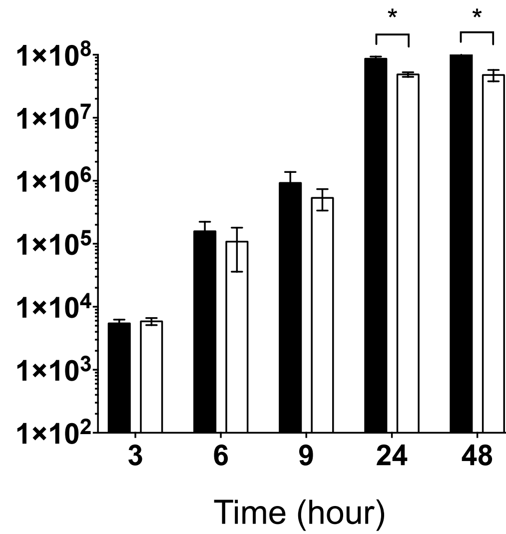


Figure S6. ***In vitro*** **transfection of A549 cells using ascending doses of His-lPEI or bPEI polyplexes incorporating either p(3NF$_1$-Luc-3NF$_2$)*m*, p(3NF$_1$-Luc-Scr$_2$)*m*, p(Scr$_1$-Luc-3NF$_2$)*m* or p(Scr$_1$-Luc-Scr$_2$)*m*.** Experiments were carried out using (A and B) His-lPEI or (C and D) bPEI polyplexes. Results are mean values of 3 wells +/- SD. The sign * denotes statistically significant differences (p-value ≤ 0.05, multiple t-test Holm-Sidak method) between p(3NF$_1$-Luc-3NF$_2$)*m* and p(Scr$_1$-Luc-Scr$_2$)*m*. Cell-survival is given as percentage compared to untreated cells.

A



B



■ p($3NF_1$-Luc-$3NF_2$)*m* □ p(Scr_1-Luc-Scr_2)*m*

Figure S7. 3NF assisted transfection using p($3NF_1$-Luc-$3NF_2$)*m* and p(Scr_1-Luc-Scr_2)*m* complexed by bPEI, a time course study. (A) C2C12 and (B) A549 cells were transfected with p($3NF_1$-Luc-$3NF_2$)*m* or p(Scr_1-Luc-Scr_2)*m*. Luminescence signals were evaluated at the indicated times after transfection. Results are mean values of 3 wells +/- SD. The sign * denotes statistically significant differences (p-value ≤ 0.05, multiple t-test Holm-Sidak method) between p($3NF_1$-Luc-$3NF_2$)*m* and p(Scr_1-Luc-Scr_2)*m*.

