Supporting Information

A light-triggerable formulation to control the stability of pro-angiogenic transcription factor hypoxia inducible factor-1α (HIF-1α)

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MATERIALS and METHODS

Synthesis of polymers. The library of poly (amido amine)s with light-cleavable moiety (NVOC) was synthesized via Michael addition reaction. Prior to synthesis, diamines (1-22), bisacrylamides A-E were diluted to 1.6 M in anhydrous DMSO (Sigma-Aldrich). Specifications of all monomers can be found in supplementary Table 1. After designing the plate layout for the 110 combinations between monomers A-E and 1-22, 100 µL aliquots of bisacrylamides and 100 µL aliquots of diamines were added to each well of a 96-deepwell plate (polypropylene (PP), VWR). The plates were sealed with aluminum foil and incubated for reaction at 60 ºC shaking for five days on an orbital shaker (250 rpm). Polymers were finally end capped with 20% molar excess (10 µL to 100 µL reaction volume) of the respective diamine 1-22 for 2 h (60ºC, 250 rpm). Next, the polymers were functionalized with 4,5-dimethoxy-2-nitrobenzyl chloroformate (NVOC, Sigma-Aldrich) in the presence of triethylamine (10% molar ratio, Sigma-Aldrich). The reaction was performed overnight under shaking at room temperature. Finally, the plates were stored at 4ºC until usage.

Determination of the best photocleavable group ratio in poly(amido amine)s. To optimize the amount of the photocleavable group in the poly(amido amine), ratios of 1:4, 1:8 and 1:12 of NVOC to diamine were used in the synthesis of A4 polymer. To obtain the degree of substitution of NVOC in the polymer, A4 was purified by precipitation in water, lyophilized, resuspended in DMSO-d_6 and analyzed by ^{1}H-NMR (Bruker Avance III 400 MHz) relative to TMS. After preparation of

nanoparticle with the polymers with different NVOC ratio, size and count decrease of the nanoparticles before and after UV irradiation (10 min, 365 nm, 100 mW/cm^2) were measured by DLS.

Evaluation of the best ratio siRNA:NP and transfection time in the gene knockdown efficiency. siRNA:NP ratio was optimized with formulation A4 to maximize GFP knockdown. A suspension of A4 NPs (200 µg/mL) was complexed for 2 h with siRNA against GFP in ratios of 1:12.5, 1:25 and 1:50 (w/w) in nuclease free sterile water under shaking on an orbital shaker (250 rpm) at room temperature. To bioactivity of the complexes were evaluated in HeLa-GFP cells which were seeded at a density of 40.000 cells/mL for 24 h prior the experiment. Cells were transfected for 4 h with NP@siRNA complexes (20 µg/mL) in starvation (DMEM), washed, fresh medium with reduced serum (DMEM, 5% FBS, 0.5% PenStrep) added and cultured for additional 48 h. After 48 h, cells were stained with H33342 and PI (both 0.25 µg/mL) and analyzed by fluorescence microscopy on a high-content microscope (In Cell Analyzer 2200). The quantification of cell viability and GFP knockdown is described in high content imaging section below.

The transfection time was optimized to identify a time relatively short that could lead to significant gene knockdown. In this way, cells were transfected with NP@siRNA complexes (20 µg/mL; 1:50 siRNA:NP (w/w)) from 10 min to 4 h. After a washing step, cells were cultured in medium with reduced serum (DMEM, 5% FBS, 0.5 % PenStrep) until 48 h. Cells were stained with H33342 and PI (both 0.25 µg/mL) and analyzed by fluorescence microscopy on a high-content microscope (In Cell Analyzer 2200) for GFP knockdown (described in high content imaging section below).

Gel permeation chromatography (GPC) analyses. Molecular weights (Mn) and molecular weight distributions (Mw/Mn) of selected polymers were measured by GPC on a HPLC Agilent 1260 system equipped with a guard column (Agilent, Aquagel, 10 mm, 10 µm) followed by three columns: (i)

Agilent, Aquagel-OH 40, 300 × 7.5 mm, 8 µm, (ii) Agilent, Aquagel-OH 50, 300 × 7.5 mm, 8 µm and (iii) Phenomenex, Polysep-GFC-P2000, 300 × 7.8 mm, range 100 – 10 k Da, connected to a UV (254 and 280 nm) and RI detector (Agilent). The GPC eluent was acetate buffer (0.5 mol/L, pH = 4.5), and the polymers were eluted at 0.7 mL/min. The temperature was set at 35 °C. Polyethylene oxide standards (EasyVial PEG/PEO, range 194 – 1000 k Da) were used to calibrate the SEC, since it has been demonstrated that such eluent composition allows PEO to be a suitable calibration standard for poly(amido amines).[1]

NP library preparation. Nanoparticle library was prepared in sterilized 96-deepwell plates by precipitation 15 µL of each polymer in water (960 µL, molecular biology grade, Fisher Bioreagents) and further addition of 25 µL of zinc sulfate (1M, Sigma-Aldrich). Plates were sealed with PP adhesive seals and left stirring on an orbital shaker (250 rpm) at 25 ºC. After a step of purification (centrifugation at 4 ºC, 8000 g for 8 min), the samples were resuspended in water (molecular biology grade) and lyophilized to determine the mass concentration of each nanoparticle. The efficiency of NP formation was calculated according to equation:

$$NP\ formation\ efficiency\ (\%) = \frac{M_{NP}}{M_{polymer}} \times 100$$

where M_{NP} denotes the weight of material recovered after NPs purification and freeze-drying and $M_{polymer}$ is the theoretical polymer weight.

Characterization of NP size and zeta potential. The diameter and zeta potential of NPs was measured by photon correlation spectroscopy (PCS) using quasi-elastic light scattering equipment (ZetaPALS analyser, Brookhaven Instruments Corp., Holtsville, NY) and ZetaPlus™ Particle Sizing Software (version 4.03). The scattered light was collected at fixed angle of 90º. To measure NPs size, a suspension of NP in water (molecular biology grade) was added to a cuvette (50 µg/mL, 2 mL), allowed to stabilize for 10 minutes and then analyzed at room temperature (3 times). To assess the percentage of NPs disassembly upon UV light irradiation (10 min, 365 nm, 100 mW/cm^2), a duplicate

of the samples was used and the values of NP diameter and NP counts (Kcps) were recorded. The zeta potential of NPs was determined in a 1 mM KCl solution at 25 ºC (50 µg/mL, 2 mL). All data were recorded as the mean of 5 measurements runs.

TEM analyses. The analysis was carried out on a FEI-Tecnai Spirit BioTwinG2 electron microscope. Aqueous dispersion of C11 NPs (500 µg/mL) was added on the surface of carbon coated 200 mesh copper grid and left air-dry for 5 h at room temperature in a closed petri dish. Digital images were acquired with coupled side mounted CCD camera MegaView III-SIS and the diameter of NPs was analysed with the Particle Tool from ImageJ.

