SUPPORTING INFORMATION

Supplementary methods

Synthesis of fluorinated short hairping (sh) RNAs

shRNA C/EBPβ and scramble shRNA (control) were synthetized *in vitro* via PCR and T7 RNA polymerase. PCR was performed in a 50 µL final reaction volume containing 1x PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 U Taq DNA polymerase (Invitrogen) and the following ssDNA primers (Eurofins MWG Operon) at 20 µM: ScrambleF 5'-TAATACGACTCACTATAAGGGCAGTAGCATGGTCCGTTGAGATTCAAGAGATCT-3'; ScrambleR 5'-AAGGCAGTAGCATGGTCCGTTGAGATCTCTTGAATC-3'; C/EBPβF 5'-TAATACGACTCACTATAGGGCGGCGACTTCCTCTCCGACCTCTTCTTCAAGAGAGAAGAGG-3'; and C/EBPβR 5'-AAAAACGACTTCCTCTCCGACCTCTTCTCTCTTGAAGAAG-3'. A single PCR cycle was run with the following protocol: 95 °C 10 min, 60 °C 1 min, 72 °C 20 min. The resulting dsDNA was purified using the Wizard® SV Gel and PCR Clean-up system (Promega) according to manufacturer's instructions.

One µg of dsDNA was used as template to transcribe shRNA molecules in a single *in vitro* transcription (IVT) reaction, using the DuraScribe® T7 transcription kit (Epicentre). IVT reaction was run overnight at 37 °C in a temperature-controlled oven. DNA template was removed by DNase I digestion and shRNAs were purified using the miRNeasy Micro Kit (Qiagen). Purified shRNAs were quantified by spectrophotometer and correct molecule length was checked by denaturing gel electrophoresis (polyacrylamide 15%, urea 7M).

Lyophilization of nanocapsules

Nanocapsules were frozen overnight at -20 °C and lyophilized in a VirTis Genesis 25L equipment (SP Industries) for their storage. Initial freezing temperature was set at -30 °C and samples were equilibrated at -40 °C before a 46 h drying process, divided in two steps. In the first drying step, temperature was slowly increased in 10°C intervals until reaching 20 °C, when a 3 h secondary drying started. Samples were then reconstituted to the initial concentration with ultrapure water by vortexing until complete resuspension, when physicochemical properties and morphology were assessed as described in the experimental section. The integrity of RNA after this process was also evaluated by gel retardation assays.

Nanocapsules uptake in RAW 264.7 macrophages

For cell uptake studies, RAW 264.7 macrophages were seeded in an 8 Chambered Borosilicate coverglass system (Lab-Tek) (0.9 x 10^5 cells/chamber) and left overnight. A dose of 1.25 µg of both free and encapsulated 5'-FAM labelled miRNA 142-3p was added to the cells diluted in 100 µL supplemented DMEM and incubated for 2 h at 37 °C. Then, media was aspirated and cells were washed with PBS. Subsequently, cells were fixed with a 4% paraformaldehyde solution and stained with DAPI and phalloidin tetramethylrhodamine B isothiocyanate (Sigma) before observation by confocal microscopy (Leica TCS SP5).

Matrigel® invasion assay

T cell invasion in response to CCL2 was analyzed by a Matrigel® invasion assay. Jurkat cells were grown in modified RPMI-1640 medium containing 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4500 mg/L glucose, and 1500 mg/L sodium bicarbonate and supplemented with 10% v/v fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin (Life Technologies). Cells were maintained at 37 °C in a humidified incubator supplied with 5% CO_2, at a density of 0.1-1 x 10^6 cells/mL. Matrigels (10 mg/mL) were prepared in 8-well chambered coverslips (Ibidi) containing CCL2 (Peprotech) (CCL2 matrigels) or medium alone (Blank matrigels). For this, an ice-cold Matrigel® stock (11.6 mg/mL) was carefully mixed with the CCL2 OptiMEM solution (60 ng) or OptiMEM alone in a final volume of 100 µL. Ice-cold mixtures were pipetted on each well creating a thin layer of material and allowed to gel in a humidified incubator at 37 °C for 30 min. Then, 300 µL of Jurkat cells (0.15 x 10^6 cells) suspended in migration buffer (DMEM supplemented with 1% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin) were added on top of each gel. After 48 h of incubation at 37 °C in a 5% CO_2 atmosphere, gels were washed carefully with PBS. Invasive cells were then fixed in paraformaldehyde and stained with DAPI before observation by confocal microscopy (Leica TCS SP5).

Supporting figures

Table S1. Physicochemical characterization of loaded nanosystems indicating: mean particle size, polydispersity index and zeta potential. Nanoemulsions were loaded with 50 µg/ml OVA. Nanocapsules (24.7 mg/mL) were loaded with 0.4 mg/mL RNA, 1 µg/mL CCL2 and 25 µg/mL DiD. Values are means ± S.D., n=6 (OVA, RNA) and n=3 (CCL2).