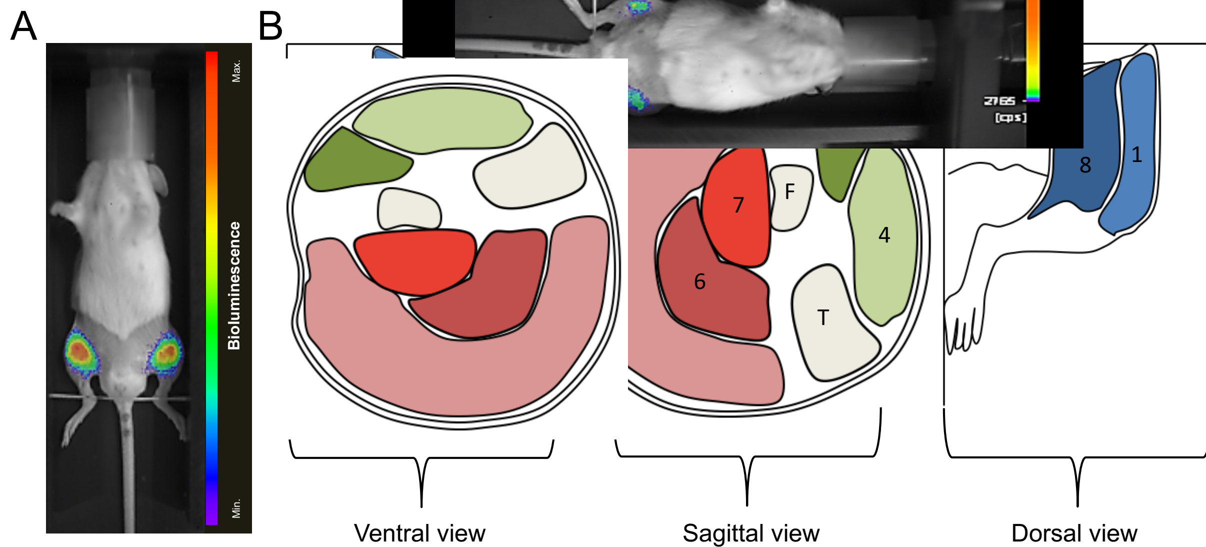


Figure S8. Bioluminescence imaging illustration and mouse hind limb simplified anatomy. (A) Illustration of bioluminescence signals obtained following *in vivo* bioluminescence imaging after HLV injection of a luciferase encoding plasmid. (B) Simplified anatomy of a mouse hind limb according to different views. Square on ventral view represents the cutting point for sagittal plan. Numbers correspond to muscles sampled with 1, quadriceps; 2, posterior muscles; 3, Extensor Digitorum Longus (EDL); 4, tibialis muscle; 5, gastrocnemius; 6, plantaris; 7, soleus and 8, hamstring. Main leg bones with F, Fibula and T, tibialis, are indicated on the sagittal view.

Figure S9. NFκB binding sites in the CMV enhancer and promoter carried by p($3NF_1$-Luc-$3NF_2$)*m* and its derivatives. NFκB binding sites are highlighted in yellow in the CMV enhancer and promoter sequences carried by p($3NF_1$-Luc-$3NF_2$)*m* and its derivatives. The sequence screened for NFκB binding sites was 5'-GGGACTTTCC-3', similar to the one found in optimized NFκB DTS termed "3NF".

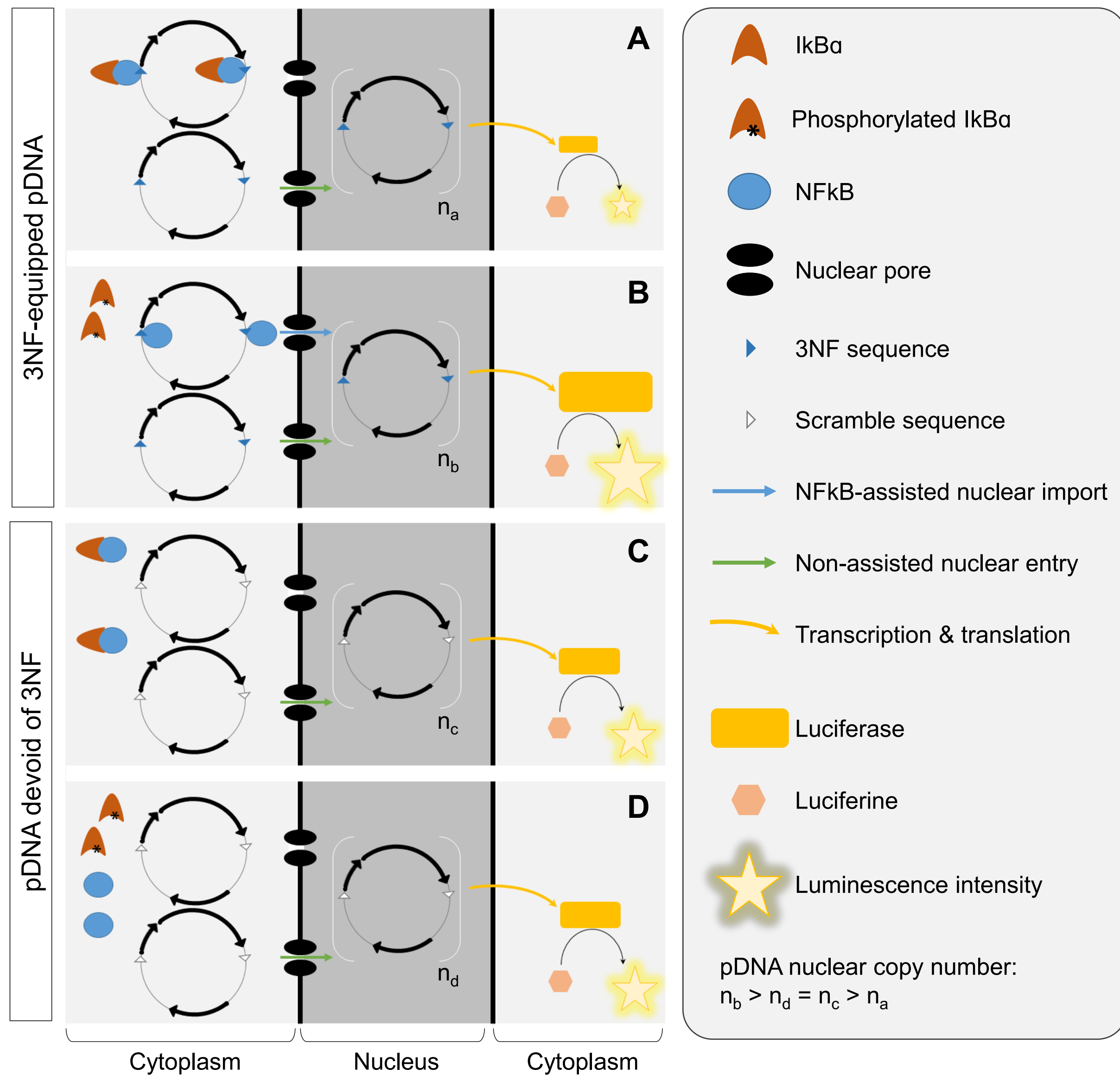
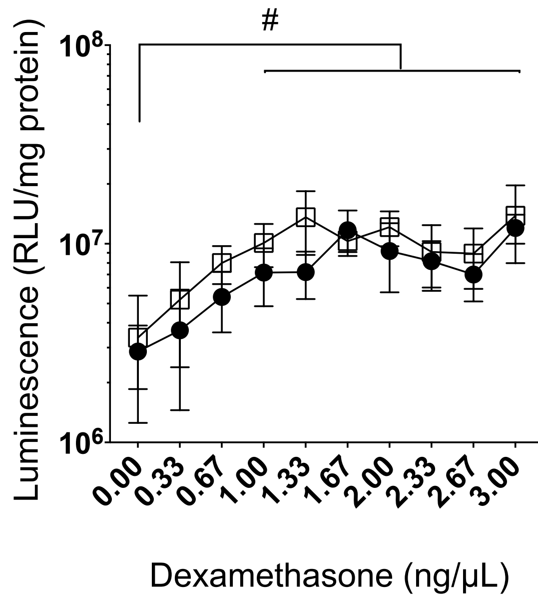