High-throughput complexation of siRNAs with NPs. In a 96-deepwell plate, the NPs (50 µL, 400 µg/mL) were complexed with siRNA against eGFP (50 µL, 4 µg/mL siRNA and 4 µg/mL Cy5-tagged siRNA, GFP Duplex I, GE Dharmacon) at a weight ratio 1:50 (siRNA:NP). As control for siRNA activity and transfection, the same procedure was followed for lipofectamine RNAiMAX (15 µL/mL; Invitrogen). The plates were sealed (PP seals) and allowed to incubate at room temperature for 2 h on an orbital shaker (250 rpm). Samples were then diluted 1:10 with DMEM to 20 µg/mL NP concentration and directly used for cell transfection or determination of complexation efficacy. Complexation efficacy was determined indirectly from Cy5 tagged-siRNA after separating NPs and non-complexed siRNA by centrifugation (4ºC, 14.000g, 15 min), quantifying Cy5 fluorescence in three replicates of the supernatant. Concentration of siRNA was determined relative to a standard curve.

High-throughput transfections with NP@siRNA. Stable transfected HeLa-GFP (CellBiolabs Inc.) reporter cells were cultured in DMEM (without phenol red) containing FBS (10%, v/v), PenStrep (0.5%, v/v, 50 µg/mL) and blasticidin (10 µg/mL). 24 hours prior to the experiment, HeLa-GFP cells were seeded in 96 well plates (Costar) with a density of 4.000 cells per well. NP@siRNA complexes

(20 µg/mL) or lipofectamine RNAiMAX (1.5 µL/ml) were prepared in DMEM as described above. Cell transfections were performed with three technical replicates and plates in duplicate. After 10 min of cell material contact for transfection, medium was replaced by DMEM containing 5% FBS (v/v), PenStrep (0.5%, v/v, 50 µg/mL) and blasticidin (10 µg/mL). One plate duplicate was used for activation of the NP using a transilluminator (10 min, 365 nm, UVP BioSpectrum 500). The second plate of the duplicate (with same sample layout) remained without NP activation, allowing comparison of the bioactivity of released siRNA with and without activation by the light trigger. 48 h post transfection, cells were stained and placed in an automated incubator (Cytomat 2, Thermo) for high-content imaging analysis with an automated fluorescence microscope (In Cell 2200, GE Healthcare).

High-content imaging analyses. To distinguish viable cells from dead cells, cell nuclei were stained with Hoechst 33342 (Sigma-Aldrich, 0.25 µg/mL) and propidium iodide (PI, Sigma-Aldrich, 0.25 µg/mL). Dead cell nuclei are permeable for PI and show staining for H33342 and PI, where live cells are stained only with H33342. 48 h and 72 h post transfection, cells were analyzed on a high-content microscope (In Cell 2200, GE Healthcare) with a 20× objective, where 4 random image fields per well were imaged. Image analysis was performed with In Cell Developer software (GE Healthcare), applying machine learning algorithms. H33342 staining was used for definition of a nuclear mask (nuclei). Dead cell masks, with PI and H33342 staining overlapping 10% (dead nuclei) were subtracted from H33342 nuclear mask, resulting in viable nuclear population (viable nuclei). That mask was then dilated to cover as much of the cell region possible (cell). Next, nuclear mask was subtracted from the cell mask, resulting in a ring that masks the cytoplasm. GFP fluorescence intensity was measured in that cytoplasm mask of live cells. GFP knockdown was calculated as percentage of fluorescence on non-treated HeLa-GFP cells (after subtracting HeLa cell fluorescence background). From the difference of the total count of nuclei and dead nuclei count, cell viability was

calculated. By quantification of the Cy5 tagged siRNA in the cytoplasm, internalization of the NP was quantified.

siRNA release from C11 NPs after UV light irradiation. The release of siRNA from C11 (50 µg/mL, weight ratio 1:50 siRNA:NP, molar ratio 1:58 siRNA:NP) after UV-irradiation (10 min, 365 nm, 1 mW/cm^2) was determined by quantification of Cy5 tagged-siRNA in the supernatant after centrifugation (4ºC, 14000g, 15 min). The concentration of siRNA-Cy5 was determined by fluorescence in a microplate reader (Synergy H1) relative to a standard curve ($y = 3978.3x – 61.87$; $R^2 = 0.9869$).

Cellular internalization profiling of NPs. Human dermal keratinocytes (HaCaT cells; CLS Cell Lines Service GmbH, Eppelheim, Germany), human normal dermal fibroblast (NHDF) or human umbilical vein endothelial cells (HUVEC, Lonza) were used to quantify internalization of NP@siRNA complexes by colocalization with the endolysosomal compartment. HaCaT and NHDF cells were cultured in DMEM medium, HUVECs were cultured in EGM-2 medium (Lonza). All media was supplemented with FBS (10%, v/v) and PenStrep (0.5%, v/v, 50 µg/mL). Twenty-four hours prior to the experiment, cells were seeded to each well in black glass bottom 96 well plates (IBIDI, Germany) coated with 0.1% gelatine (Sigma) with densities of HaCaT and HUVECs at 20.000 cells/well, NHDF cells at 10.000 cells/well. Cells were stained with CellTrace™ CFSE 488 (5 µM; Molecular Probes, Life Technologies) according to manufacturer's instructions prior to the experiment. For cell transfection, cells were incubated for 1 h with NP@siRNA-Cy5 or lipofectamine@siRNA-Cy5 complexes in DMEM or EGM-2 media. Lysotracker Red (100 nM; Molecular Probes, Life Technologies) staining was added for 30 min during cell transfection. Next, complexes were removed, and cells were washed twice with PBS. After fixation with 4% (v/v) paraformaldehyde (Alfa Aesar) in PBS for 10 min at room temperature, cell nuclei were stained with

H33342 (2 μg/mL) for 10 min and subsequent washes with PBS. Cells were analysed by confocal microscopy (Zeiss LSM710) using a 40× immersion oil objective with two technical replicates per condition and a minimum of four representative image fields per replicate. Colocalization of NP@siRNA-Cy5 with Lysotracker red was analysed using JaCoP on ImageJ.

Complexation of PHD2 to the NPs. The complexation of PHD2 (GE Dharmacon) to C11 NPs followed the same procedure previously described for siRNA. Briefly, PHD2 and C11 NPs were mixed in molecular grade nuclease free, sterile water (Fisher Bioreagents) in a ratio of 1:50 (w/w, siRNA to NPs), and the suspension agitated on an orbital shaker for 2 h at room temperature. After complexation, the NP suspension was suspended in cell culture medium before use.