Systems	Loading	Size (nm)	Polidispersity Index	Zeta potential (mV)
Nanoemulsions	*OVA*	172 ± 6	0.1	- 13 ± 1
Nanocapsules	*miRNA control*	186 ± 27	0.1	- 25 ± 7
	shRNA control	174 ± 13	0.1	-
	CCL2 freeze-dried	160 ± 21	0.1	26 ± 6
	DiD	170 ± 11	0.1	34 ± 5

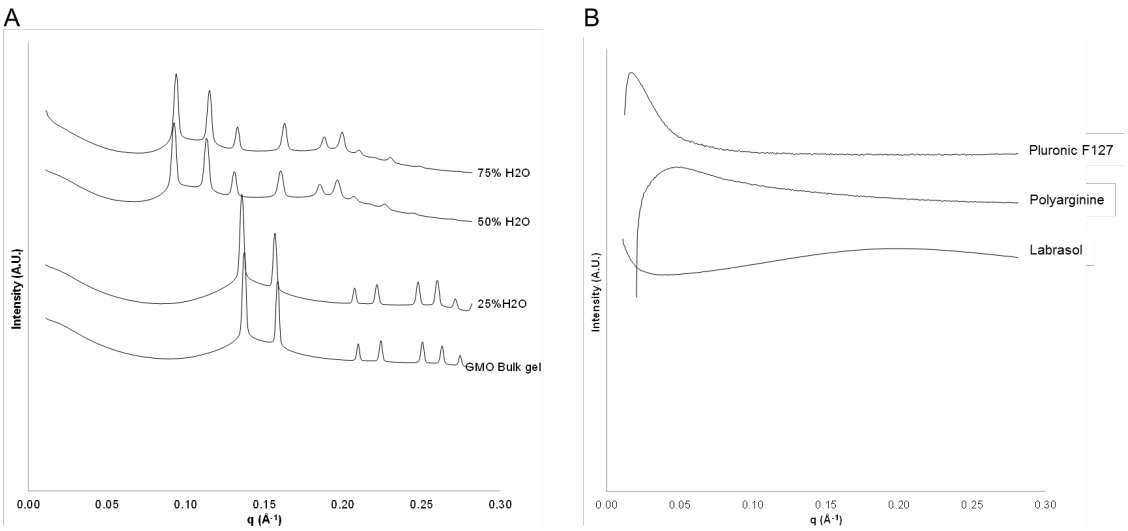

Fig. S1. SAXS characterization of the precursor materials. A) SAXS analysis of GMO precursor gel, showing the transition from a Ia3d to a Pn3m phase arrangement upon increasing hydration. B) SAXS profile of raw materials.