Figure S10. Hypothetical mechanistic model describing differential nuclear uptake of either 3NF-pDNA or pDNA devoid of such DTS and their resulting transgene expression, depending on whether NFκB is activated or not. In our study, 3NF-pDNA and 3NF-devoid-pDNA as illustrated in this Figure correspond to p($3NF_1$-Luc-$3NF_2$)*m* and p(Scr_1-Luc-Scr_2)*m*, respectively. (A) In absence of any signal activating the NFκB pathway, NFκB is sequestrated in the cytoplasm by its inhibitor IκBα. In this situation, copies of 3NF-pDNA bound to the inhibited NFκB are retained in the cytoplasm and only the non-titrated pDNA copies may enter into the nucleus, independently of NFκB. (B) On the contrary, when a stimulus efficiently triggers the NFκB pathway, IκBα is phosphorylated and no longer interacts with NFκB. Copies of 3NF-pDNA bound with NFκB can thus be actively shuttled thanks to the latter into the nucleus. This assisted nuclear import may still occur concomitantly with some nuclear entry independent of NFκB. (C and D) For pDNA devoid of 3NF, since the pDNA nuclear import cannot rely on NFκB translocation, the number of nuclear pDNA copies is similar, regardless of any NFκB activation. As a result, under experimental conditions where only the pDNA used (either 3NF-pDNA or 3NF-devoid-pDNA) and NFκB activation vary, the overall pDNA nuclear copy number is different, as follows $n_b>n_d=n_c>n_a$. Assuming that transcription and translation are constant, the amount of luciferase (denoted by the size of the yellow rectangle) is proportional to the nuclear content of each pDNA. Thus, luminescence intensity can inform on nuclear import of each pDNA considered.