Proliferation of endothelial cells. To assess cell proliferation, EOMA-GFP cells cultured in DMEM medium containing FBS (10%, v/v) and PenStrep (0.5%, v/v, 50 μg/mL), were seeded in 24 well plates (7.500 cells/well; pre-coated for 10 min with 0.1% gelatine) and allowed to adhere overnight. Cells were transfected with C11@PHD2 complexes (20 μg/mL) for 12 h in DMEM with reduced FBS (2.5%, v/v and PenStrep). We have chosen 12 h because shorter transfection times did not translate in measurable functional activity (data not shown). Non-internalized NPs were removed, and medium was replaced by complete medium with 10% FBS (v/v) and PenStrep. NPs were activated with UV light, using a transilluminator (365 nm, 10 min, 1 mW/cm^2; UVP BioSpectrum 500). Cell growth was analyzed at 36 h post transfection by staining the cells with H33342 (1 μg/mL, Sigma) and fluorescence imaging (In Cell 2000). Nuclei were counted from H33342 nucleus staining on In Cell Developer. Each experimental condition was performed with at least three technical replicates. Six images per well were analyzed.

***In vitro* wound healing assay.** EOMA-GFP cells (15.000 cells/well) were seeded in 96 well plates (pre-coated for 10 min with 0.1% gelatine) 24 h prior to experiment to allow cells to grow to a complete monolayer in full medium (DMEM with FBS (10%, v/v) and PenStrep (0.5%, v/v, 50 µg/mL)). Cells were then inhibited by mitomycin (5 µg/mL, in cell culture medium, Tocris Bioscience) for 2 h and then transfected for 12 h with C11@PHD2 NPs (20 µg/mL) in DMEM with FBS (2.5%, v/v) and PenStrep (0.5%, v/v, 50 µg/mL). We have chosen 12 h because shorter transfection times did not translate in measurable functional activity (data not shown). NPs were then removed from the cells and washed with PBS. The monolayer was wounded, by scratching the cells with a yellow (200 µL) pipette tip. Cell debris and detached cells were removed by gently washes with PBS. Fresh medium (DMEM with FBS (1%, v/v) and PenStrep (0.5%, v/v, 50 µg/mL)) was added. NPs were then activated with UV light, using a transilluminator (365 nm, 10 min, 1 mW/cm^2; UVP BioSpectrum 500). Wound healing was monitored until 36 h post-scratch using brightfield microscopy (4x objective) on an automated microscope (In Cell 2000). Wound area was quantified by measuring cell free area with ImageJ. Relative wound closure was calculated at 36 h post wounding relative to time 0 h and normalized to control condition.

Quantitative analysis of PHD2 transfection by qRT-PCR. EOMA-GFP cells were seeded at 30.000 cells/well to 24 well plate pre-coated for 10 min with 0.1% gelatine. Cells were transfected for 12 h with C11-PHD2 (20 µg/mL) in DMEM with reduced FBS (2.5%, v/v) and PenStrep (0.5%, v/v, 50 µg/mL). Non-internalized NPs were removed by washing with PBS and further incubation with fresh medium (DMEM, 10% FBS, v/v 0.5% PenStrep). NPs were activated with UV light, using a transilluminator (365 nm, 10 min, 1 mW/cm^2; UVP BioSpectrum 500). After 24 h, cells from each condition were harvested after application of lysis buffer. RNA extraction was performed using RNeasy Plus Micro Kit (Quiagen) following manufacturers instruction. RNA was quantified on NanoDrop (Thermo Scientific). 1 µg total RNA was used to synthesize cDNA with qScript cDNA SuperMix (Quantabio). Quantitative RT-PCR was performed using NZYSpeedy qPCR Green Master

Mix (NZYTech, Portugal) on a RT-PCR (CFX Connect Real-Time System, BioRad). Quantification of the target gene (PHD2) was analyzed relative to GAPDH as housekeeping gene: $relative\ expression = 2^{\left[-(C_{T\ Sample} - C_{T\ GAPDH})\right]}$ (Supplementary Table 2). Minimal cycle threshold values (C_T) were calculated from at least 3 independent reactions. $\Delta\Delta C_T$ was calculated to determine relative PHD2 expression.

C11 NPs disassembly after UV light irradiation through a skin barrier. To demonstrate that NPs placed under a skin biopsy can be disassembled by UV light, a skin fragment (taken from the back of the mouse) was placed in a 1 cm^2 hole of a cardboard and a cuvette with a suspension of C11@siRNA (50 μg/mL) placed beneath the skin. The cuvette was irradiated with UV light (365 nm, 10 min, 1 mW/cm^2) and the number of NPs monitored by DLS. As control, a cuvette with C11@siRNA (50 μg/mL) was used in the same set up but without skin.

1. Xing, H.; Lu, M.; Xian, L.; Zhang, J.; Yang, T.; Yang, L.; Ding, P. *Asian Journal of Pharmaceutical Sciences* **2017,** 12, (3), 292-298.