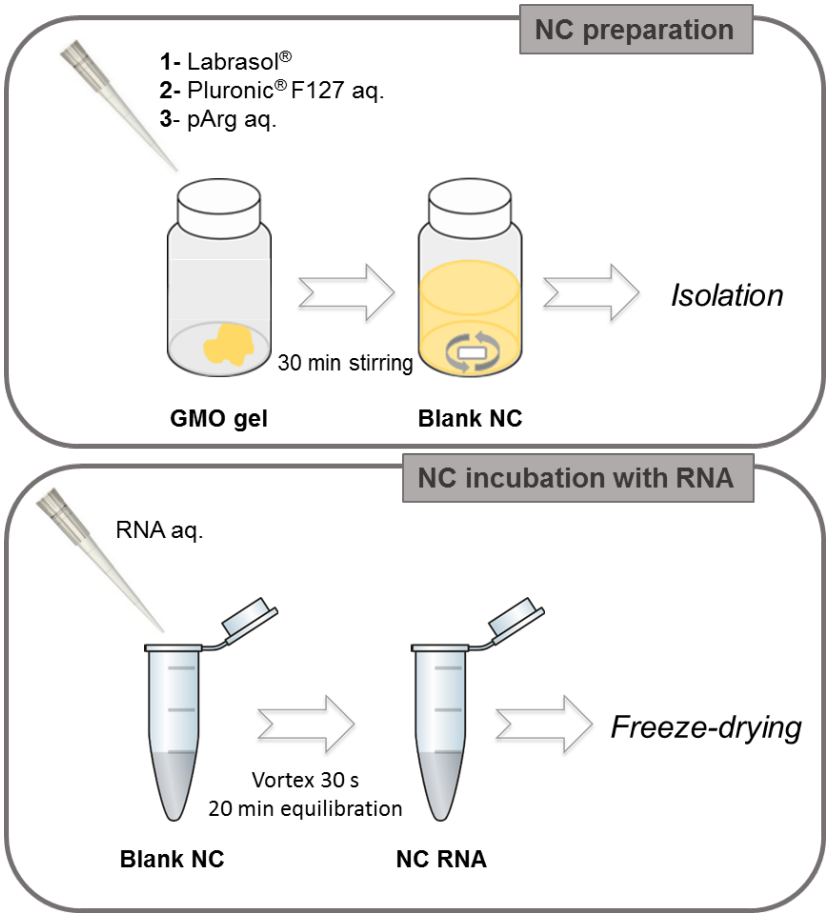

Fig. S2. Schematic representation of the method used for nanocapsules preparation. Objects in figure are not drawn to scale. pArg: polyarginine; aq.: aqueous; GMO: glyceryl monooleate; NC: nanocapsules.

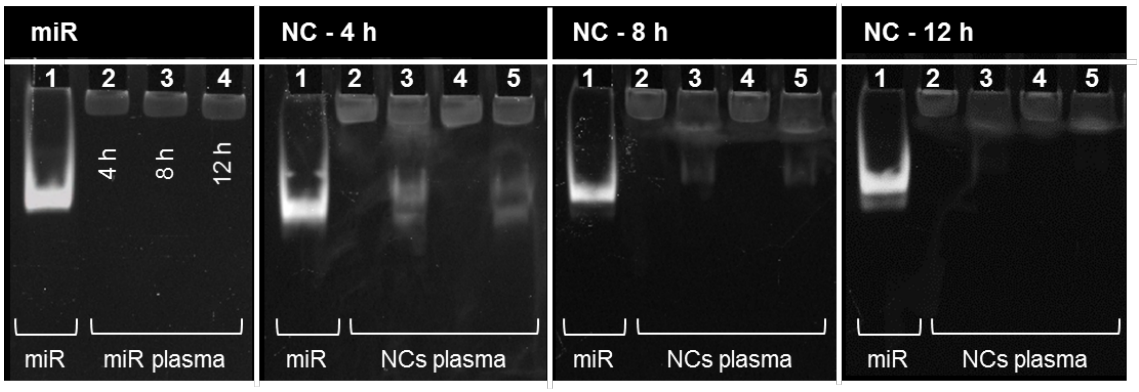

Fig. S3. miR 142-3p stability and release from NCs in human plasma after 4, 8 and 12 h incubation. NCs were diluted 1/10 in plasma and at the desired time points samples were loaded in a 15% polyacrylamide gel with a theoretical miR amount of 400 ng per well. Lanes miR sequences: (1) miR 142-3p in solution; (2), (3) and (4) miR 142-3p incubated 4, 8 and 12 h in plasma, respectively. Lanes 4 h assay: (1) miR 142-3p in solution; (2) and (4) NCs miR 142-3p incubated 4 h in plasma; (3) and (5) NCs miR 142-3p incubated with heparin. Lanes 8 h assay: (1) miR 142-3p in solution; (2) and (4) NCs miR 142-3p incubated 8 h in plasma; (3) and (5) NCs miR 142-3p incubated with heparin. Lanes 12 h assay: (1) miR 142-3p in solution; (2) and (4) NCs miR 142-3p incubated 12 h in plasma; (3) and (5) NCs miR 142-3p incubated with heparin.