A



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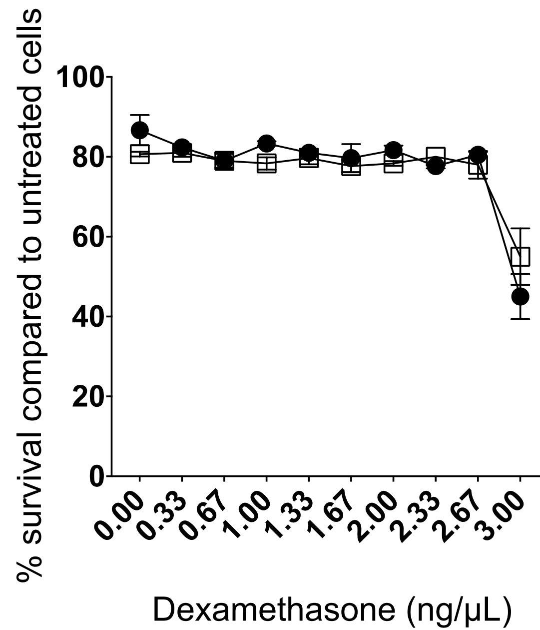
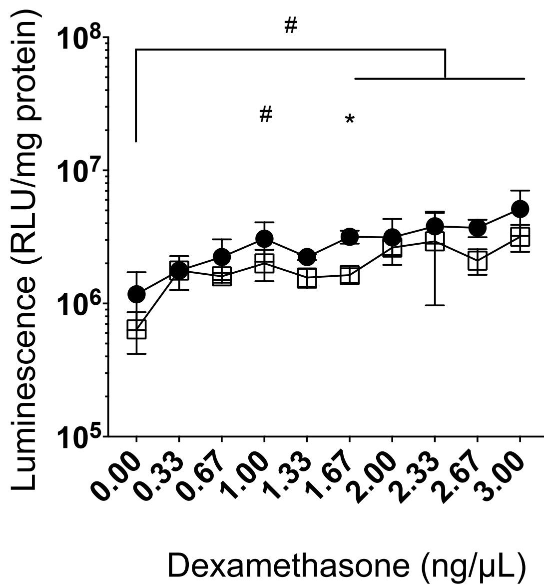


● p($3NF_1$-Luc-$3NF_2$)*m* ⊟ p(Scr_1-Luc-Scr_2)*m*

Figure S11. *In vitro* effect of dexamethasone on transfection using p($3NF_1$-Luc-$3NF_2$)*m* and p(Scr_1-Luc-Scr_2)*m* complexed with bPEI in C2C12 cells. (A) Luminescence signals of C2C12 cells transfected with p($3NF_1$-Luc-$3NF_2$)*m* or p(Scr_1-Luc-Scr_2)*m* in presence of ascending doses of dexamethasone. For p($3NF_1$-Luc-$3NF_2$)*m* and p(Scr_1-Luc-Scr_2)*m* comparisons, results are mean values of 3 wells +/- SD. The sign * denotes statistically significant differences between plasmids (*p*-value ≤ 0.05, multiple t-test Holm-Sidak method). For non-specific corticoid effect on plasmids, results are mean values of 6 wells +/- SD. The character $^{\#}$ denotes *p*-value ≤ 0.05 for two ways ANOVA used to compare the plasmids without *versus* with dexamethasone at indicated doses. (B) Cell-survival is given as percentage compared to untreated cells.

A



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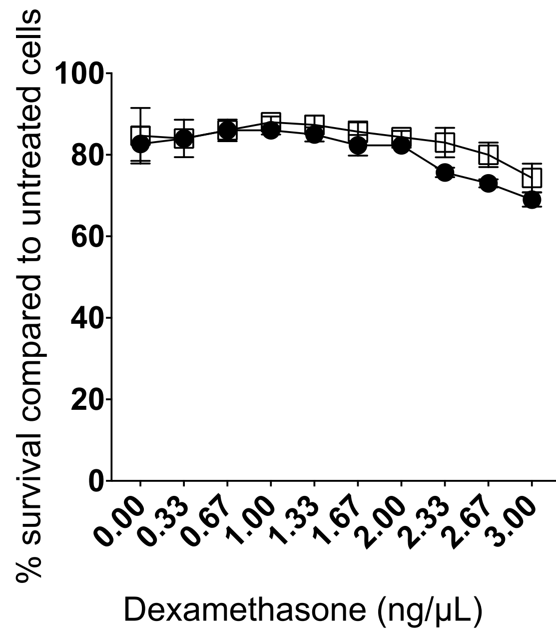