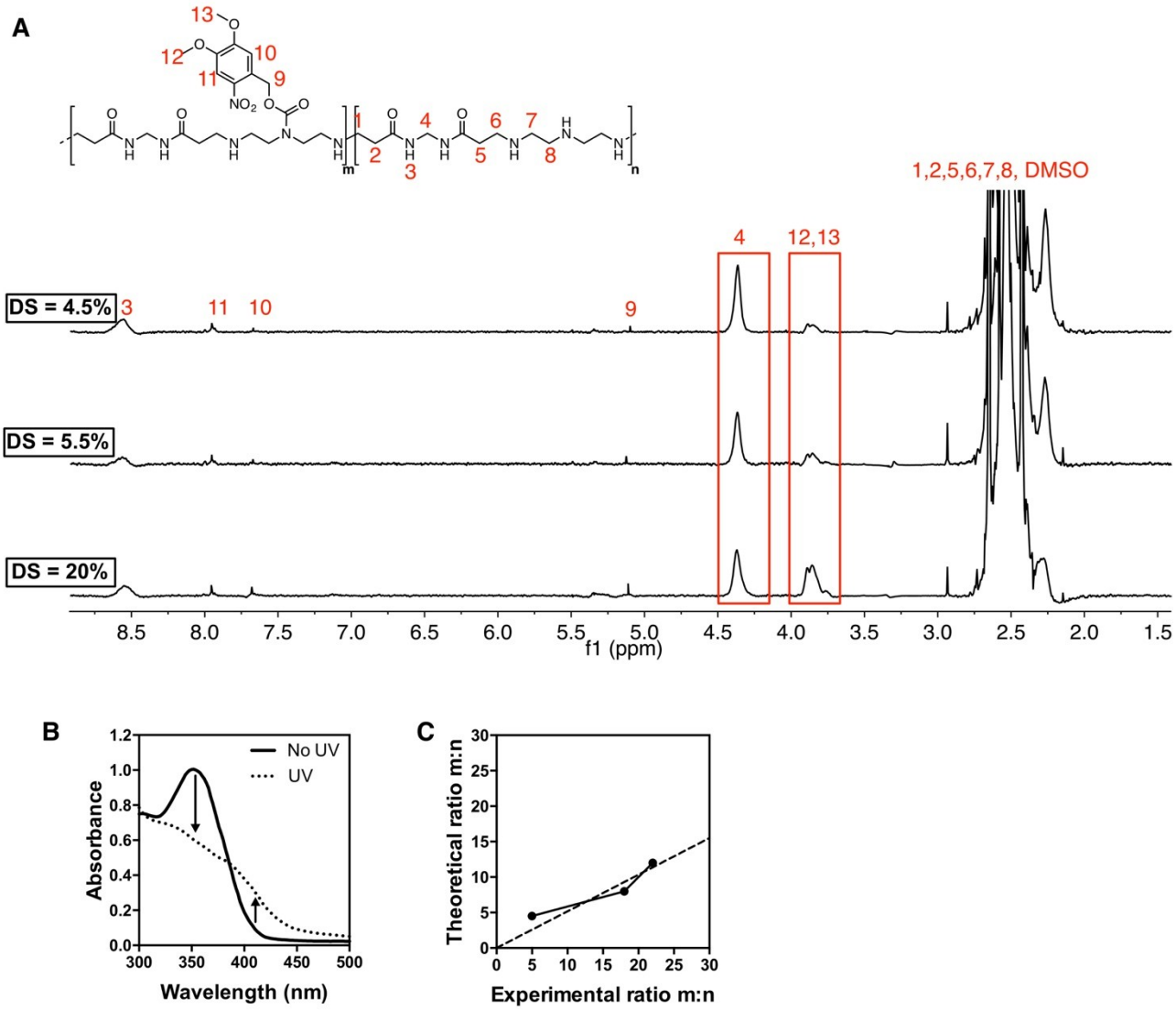
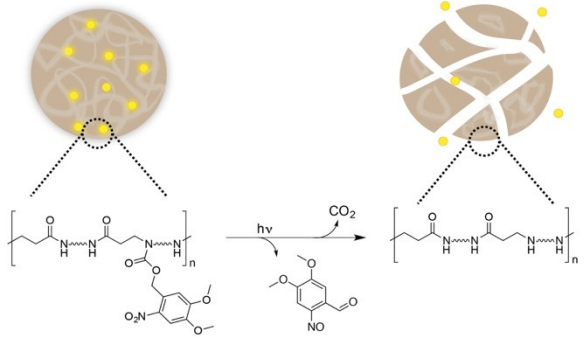


Figure S1. Optimisation of the light-cleavable moiety (NVOC) ratio in the polymer A4. After the synthesis, the polymer was reacted with NVOC at the following theoretical molar ratio (NVOC:diamine): 1:4, 1:8 and 1:12. To calculate the ratio of incorporation of NVOC into the polymer, polymers were precipitated in water, lyophilized, resuspended in DMSO-d_6 and analyzed by ^{1}H-NMR. (A) NMR spectra (in DMSO-d6) of A4 polymers with different NVOC-Cl:diamine molar ratios. The results show a degree of substitution of 20 %, 5.5% and 4.5%. (B) Effect of UV light (10 min, 365 nm, 100 mW/cm^2) in the absorbance of the polymer A4 (DS_{exp} = 20%). The decrease in absorption at 350 nm (NVOC) and the increase at 420 nm (nitroso product) indicate the photo-cleavage of NVOC. (C) Theoretical and experimental NVOC:diamine molar ratio´s ratio by NMR.

A



B

	UV	Diameter (nm)	Count (kcps)	C.decrease (%)
DS = 20%	No	165.7	124.0	87.7
	Yes	2.2	15.2	
DS = 5.5%	No	140.2	170.8	85.1
	Yes	176.1	25.4	
DS = 4.5%	No	306.8	230.5	80.8
	Yes	579.5	44.2	

Figure S2. (A) Schematic illustration of NPs disassembly upon UV light irradiation. (B) Optimisation of the NVOC to amine ratio in the nanoparticle A4, to obtain the higher nanoparticle count decrease after UV irradiation. The formulation (50 μg/mL) was irradiated with UV light (365 nm, 100 mW/cm^2) for 10 min.