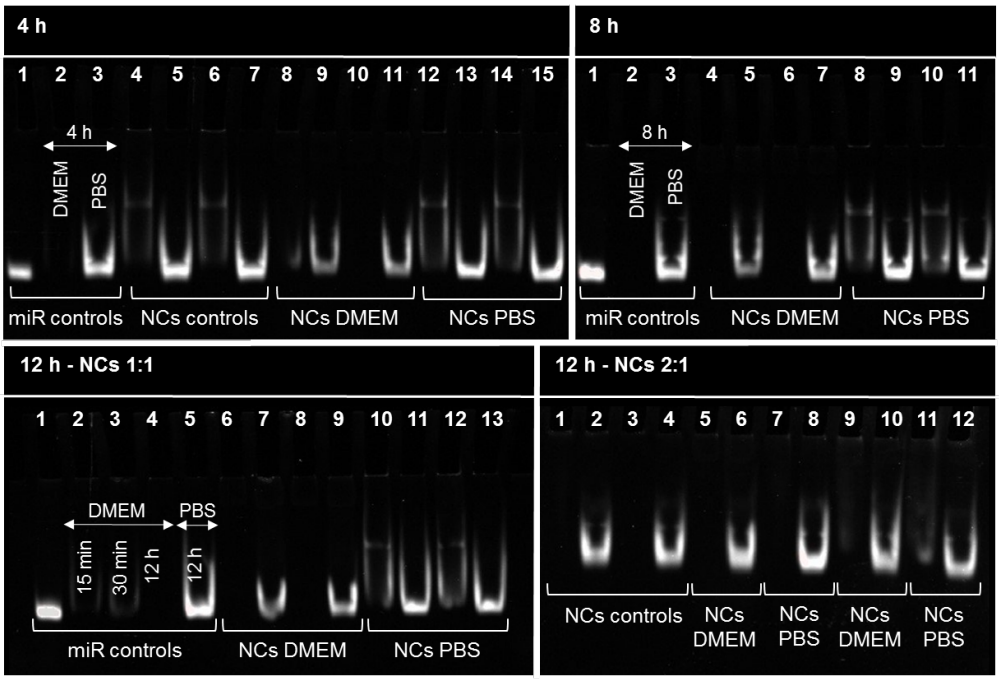

Fig. S4. miR release assay in supplemented DMEM (10% FBS + 1% Pen/Strep) and PBS pH 7.2 after 4, 8 and 12 h incubation. NCs were diluted 1/10 in the correspondent media and at the desired time points samples were loaded in a 15% polyacrylamide gel with a theoretical miR amount of 800 ng per well. Lanes 4 h assay: (1) miR 142-3p in solution; (2) and (3) miR sequence in DMEM and PBS after 4 h incubation, respectively; (4) and (6) fresh NCs; (5) and (7) fresh NCs incubated with heparin; (8) and (10) NCs incubated 4 h in DMEM; (9) and (11) NCs DMEM incubated with heparin; (12) and (14) NCs incubated 4 h in PBS; (13) and (15) NCs PBS incubated with heparin. Lanes 8 h assay: (1) miR 142-3p in solution; (2) and (3) miR sequence in DMEM and PBS after 8 h incubation, respectively; (4) and (6) NCs incubated 8 h in DMEM; (5) and (7) NCs DMEM incubated with heparin; (8) and (10) NCs incubated 8 h in PBS; (9) and (11) NCs PBS incubated with heparin. Lanes 12 h assay polyarginine:miR ratio 1:1: (1) miR 142-3p in solution;

(2) and (3) miR in DMEM after 15 and 30 min incubation, respectively; (4) and (5) miR in DMEM and PBS after 12 h incubation, respectively; (6) and (8) NCs incubated 12 h in DMEM; (7) and (9) NCs DMEM incubated with heparin; (10) and (12) NCs incubated 12 h in PBS; (11) and (13) NCs PBS incubated with heparin. Lanes 12 h assay ratio 2:1: (1) and (3) NCs miR; (2) and (4) NCs incubated with heparin; (5) and (9) NCs incubated 12 h in DMEM; (6) and (10) NCs DMEM incubated with heparin; (7) and (11) NCs incubated 12 h in PBS; (8) and (12) NCs PBS incubated with heparin.