● p(3NF$_1$-Luc-3NF$_2$)*m* ⊟ p(Scr$_1$-Luc-Scr$_2$)*m*

Figure S12. ***In vitro*** **effect of dexamethasone on transfection using p(3NF$_1$-Luc-3NF$_2$)*m* and p(Scr$_1$-Luc-Scr$_2$)*m* complexed with bPEI in A549 cells.** (A) Luminescence signals of A549 cells transfected with p(3NF$_1$-Luc-3NF$_2$)*m* or p(Scr$_1$-Luc-Scr$_2$)*m* in presence of ascending doses of dexamethasone. For p(3NF$_1$-Luc-3NF$_2$)*m* and p(Scr$_1$-Luc-Scr$_2$)*m* comparisons, results are mean values of 3 wells +/- SD. The sign * denotes statistically significant differences between plasmids (*p*-value ≤ 0.05, multiple t-test Holm-Sidak method). For non-specific corticoid effect on plasmids, results are mean values of 6 wells +/- SD. The character # denotes *p*-value ≤ 0.05 for two ways ANOVA used to compare the plasmids without *versus* with dexamethasone at indicated doses. (B) Cell-survival is given as percentage compared to untreated cells.

Supplemental tables

Table S1. Oligonucleotides and primers used for p($3NF_1$-Luc-$3NF_2$)*m* and its derivatives

Oligonucleotides	Sequences
Forward Scr_1	5'-CATTCTAGAGAGCATGCTAATGGGAGCCGGTTAACCGCGTATCATTTGAGCATCAATCCTCCTGTCACGTATGGTCTCGCTACCCCTGAGTGTGCTATCG-3'
Reverse Scr_1	5'-AATTCGATAGCACACTCAGGGGTAGCGAGACCATACGTGACAGGAGGATTGATGCTCAAATGATACGCGGTTAACCGGCTCCCATTAGCATGCTCTCTAGAATGGTAC-3'
Primer F1	5'-CGACTGCATCTGCGTGTTC-3'
Primer F1-Scr_1	5'-CTACCCCTGAGTGTGCTATC-3'
Primer R1	5'-GAAAGTCCCTATTGGCGTTAC-3'
Forward Scr_2	5'-GATCCTACTCAACATCGTAGCGTCTCTTGTGTGCAGGAGTATAATAGACCCGGTCGGCGTACCG-3'
Reverse Scr_2	5'-TCGACGGTACGCCGACCGGGTCTATTATACTCCTGCACACAAGAGACGCTACGATGTTGAGTAG-3'
Primer F2	5'-TTGTGATGCTATTGCTTTATTTG-3'
Primer R2	5'-GGAAAGTCCCCAGGCTAG-3'
Primer R2-Sc_{r2}	5'-AGAGACGCTACGATGTTGAG-3'

Table S2. Physicochemical properties of bPEI and His-lPEI polyplexes at mass ratio 5 with p($3NF_1$-Luc-$3NF_2$)*m*

Polyplexes	Average size (nm)	PdI	Mean zeta potential (mv)
bPEI/p$3NF_1$-Luc-$3NF_2$	113,6 +/- 10,1	0,3 +/- 0,1	20 +/- 13,1
His-lPEI/p$3NF_1$-Luc-$3NF_2$	164,3 +/- 2,9	0,2 +/- 0,0	9,8 +/- 0,5

Table S3. Summary of the *in vivo* data and statistical analyses

HLV injection of 0.5 µg of $p(3NF_1\text{-}Luc\text{-}3NF_2)m$ *versus* $p(Scr_1\text{-}Luc\text{-}Scr_2)m$					
Muscle	Mean *ex vivo* luminescence value (RLU/mg muscle)		$p(3NF_1\text{-}Luc\text{-}3NF_2)m$ / $p(Scr_1\text{-}Luc\text{-}Scr_2)m$ ratio	Mann-Whitney *p*-value	Mean of ratio
	$p(3NF_1\text{-}Luc\text{-}3NF_2)m$	$p(Scr_1\text{-}Luc\text{-}Scr_2)m$			
Soleus	503,000	325,000	1.5	0.8857	2.4
Plantaris	256,000	61,500	4.2	0.1143	
Tibialis	55,750	24,475	2.3	0.0571	
Gastrocnemius	140,500	110,250	1.3	0.6857	
EDL	17,007.5	8,950	1.9	0.3429	
Posterior muscle	248,500	115,000	2.2	0.9429	
Hamstring	80,500	51,075	1.6	0.4857	
Quadriceps	71,000	16,750	4.2	0.5143	