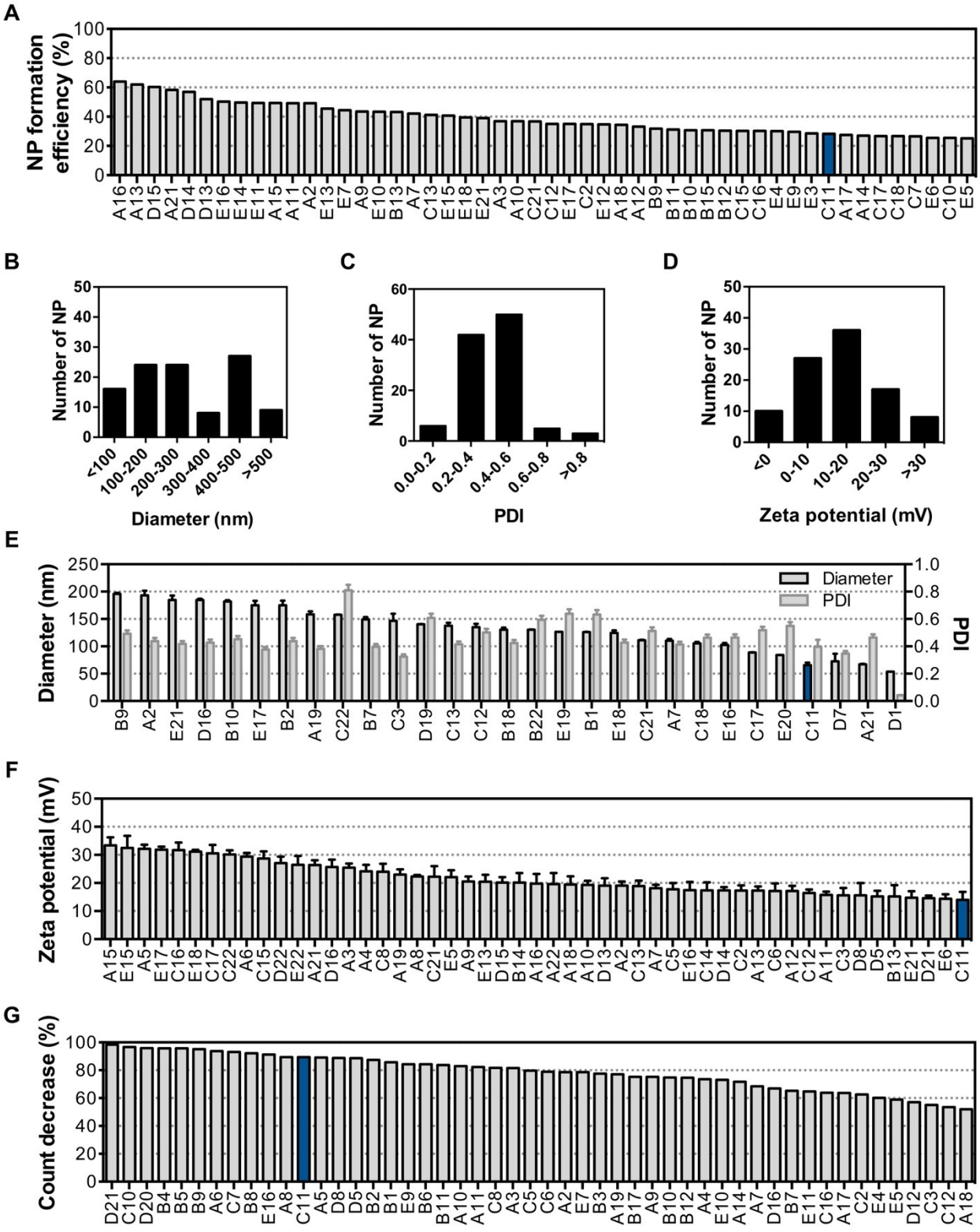


Figure S3: Physicochemical properties of the 50 top NP formulations. (A) Efficiency of NP formation calculated from the ratio of theoretical polymer weight and weight of NP after purification. (B) Diameter frequency distribution. (C) PDI frequency distribution. (D) Zeta potential frequency distribution. (E) Size and PDI of nanoparticles within size range between 50 and 200 nm. (F) Zeta potential of the NPs measured by DLS. In E and F, results are Mean ± SEM (n = 3). (G) NP disassembly by light. Count decrease was determined by DLS after 10 min UV irradiation (365 nm, 100 mW/cm^2).

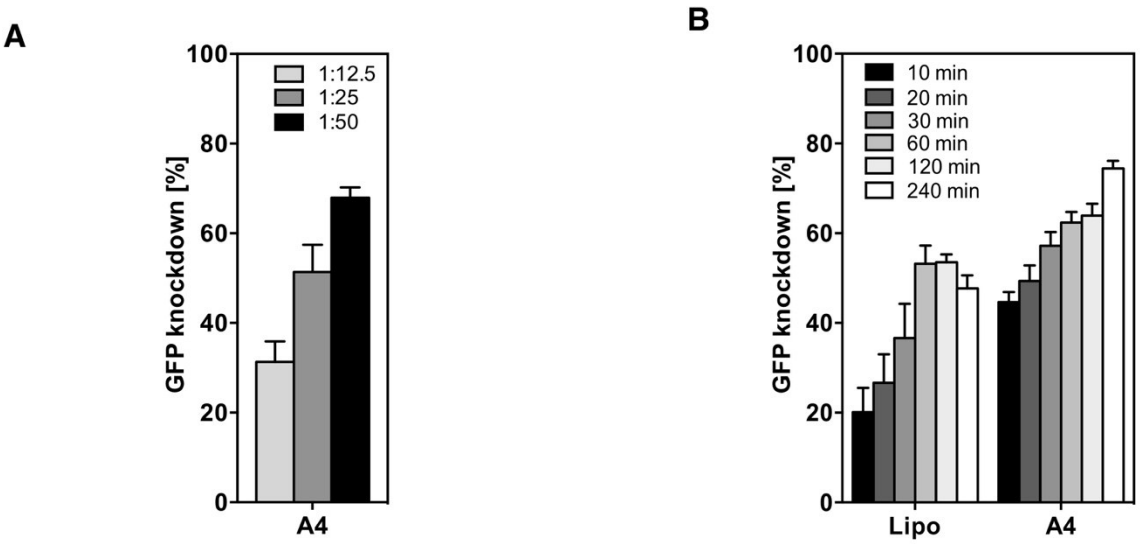


Figure S4. Evaluation of siRNA:NP ratio and transfection time in the knock down efficiency of the formulations. (A) Effect of the ratio siRNA:NP in the knock down efficiency. HeLa-GFP cells were transfected for 4 h with a NP formulation (formulation A4; 20 µg/mL) containing a siRNA-Cy5 against GFP at different ratio´s siRNA:NP (1:12.5; 1:25 and 1:50, w/w). GFP knockdown was quantified at 48 h post transfection. (B) Effect of transfection time in the knock down efficiency. Cells were transfected for various times (between 10 and 240 min) with A4 NP@siRNA-Cy5 complexes (20 µg/mL; 1:50 siRNA:NP, w/w). Lipofectamine RNAiMAX (Lipo) was used as a control transfection agent. GFP knockdown was analysed at time 48 h after transfection by high content microscopy. All results are presented as Mean ± SEM (n = 3).