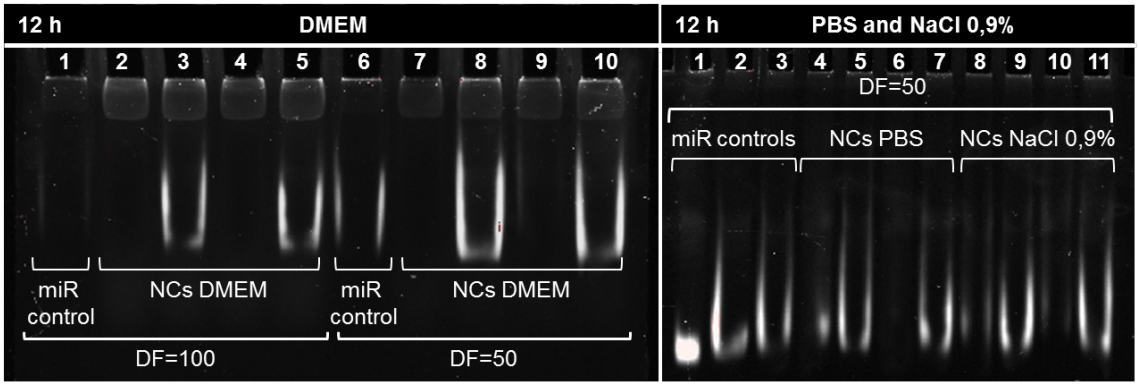

Fig. S5. miR release assay in supplemented DMEM (10% FBS + 1% Pen/Strep), PBS pH 7.2 and saline solution after 12 h incubation. NCs (ratio 1:1) were diluted 1/100 and 1/50 in DMEM and 1/50 in PBS and saline solution. After 12 h incubation, samples were loaded in a 15% polyacrylamide gel with a theoretical miR amount of 400 ng per well. Lanes DMEM assay: (1) miR 142-3p sequence incubated 12 h in DMEM with a 1/100 dilution; (2) and (4) NCs incubated 12 h in DMEM with a 1/100 dilution; (3) and (5) NCs DMEM 1/100 incubated with heparin; (6) miR 142-3p sequence incubated 12 h in DMEM with a 1/50 dilution; (7) and (9) NCs incubated 12 h in DMEM with a 1/50 dilution; (8) and (10) NCs DMEM 1/50 incubated with heparin. Lanes PBS and saline assay: (1) miR 142-3p sequence in solution; (2) and (3) miR 142-3p sequence incubated 12 h in PBS and saline with a 1/50 dilution, respectively; (4) and (6) NCs incubated 12 h in PBS with a 1/50 dilution; (5) and (7) NCs PBS 1/50 incubated with heparin; (8) and (10) NCs incubated 12 h in saline solution with a 1/50 dilution; (9) and (11) NCs saline 1/50 incubated with heparin.

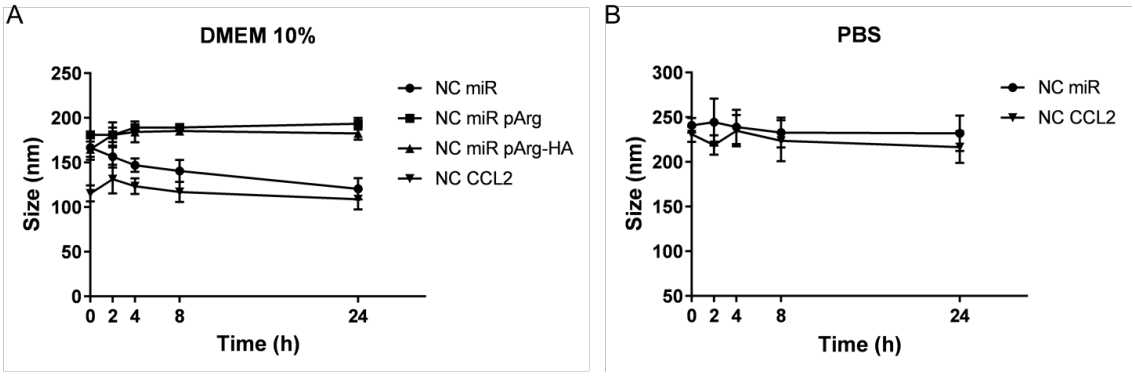

Fig. S6. Colloidal stability of polyarginine NCs in supplemented DMEM (10% FBS + 1% Pen/Strep) and PBS pH 7.2.