HLV injection of 5 µg of $p(3NF_1\text{-}Luc\text{-}3NF_2)m$ *versus* $p(Scr_1\text{-}Luc\text{-}Scr_2)m$					
Muscle	Mean *ex vivo* luminescence value (RLU/mg muscle)		$p(3NF_1\text{-}Luc\text{-}3NF_2)m$ / $p(Scr_1\text{-}Luc\text{-}Scr_2)m$ ratio	Mann-Whitney *p*-value	Mean of ratio
	$p(3NF_1\text{-}Luc\text{-}3NF_2)m$	$p(Scr_1\text{-}Luc\text{-}Scr_2)m$			
Soleus	5,926,000	3,553,333	1.7	>0.9999	3.6
Plantaris	877,000	630,667	1.4	0.7922	
Tibialis	836,400	89,950	9.3	0.1255	
Gastrocnemius	1,058,000	419,833	2.5	0.0823	
EDL	331,000	109,900	3.0	0.4286	
Posterior muscle	794,000	670,000	1.2	>0.9999	
Hamstring	1,854,000	344,667	5.4	0.3550	
Quadriceps	183,800	44,483	4.1	0.2468	

HLV injection of 0.5 µg of $p(3NF_1\text{-}Luc\text{-}3NF_2)c$ *versus* $p(Scr_1\text{-}Luc\text{-}Scr_2)c$					
Muscle	Mean *ex vivo* luminescence value (RLU/mg muscle)		$p(3NF_1\text{-}Luc\text{-}3NF_2)c$ / $p(Scr_1\text{-}Luc\text{-}Scr_2)c$ ratio	Mann-Whitney *p*-value	Mean of ratio
	$p(3NF_1\text{-}Luc\text{-}3NF_2)c$	$p(Scr_1\text{-}Luc\text{-}Scr_2)c$			
Soleus	198,155	195,036	1.0	0.8413	3.6
Plantaris	54,379	99,048	0.5	0.8413	
Tibialis	45,508	6,537	7.0	0.2857	
Gastrocnemius	210,016	65,942	3.2	0.0952	
EDL	17,517	9,061	1.9	0.1111	
Posterior muscle	132,121	31,487	4.2	0.0317*	
Hamstring	132,216	17,046	7.8	0.0159*	
Quadriceps	26,400	9,190	2.9	0.0159*	

*p-value ≤ 0.05, Mann-Whitney test (with n=4-6 for each muscle).

Supplemental materials and methods

Construction of p(3NF$_1$-Luc-3NF$_2$)$_m$ derivatives

Five pDNA derived from p(3NF$_1$-Luc-3NF$_2$)*m* (5,556 bp) were constructed by replacing either 3NF$_1$, 3NF$_2$ or both, by scramble sequences of the same length. The scramble sequence 1 (Scr$_1$) insert, for 3NF$_1$ replacement was obtained by hybridization of oligos "Forward Scr$_1$" and "Reverse Scr$_1$" (Table S1). For this purpose, 2 µg of each simple strand oligos were mixed in 50 µL of 1X NEB4 buffer (New England Biolabs, Evry, France) and incubated for 2 min at 94°C prior to be progressively cooled down at 25°C. The same protocol was applied for oligos "Forward Scr$_2$" and Reverse Scr$_2$" forming the Scr2 insert (Table S1). p(Scr$_1$-Luc-3NF$_2$)*m* (5,556 bp) was obtained by digestion of p(3NF$_1$-Luc-3NF$_2$)*m* with KpnI and EcoRI (New England Biolabs, Evry, France) (Figure S1) for 2 h at 37°C followed by electrophoresis of the products (50 V for 1 h 30 in a 0.8 % agarose gel stained with ethidium bromide (EtB)(Dutscher, Brumath, France)). Lane corresponding to the plasmid depleted from 3NF$_1$ was isolated and purified thanks to the NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany) following the manufacturer instructions. Plasmid was then ligated with Scr$_1$ insert. p(3NF$_1$-Luc-Scr$_2$)*m* (5,556 bp) was obtained following a similar procedure using BamHI and SalI enzymes and NEB3 buffer with Scr$_2$ insert. Finally, p(Scr$_1$-Luc-Scr$_2$)*m* (5,556 bp), was obtained by digestion of 100 ng of p(3NF$_1$-Luc-Scr$_2$)*m* using KpnI and EcoRI for 4 h at 37°C. Digestion mix was finally heated at 70°C for 15 min to inactivate the restriction enzymes. Ten nanograms of the digested plasmid were then incubated for 30 min at 22.5°C with 1 ng of Scr$_1$ insert in a mix containing 1 µL of T4 ligase buffer and 0.5 µL of T4 ligase enzyme (Invitrogen, Paris, France). At the end of this incubation step, the mix was heated at 65°C for 10 min.