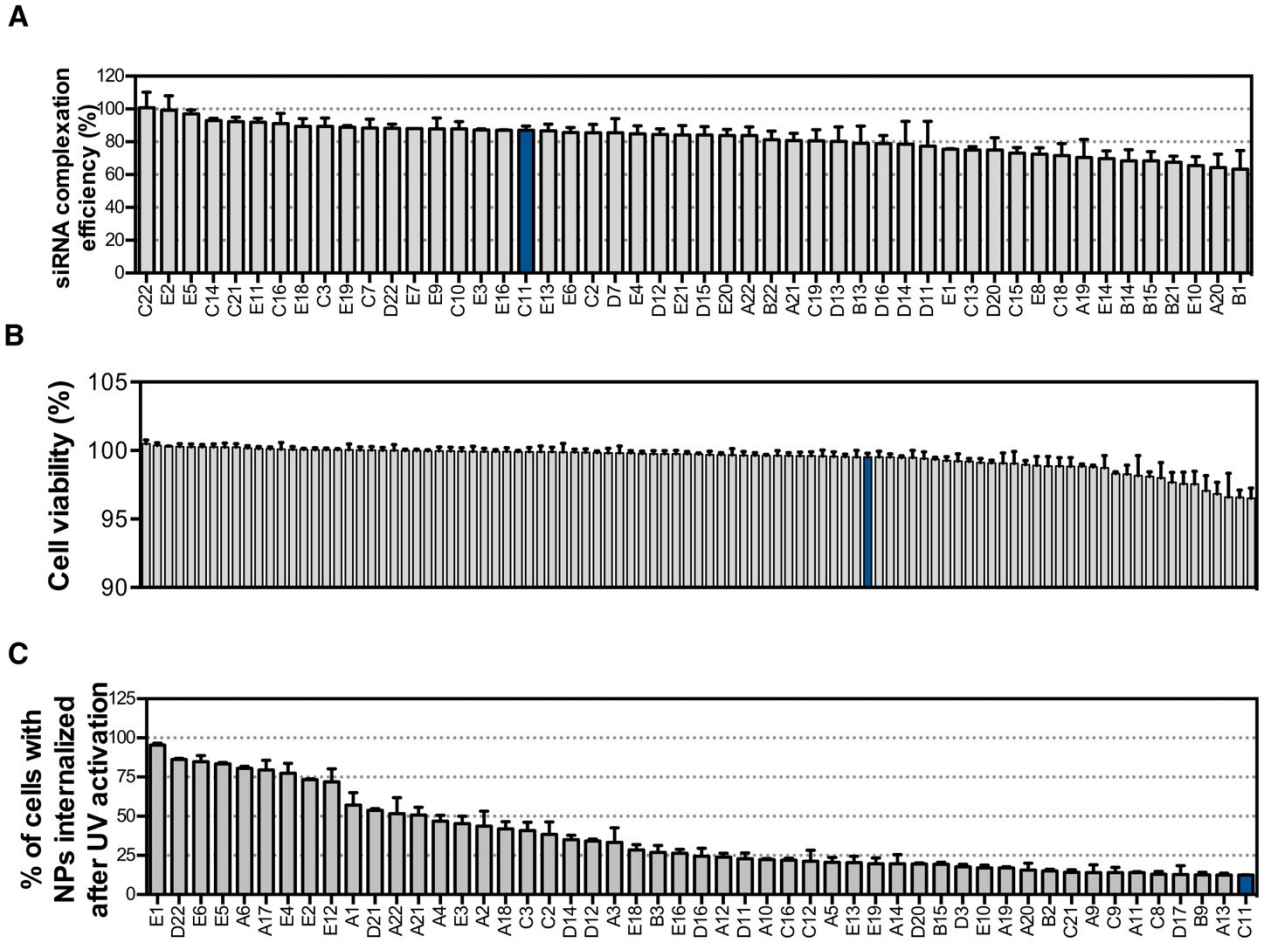


Figure S5. Complexation capacity of the NPs for siRNA as well as cytotoxicity and cellular internalisation of NP@siRNA complexes. NPs complexed with siRNA (siRNA:NP ratio = 1:50, w/w) were used for toxicity and cell internalisation studies (in HeLa cells). The concentration of NPs@siRNA-Cy5 was 20 µg/mL and the transfection time of 10 min. (B) Efficiency of siRNA complexation in the top 50 formulations. (B) Cell viability at 48 h post transfection without UV irradiation. Cell nuclei were stained with Hoechst H33342 and dead cells with propidium iodide. Cell viability was calculated as the % of dead nuclei from the total count of nuclei. (C) Percentage of cells stained for NPs@siRNA-Cy5 (siRNA:NP ratio = 1:50, w/w) at 48 h post-transfection. The graph shows the top 50 NP formulations with high cell internalization. In A, B and C, results are Mean ± SEM (n=3).

