Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information for:

Enhancement of nucleic acid delivery to hard-to-transfect human colorectal cancer cells by magnetofection at laminin coated substrates and promotion of the endosomal/lysosomal escape

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Fig.S1: TEM images of magnetic nanoparticles

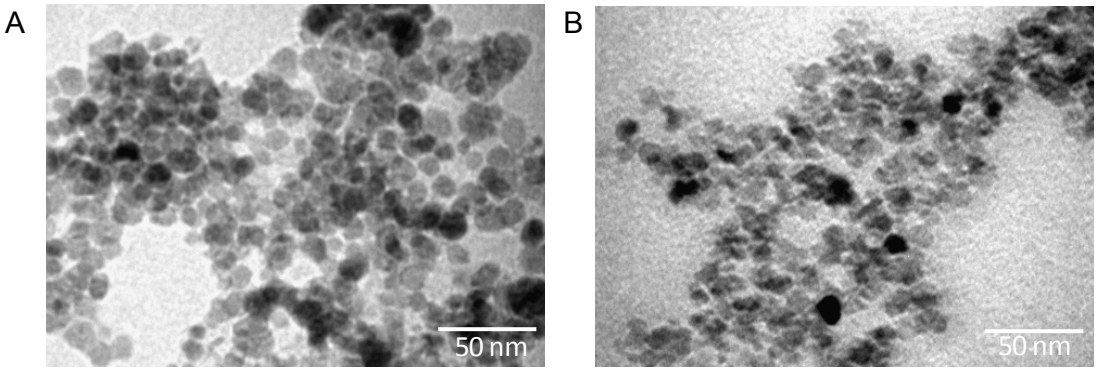

Fig. S1. TEM images of magnetic nanoparticles. TEM images of SOMag5 (A) and PEI-Mag2 (B) magnetic nanoparticles. (A) TEM image adapted from Ref. 1

Fig. S2: Enhancement of the luciferase down-regulation in HT29Luc by magnetic complex modified with INF-7 peptide.

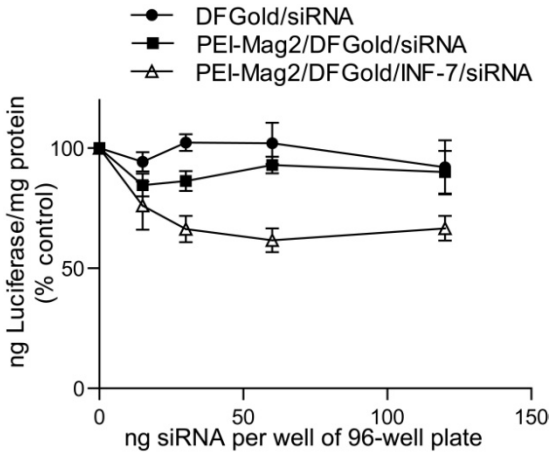

Fig.S2. Enhancement of the luciferase down-regulation in HT29Luc by magnetic complex modified with INF-7 peptide. The HT29Luc cells were transfected with lipoplex, magnetic complex and magnetic complex modified with INF-7 peptide (INF-7-to-siRNA ratio of 50:1 w/w). Luciferase expression was measured 72 h post-transfection and expressed as ng luciferase per mg total protein (%) normalized to the data for transfection with siRNA control complexes. The values represent the mean ± SD, n=3.

Abbreviations: siRNA, small interference RNA; DFGold, DreamFectGold reagent

Fig. S3: Luciferase expression in HT29Luc cells after lipofection and magnetofection of siRNA using different lipid reagents and SOMag5 or PEI-Mag2 nanoparticles.

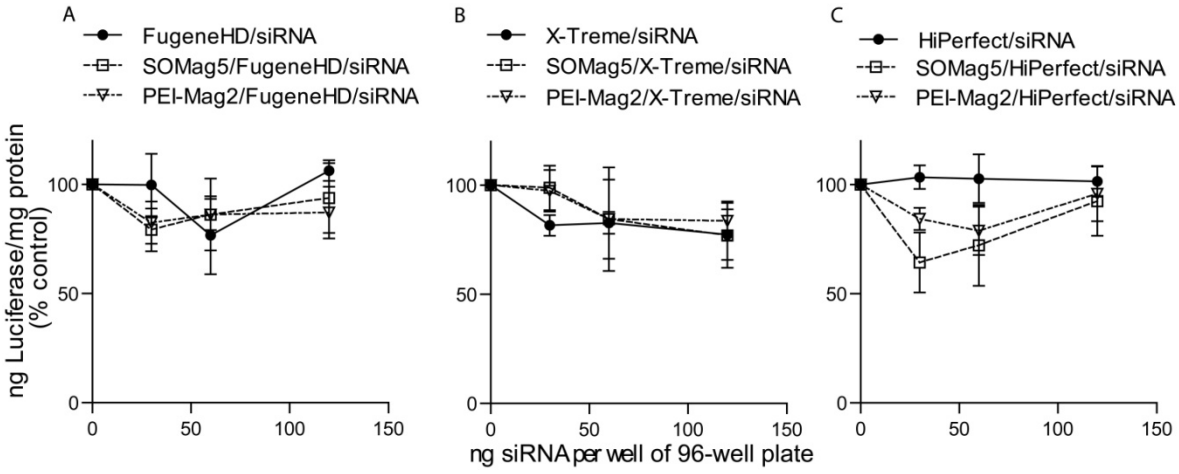

Fig. S3. Luciferase expression in HT29Luc cells after lipofection and magnetofection of siRNA using different lipid reagents and SOMag5 or PEI-Mag2 nanoparticles. HT29Luc were transfected using siRNA lipoplexes with FugeneHD (A), X-Treme (B) or HiPerfect (C), or with magnetic lipoplexes comprising SOMag5 or PEI-Mag2 MNPs (at an iron-to-siRNA ratio of 0.5:1). Luciferase expression was measured 72 h after transfection and the data were calculated as ng luciferase per mg total protein (%) normalized to the data for transfection with siRNA control complexes. The values represent the mean ± SD, n=3.