Fusion of pDNA ("concatemerisation")

To obtain p(3NF$_1$-Luc-3NF$_2$)*c* (11,112 bp), 120 ng of p(3NF$_1$-Luc-3NF$_2$)*m* were digested using KpnI (Figure S1), in a 40 µL mixture of 1X BSA (Promega, Charbonnières-les-Bains, France) and 1X NEB2 buffer. Digestion took place at 37°C for 4 h and was followed by heat inactivation of the enzyme. Then, 100 ng of digested plasmids were mixed in a final volume of 50 µL containing 1 µL of T4 ligase. This mix was incubated for 2 h at 16°C prior to be cooled down and kept at 4°C for 48 h. pDNA from the ligation mixture was run for 2 h, 100 V in a 0.8% agarose gel stained with Sybr gold (Invitrogen, Paris, France). Gel was quickly illuminated on a UV bench and the band, corresponding in size to the concatemerized plasmid, isolated and purified thanks to the Nucleospin® gel and PCR clean-up kit following the manufacturer instructions. The same procedure was applied to p(Scr$_1$-Luc-Scr$_2$)*m* to obtain p(Scr$_1$-Luc-Scr$_2$)*c* (11,112 bp).

Bacterial transformation and PCR

For bacterial transformation, 5 ng of plasmid of interest were mixed with 50 µL of chemically competent *E. coli*. The mixture was incubated on ice for 30 min prior to be dived in a water bath for 30 sec at 42°C, followed by an incubation on ice for 2 min. Then, 250 µL of SOC medium (Super Optimal Broth with Catabolite repression) (Invitrogen, Paris, France) were added and bacteria were grown for 1h at 37°C, 230 rpm. After outgrowth, 100 µL of the culture were spread and grown overnight at 37°C on LB agar medium (Invitrogen, Paris, France) containing ampicillin (100 µg/mL) (AppliChem, Darmstadt, Germany). Obtained colonies were screened by PCR to discriminate in between the different p(3NF$_1$-Luc-3NF$_2$)*m* derivatives. Each colony was picked in 50 µL of sterile water (Fresenius, Sèvres, France) and incubated for 10 min at 95°C prior to be quickly centrifuged (short spin). For PCR reaction, PCR Mastermix (Invitrogen, Paris, France) was supplemented with specific primers of each 3NF or Scr motifs (Table S1). To identify the 3NF$_1$ and 3NF$_2$ sequences, primers F1/R1 and F2/R2 were used, respectively. Scr$_1$ and Scr$_2$ were identified using F1-Scr$_1$/R1 and F2/R2-Scr$_2$ primers. A typical PCR mix was composed by 22.5 µL of Mastermix mixed with 0.5 µL (10 µM) of forward and reverse primers completed by 0.5 µL of colony lysate. PCR were performed thanks to a Geneamp PCR system 9700 thermocycler (Applied Biosystems, Foster City, USA). The program was composed of an initial denaturation step of 5 min at 94°C followed by 30 cycles as stated: 94°C for 30 sec / 63°C for 30 sec / 72°C for 30 sec. Amplification products were run for 22 min, 100 V in a 2% agarose gel stained with 0.005% of EtB prior to be imaged using a trans-illuminator (Fischer Scientific, Illkirch, France). To assay pDNA sizes, p(3NF$_1$-Luc-3NF$_2$)*m* and its derivatives were also run at 140 V for 40 min in a 0.8% agarose gel stained with EtB.

DNA sequencing

The GATC "Lighrun tube" service (Eurofins genomics, Les Ulis, France) performed DNA sequencing of p(Scr$_1$-Luc-Scr$_2$)*m* and p(3NF$_1$-Luc-3NF$_2$)*m* using the following primers: 5'-TGACGTTGGAGTCCACG-3' (for NF$_1$ and Scr$_1$ areas) and 5'-CCACAACTAGAATGCAGTG-3' (for NF$_2$ and Scr$_2$ areas).