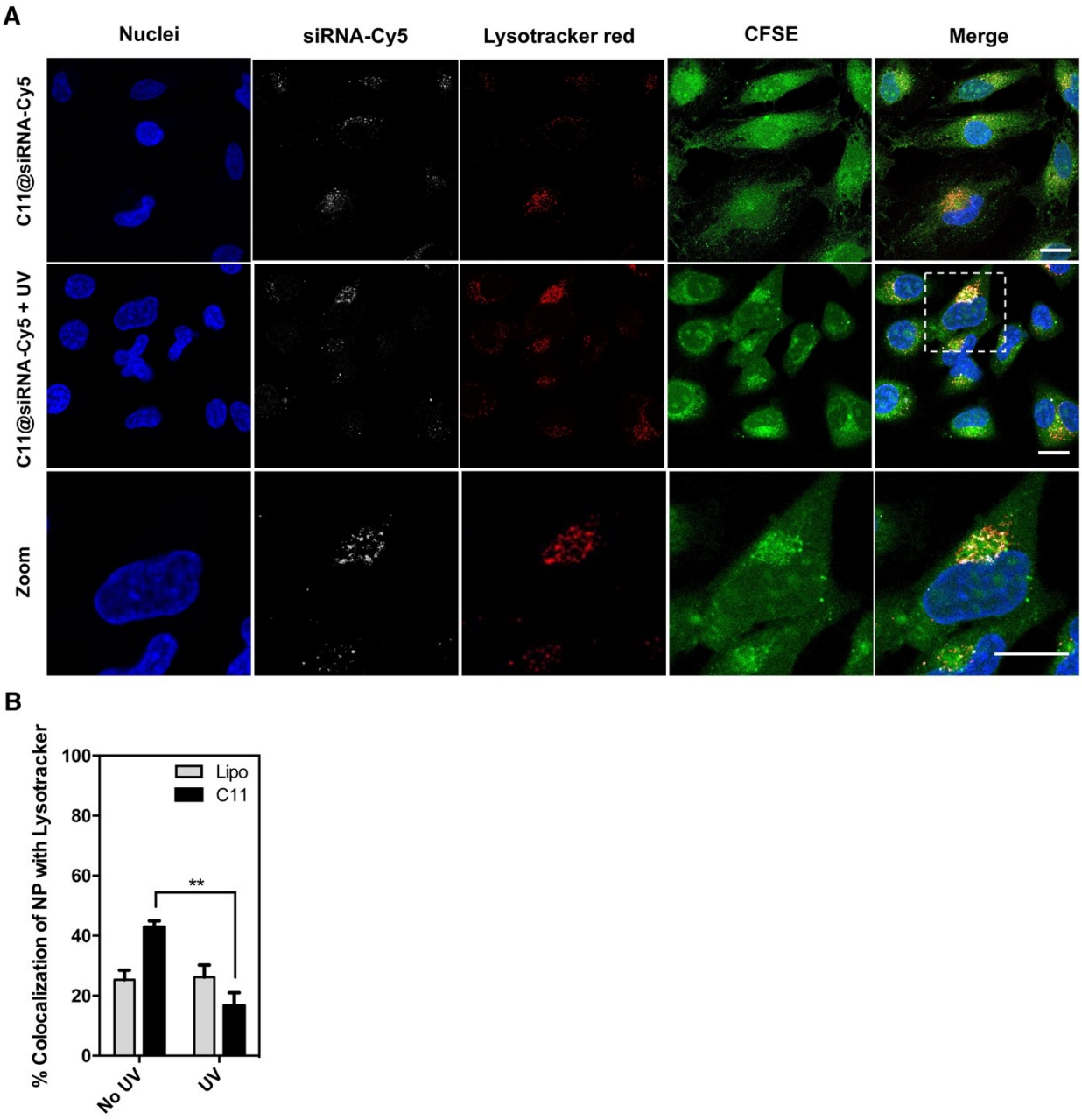
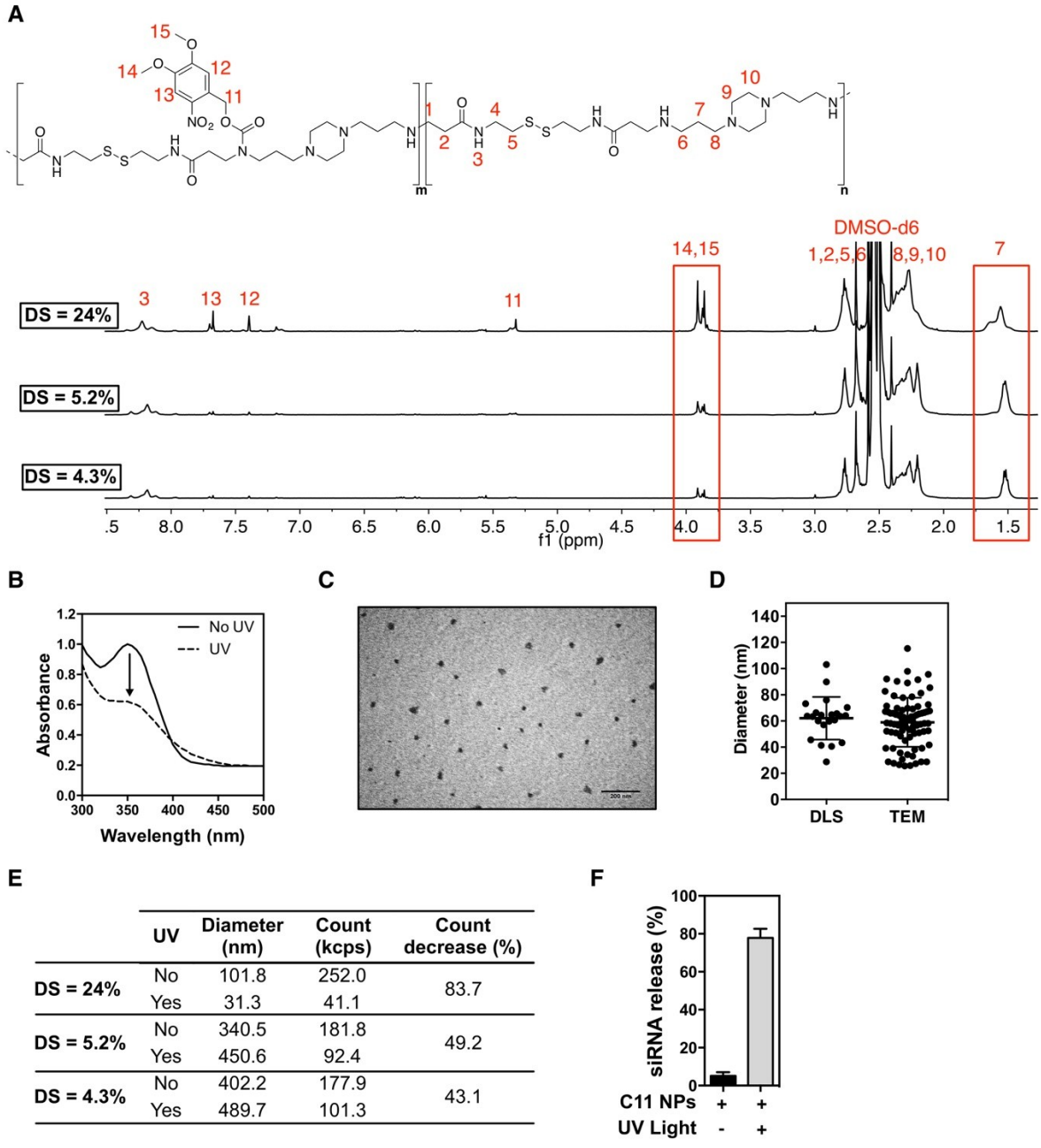


Figure S6. C11@siRNACy5 colocalization with LysoTracker Red. HeLa cells were transfected with C11@siRNA-Cy5 (20 µg/ml) or Lipo@siRNA-Cy5 for 10 minutes. Cells were then washed and NPs were activated with UV light (10 min, 1 mW/cm^2). The cells were stained with CFSE for cell membrane, LysoTracker red for endolysosome, H333342 for cell nuclei and analysed 1 h after transfection by confocal microscopy. (A) Representative confocal microscopy images showing the colocalization of C11@siRNA-Cy5 formulation irradiated or not with UV light with Lysotracker Red. White scale bar is 20 µm. (B) Colocalization of Lysotracker Red with siRNA-Cy5 expressed as the Manders' overlap coefficient quantified using JACoP on ImageJ. Results are resented as Mean ± SEM. Statistical analysis was assessed by unpaired student t-test with Welch's correction. ** P <0.01.



	UV	Diameter (nm)	Count (kcps)	Count decrease (%)
DS = 24%	No	101.8	252.0	83.7
	Yes	31.3	41.1	
DS = 5.2%	No	340.5	181.8	49.2
	Yes	450.6	92.4	
DS = 4.3%	No	402.2	177.9	43.1
	Yes	489.7	101.3	

Figure S7. Characterization of C11 polymer and NPs. (A) NMR spectra (in DMSO-d6) of C11 polymers with different NVOC:diamine molar ratios. The results show a DS_{exp} of 24.0 %, 5.2% and 4.3% (DS_{theo} = 25%, 12.5% and 8.3%, respectively). (B) Effect of UV light (10 min, 365 nm, 100 mW/cm^2) in the absorbance of the polymer C11 (DS_{exp} = 24%). The decrease in absorption at 350 nm (NVOC) and the increase at 420 nm (nitroso product) indicate the photo-cleavage of NVOC. (C) Representative image of NPs obtained by TEM (DS_{exp} = 24%). Bar corresponds to 200 nm. (D) Distribution of NP diameters as evaluated by TEM and DLS analyses. For DLS analyses, a suspension of NPs at a concentration of 50 µg/mL was used. For TEM analyses, a NP suspension at a concentration of 500 µg/mL was applied on carbon coated 200 mesh copper grids, left to air dry and analyzed (FEI-Tecnai Spirit BioTwinG2). The images were acquired and analysed on ImageJ. Results are Mean ± SEM (n = 2-5, up to 5 images per replicate). (E) Disassembly of C11 NPs with different ratios of NVOC after UV irradiation. The formulations (50 µg/mL) were irradiated with UV light (365 nm, 10 min, 100 mW/cm^2) for 10 min and analysed by DLS. (F) siRNA-Cy5 release from C11 NPs (50 µg/mL) after UV light irradiation (10 min, 1 mW/cm^2). siRNA-Cy5 was determined in the supernatant relative to a standard curve. Results are presented as Mean ± SEM (n = 3).