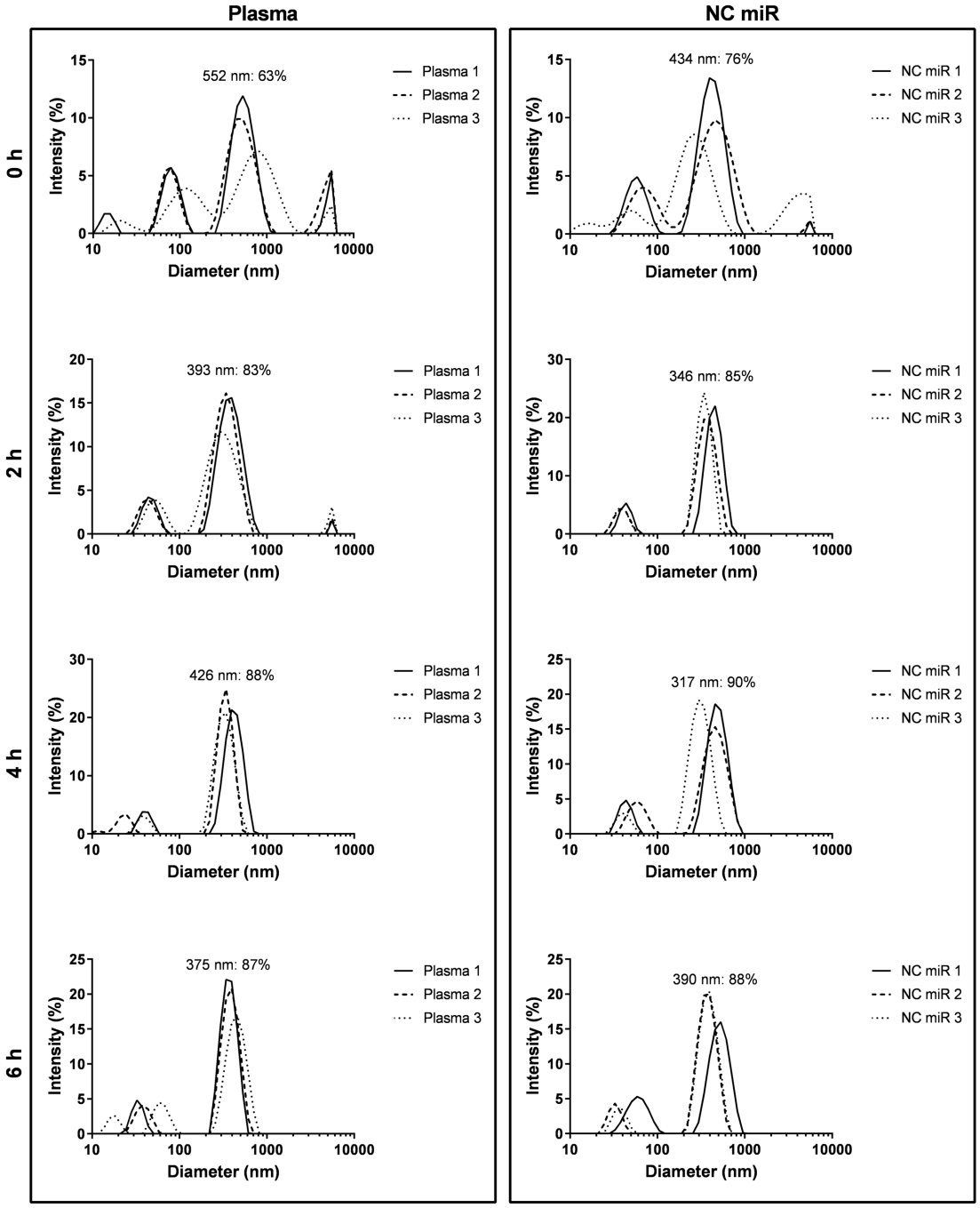

Fig. S7. Colloidal stability of miR-loaded polyarginine NCs after 0, 2, 4 and 6 h incubation in human plasma at 1/10 dilution. Data show the frequency distributions of sizes as assessed by DLS. Size values and percentages of the most abundant population are highlighted. Plasma samples were treated in the same way and included as controls.