Amplification and purification of pDNA

For amplification, transformed *E. coli* were outgrowth at 37°C, 220 rpm for 7 h in 5 mL of liquid LB medium containing ampicillin (100 µg/mL) prior to be cultured for 16 h in 1.8 L of the same medium at 37°C, 180 rpm. Plasmids were purified thanks to the Macherey-Nagel NucleoBond® PC 10 000 kit (Macherey-Nagel, Düren, Germany) following the manufacturer instructions. Plasmids batch purity were assayed by measurement of the A260/A280 ratio. Only batches showing values between 1.8 and 2.0 were used in subsequent experiments.

Complexation assay of p($3NF_1$-Luc-$3NF_2$)*m* by bPEI and His-lPEI

To determine a polymer:pDNA mass ratio (MR) at which p($3NF_1$-Luc-$3NF_2$)*m* and its derivatives were fully complexed by bPEI (Sigma-Aldrich, Saint-Quentin-Fallavier, France) or His-lPEI, polyplexes of p($3NF_1$-Luc-$3NF_2$)*m* with the different polymers were formed as stated in the dedicated materials and methods section. MR assayed were 0.25; 0.5; 1; 2.5; 10 and 15. Ten microliters of each polyplexes mixture were run with 2 µL of loading buffer following agarose gel electrophoresis (0.8% agarose gel stained with EtB) for 15 min, 100 V in 1X TBE (Biosolve Chimie, Dieuze, France). Gel was imaged thanks to a UV trans-illuminator device.

Characterization of His-lPEI and bPEI complexes

Physicochemical characteristics (size and zeta potential) of His-lPEI and bPEI complexes at MR5 with 0.25 µg of p($3NF_1$-Luc-$3NF_2$)*m* were obtained using a Zetasizer nano Zen3600 (Malvern Panalytical, Malvern, UK). Briefly, polyplexes were diluted 1:100 in sterile water prior to be measured at 25°C.

Cell culture

A549 (adenocarcinomic human alveolar basal epithelial cells)(CCL-185, ATCC, Rockville, MD, USA) and 16HBE (human bronchial epithelial cells) were respectively cultured at 37°C, 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) and in Eagle's Minimum Essential Medium (EMEM) (Ozyme, Saint-Cyr-l'École, France) containing 10% of heat inactivated serum (Lonza, Levallois-Perret, France), 1% of antibiotics (10,000 U mL-1 Penicillin, 10,000 µg mL-1 Streptomycin) (PAA laboratories, Les Mureaux, France) and a supplementation of 1% L-Glutamine (Ozyme, Saint-Cyr-l'École, France).

Luciferase expression time course study of p($3NF_1$-Luc-$3NF_2$)*m* and p(Scr_1-Luc-Scr_2)*m* in C2C12 and A549 cells

For luciferase expression time course study of p($3NF_1$-Luc-$3NF_2$)*m* and p(Scr_1-Luc-Scr_2)*m*, C2C12 and A549 cells were plated 24 h prior transfection in 96 wells plates (in 200 µL of DMEM) to reach 80% of confluency the experiment's day. Cells were transfected with 0.25 µg of p($3NF_1$-Luc-$3NF_2$)*m* or p(Scr_1-Luc-Scr_2)*m* complexed by bPEI at MR 5 and were then lysed 3, 6, 9, 24 and 48 h after transfection to evaluate the different luminescence signals obtained.

***In vitro* transfection in presence of dexamethasone**

To assay the influence of corticoid on transfection using p($3NF_1$-Luc-$3NF_2$)*m* and p(Scr_1-Luc-Scr_2)*m*, cells were plated 24 h prior to the experiment to reach 80% confluency the transfection's day. Dexamethasone (Mylan, Saint-Priest, France) was diluted in DMEM at desired concentration to reach 0.33 ng/µL, 0.67 ng/µL, 1 ng/µL, 1.33 ng/µL, 1.67 ng/µL, 2 ng/µL, 2.33 ng/µL, 2.67 ng/µL or 3 ng/µL. Dexamethasone was added to cells 30 min prior transfection with 0.25 µg of p($3NF_1$-Luc-$3NF_2$)*m* or p(Scr_1-Luc-Scr_2)*m* complexed by bPEI at MR 5. The cells were then incubated for 24 h at 37°C, 5% CO_2 prior to be lysed.