Abbreviations: siRNA, small interference RNA; DFGold, DreamFect Gold reagent; X-Treme, X-TremesiRNA Reagent; MNPs, magnetic nanoparticles.

Fig. S4: Stability of DNA magnetic complexes

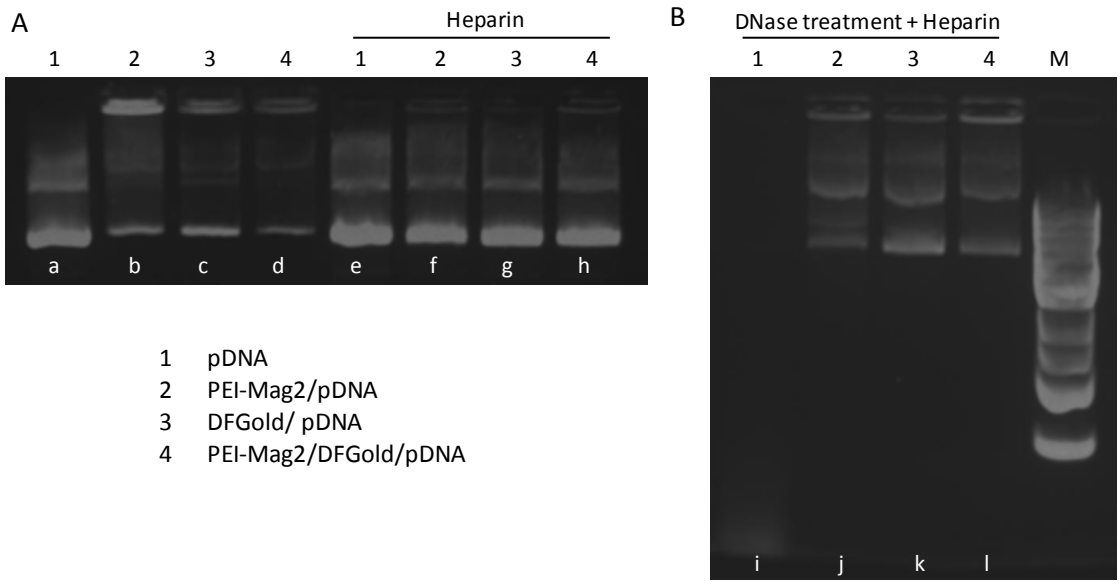

Fig. S4. Stability of DNA magnetic complexes. (A) Gel retardation assay of lipoplex and magnetic complexes (lane b-d) and heparin displacement assay (lane e-h). (B) DNase protection assay (lane i-l). Naked pDNA (line a, e and i) was used as control. 200 ng of pDNA was used en each well. Lane M: 1 Kb DNA marker ladder.

Methods and Results of Fig. S4

The stability of nucleic acids in magnetic complexes was evaluated by an agarose gel retardation assay and the protection of nucleic acids was assessed by nuclease resistance assay. The following complexes were performed: lipoplex pDNA/DFGold (4 µl DFGold/µg pDNA), duplex magnetic complex PEI-Mag2/pDNA (Fe/pDNA w/w of 0.5:1) and ternary magnetic complex PEI-Mag2/DFGold/pDNA (4 µl DFGold/µg pDNA and Fe/pDNA w/w of 0.5:1). Electrophoresis was carried out on 1% agarose gel at 70 V for 40 min in TAE buffer solution (40 mM Tris–HCl, 1 % v/v acetic acid, and 1 mM EDTA). The retardation of the pDNA was visualized by staining with GelRed (Biodynamics, Buenos Aires, Argentina) and by imaging under a UV lamp (lane a-d), the pDNA was released from de complexes by incubation with heparin 0.4 % w/v (Sigma, Buenos Aires, Argentina) (lane e-h). To evaluate nuclease resistance, the complexes were incubated with DNase I (Invitrogen, Buenos Aires, Argentina) in a final concentration of 0.1U/µg DNA for 15 min at room temperature, followed by 10 min at 65°C to inactivate the DNase I. Thereafter the pDNA

was released from complexes by addition of heparin 0.4% w/v and analyzed by agarose gel electrophoresis (lane i-l). As shown in Fig. S4, free pDNA is completely degraded while pDNA in poliplex or magnetic complexes were protected from nuclease degradation.

Ref. 1 Conde AJ, Batalla M, Cerda B, Mykhaylyk O, Plank C, Podhajcer P, Cabaleiro JM, Madrid RE and Policastro L. Lab Chip. 2014; 14: 4506-12.