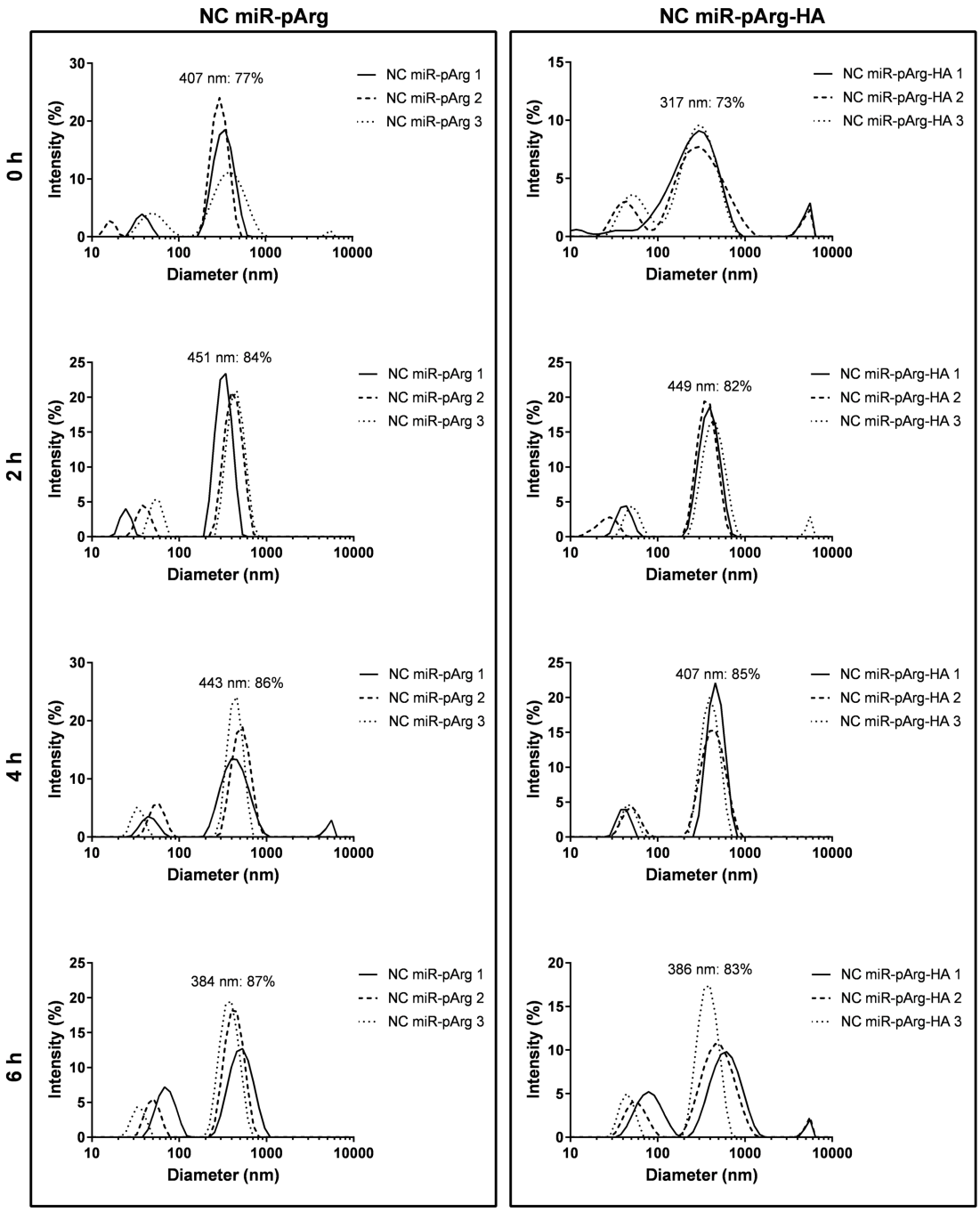

Fig. S8. Colloidal stability of polymer coated miR-loaded polyarginine NCs after 0, 2, 4 and 6 h incubation in human plasma at 1/10 dilution. Data show the frequency distributions of sizes as assessed by DLS. Size values and percentages of the most abundant population are highlighted.

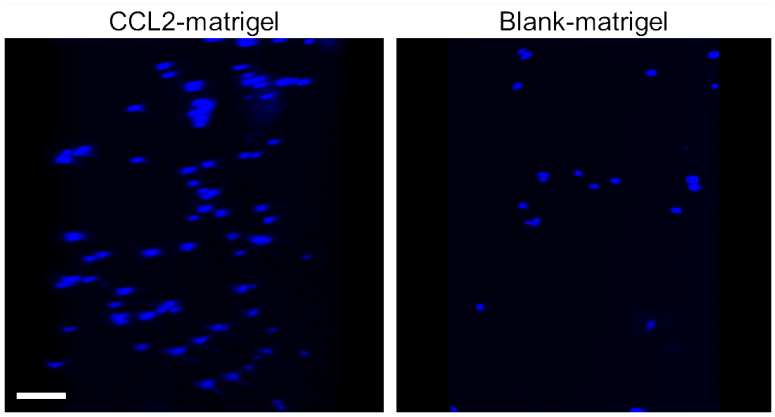

Fig. S9. Jurkat cells invade the tumor-mimicking Matrigel® system in response to CCL2. Fluorescence images of DAPI stained-cells associated to the matrigels after a 48 h invasion assay. Representative z-stacks for CCL2-loaded and blank-matrigels. Scale bar = 100 µm for all the images.

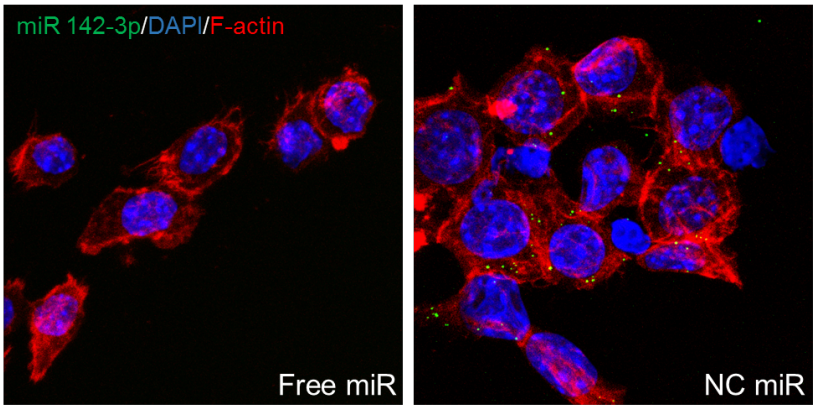

Fig. S10. Polyarginine NCs promote RNAi uptake in macrophages. Confocal laser microscopy images of RAW264.7 cells incubated with either fluorescently labelled free RNAi or with the same RNAi loaded in polyarginine NCs.

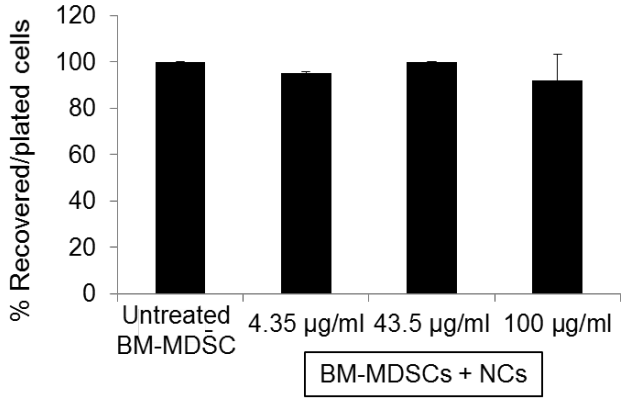

Fig. S11. Polyarginine blank NCs do not affect the viability of the BM-MDSCs culture. Cells were treated with different concentrations of blank NCs and cytotoxicity was assessed by calculating the percentage of viable recovered cells on plated cells. Values are means ± SD of two independent experiments.