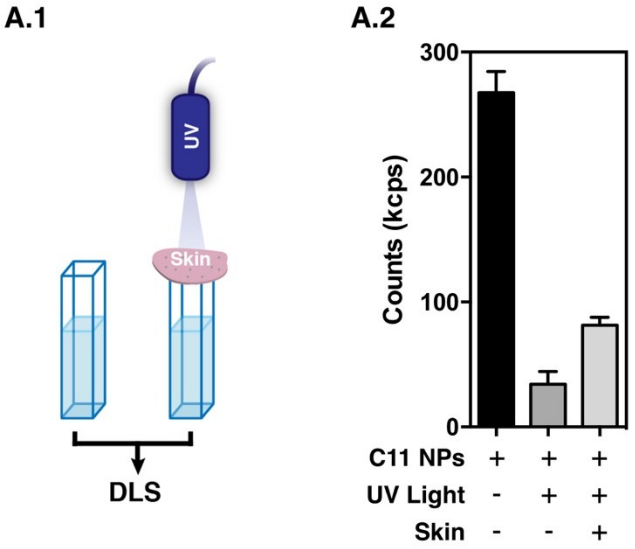


Figure S8. Photo-disassembly of C11@siRNA NPs. (A.1) Schematic representation of the methodology used to evaluate the photo-disassembly of C11@siRNA NPs beneath a mouse skin biopsy. A skin fragment (diameter ~1.7 cm placed on a cardboard with a 1 cm^2 hole; thickness of 200-230 µm as measured by a caliper) was placed above a cuvette containing a suspension of C11@siRNA (50 µg/mL, 2 mL). The cuvette was irradiated with UV light at 1 mW/cm^2 during 10 min and then analysed by DLS to determine NP count (A.2). In A.2, results are presented as Mean ± SEM (n = 3).

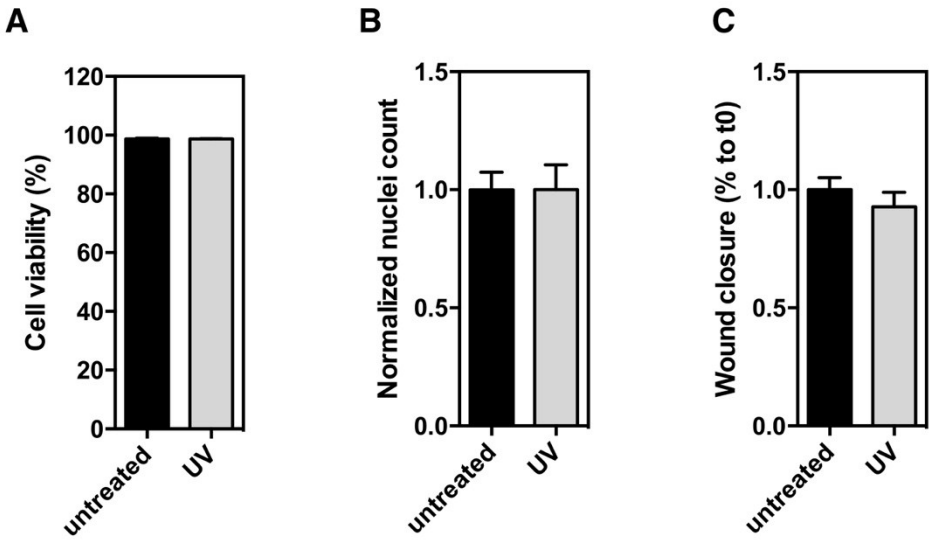


Figure S9. Influence of UV light on cell viability and activity. Cells were irradiated or not with UV light (10 min, 1 mW/cm^2) and analysed at 48 h. (A) Cell viability of HeLa-GFP cells. Cell nuclei were stained with Hoechst H33342 and propidium iodide at 48 h, and cell viability calculated as the % of dead nuclei from the total count of nuclei. (B) Endothelial cell proliferation. Nuclei were stained with H33342 and analysed by high content imaging at time 48 h. The number of cells at time 48 h was normalized by the one at time 0. (C) Endothelial cell migration. Wound area was quantified in ImageJ and normalised to the initial wound area. All results are presented as Mean ± SEM (n = 3).

Supplementary Table 1. Detailed description of name, CAS, Vendor, structure of library monomers

Monomer	Chemical Name IUPAC	CAS	Vendor
A	Methylenebisacrylamide	110-26-9	Aldrich
B	Hexamethylenebisacrylamide	16069-15-1	Aldrich
C	Cystaminebisacrylamide	6098-457-8	Polyscience
D	Dihydroxyethylenebisacrylamide	868-63-3	Aldrich
E	Bisacryloylpiperazin	6342-17-2	Sigma
1	Ethylenediamine	107-15-3	Merck
2	1,4-Diaminobutan	110-60-1	Aldrich
3	1,6-Diaminohexan	124-09-4	Alfa Aesar
4	Diethylenetriamine	111-40-0	Alfa Aesar
5	Triethylenetetraamine	112-24-3	Acros Organics
6	Pentaethylenehexamine	4067-16-7	Aldrich
7	3,3'-Diamino-N-methyldipropylamine	105-83-9	Aldrich
8	1,2-Diaminocyclohexane	694-83-7	Aldrich
9	1,8-Diamino-3,6-dioxaoctane	929-59-9	Acros Organics
10	1,13-Diamino-4,7,10-trioxatridecane	4246-51-9	Aldrich
11	1,4-Bis(aminopropyl)piperazine	7209-38-3	Aldrich
12	1,4-Phenylenedimethanamine	539-48-0	Merck
13	1,5-Diaminonaphthalene	2243-62-1	Aldrich
14	4,4'-methylenedianiline	101-77-9	Aldrich
15	1,3-Phenylenediamine	108-45-2	TCI Chemicals
16	1,3-Diaminopropane	109-76-2	TCI Chemicals
17	2,2-Dimethyl-1,3-propanediamine	7328-91-8	TCI Chemicals
18	1,3-Diaminopentane	589-37-7	TCI Chemicals
19	2,2'-Diamino-N-methyldiethylamine	4097-88-5	TCI Chemicals
20	Agmatine sulfate	2482-00-0	TCI Chemicals
21	1,4-Bis(aminomethyl)cyclohexane	2579-20-6	TCI Chemicals
22	4,4'-Methylenebis(cyclohexylamine)	1761-71-3	Aldrich

Supplementary Table 2. Sequence of primers used in qRT-PCR experiments.

Gene		Sequence	species
m-PHD2	FW	CCACTGGCACTCAACTAACTCA	mouse
	RW	CCGAGTTCATTTAGTGCCCGTCA	
m-GAPDH	FW	AAGGTCATCCCAGAGCTGAA	mouse
	RW	CTGCTTCACCACCTTCTTGA	