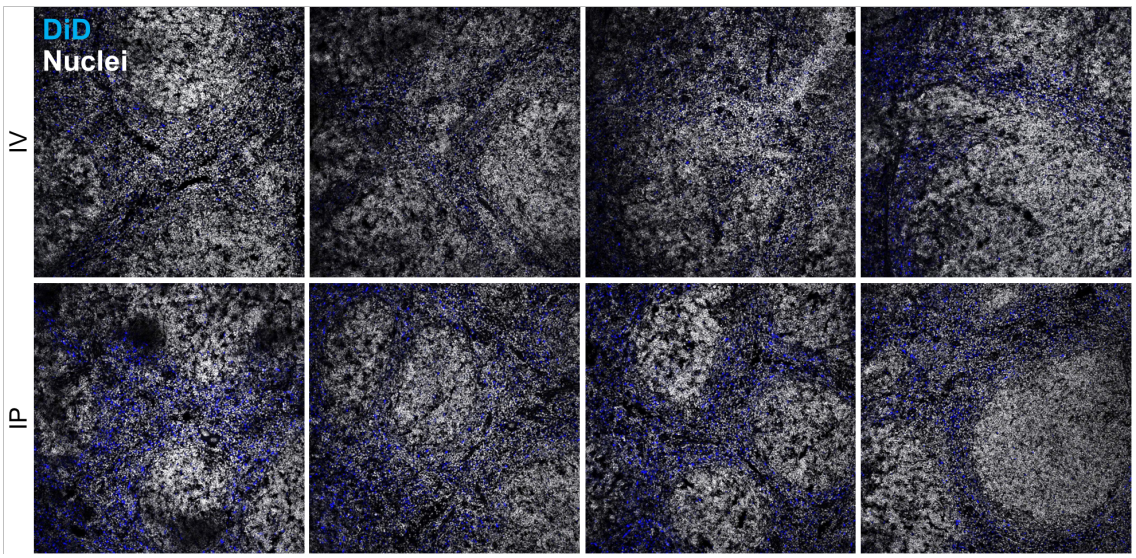

Fig. S12. Spleen biodistribution of DiD-labeled NCs in an EG7 lymphoma mouse model. Mice were sacrificed 12 h after the intravenous (IV) or intraperitoneal (IP) administration of DiD-loaded NCs (25 µg/mL DiD) and spleens were harvested for confocal imaging. Images correspond to individual mice shown at 20x magnification.

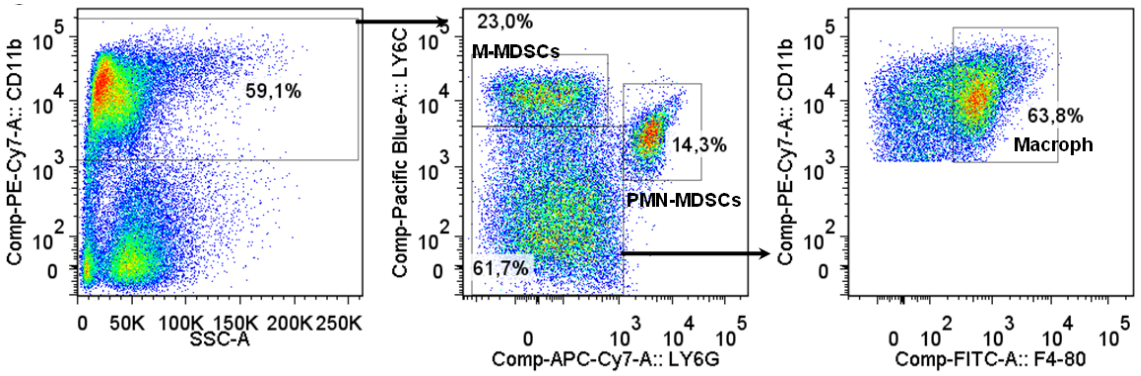

Fig. S13. Gating strategy for the identification of PMN-MDSCs ($CD11b^{+}Ly6G^{+}Ly6C^{int}$), M-MDSCs ($CD11b^{+}Ly6G^{-}Ly6C^{high}$) and macrophages ($CD11b^{+}Ly6G^{-}$ $Ly6C^{low/-}$ $F4/80^{+}$) in the tumor. All analysis were performed after selection of living single cells by morphologic gating, doublets exclusion and dead cell exclusion by LIVE/DEAD® dye staining.