Supplementary information

Tripartite polyionic complex (PIC) micelles as non-viral vectors for mesenchymal stem cells siRNA transfection

Sophie Raisin[1], Marie Morille[1], Claire Bony[2], Danièle Noël[2], Jean-Marie Devoisselle[1], Emmanuel Belamie[1,3]

[1] Institut Charles Gerhardt Montpellier, UMR 5253 CNRS-ENSCM-UM2-UM1, Equipe Matériaux Avancés pour la Catalyse et la Santé, 8 rue de l'Ecole Normale, 34296 Montpellier Cedex 5, France

[2] INSERM U1183, Institut de Médecine Régénérative et Biothérapies, Centre Hospitalier Universitaire (CHU) Saint Eloi, 80 Rue Augustin Fliche, 34295 Montpellier Cedex 5, France

[3] Ecole Pratique des Hautes Etudes, PSL Research University, 75014 Paris, France

Correspondence should be addressed to E.B. (emmanuel.belamie@enscm.fr, +33 4 67 16 34 44)

S1 : Characterization of bone marrow-derived primary murine mesenchymal stem cells

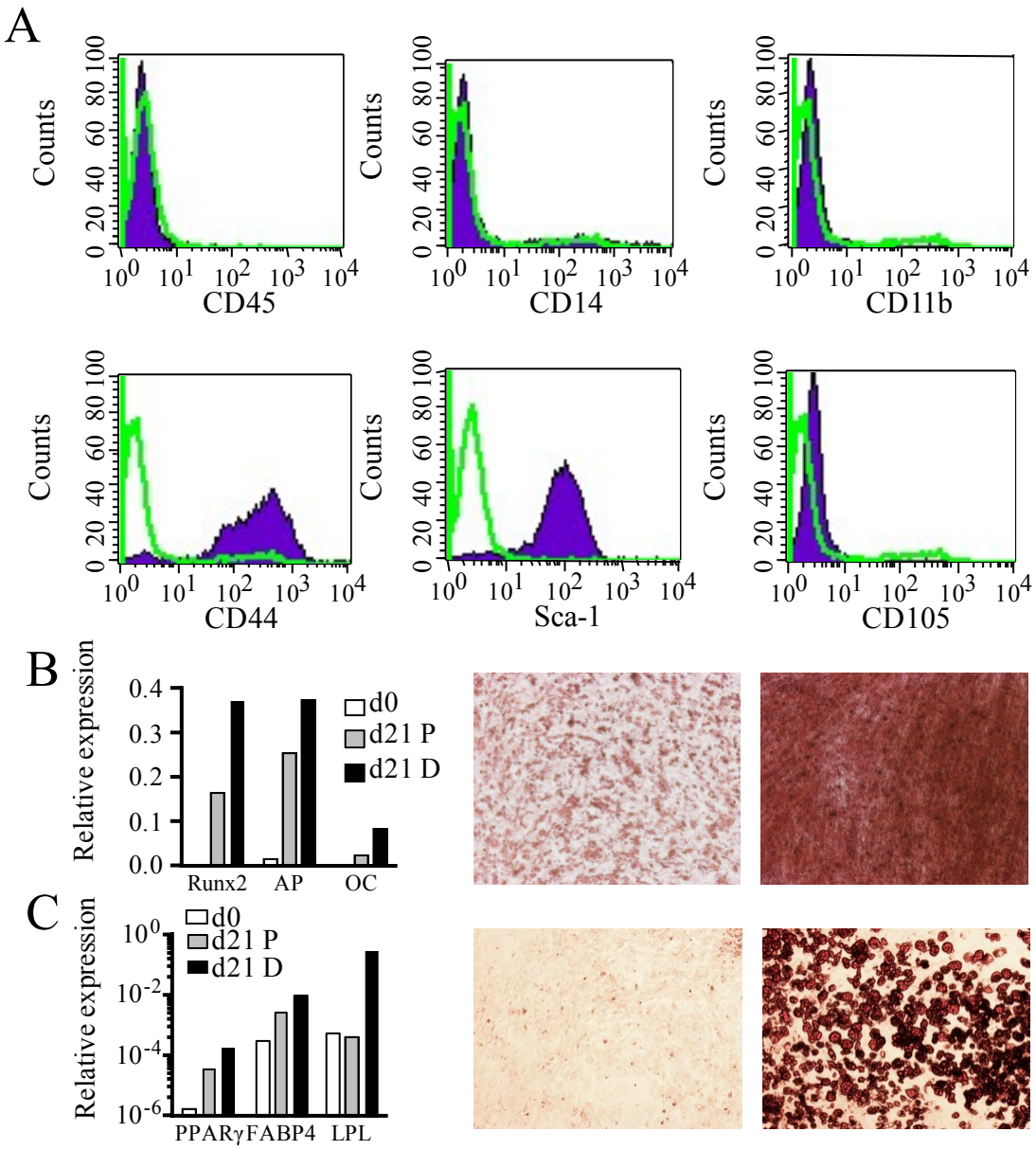

Figure S1. Characterization of bone marrow-derived primary murine mesenchymal stem cells expressing GFP Cells were characterized by their phenotype (expression of CD44, Sca-1 and lack of hematopoietic markers CD11b, CD14, CD45, see Fig S1A) as previously described (Luz-Crawford, Stem Cells, 2016, 34:2, 456-469). Their ability to differentiate into osteoblasts expressing Runx2, AP and OC (Fig S1B) and adipocytes expressing PPARγ, LPL, FABP4 (Fig S1C) was tested as already reported (Luz-Crawford, Stem Cells, 2016, 34:2, 456-469).

S2 – Release of SiRNA from M2 micelles at acidic pH studied by spectrofluorimetry

Protocol: The release of siRNA from micelles was evaluated by spectrofluorimetry with the Quant-iT™ RiboGreen® RNA assay. Shortly, calibration curves were established at pH 7 and pH 5. The standard solution (100 µg/mL) was diluted with sterile MilliQ water adjusted to the desired pH with NaOH 0.1 M or HCl 0.05 M, to a concentration of 2 µg/mL. RNA concentrations for the calibration curves ranged between 20 and 1000 ng/ml. One mL of Quant-iT™ RiboGreen® diluted 200-fold was added to 1 mL of sample RNA solution placed in a 10 mm-wide quartz cuvette. The fluorescence was measured on a RF-5301PC (Shimadzu) spectrofluorimeter (excitation max : 480 nm, emission max : 520 nm). The titration of RNA with micelles (M2) loaded with 1.5 µM RNA was performed following the same procedure at the two pH.

All manipulations were performed with RNAse-free disposable plastic-ware.

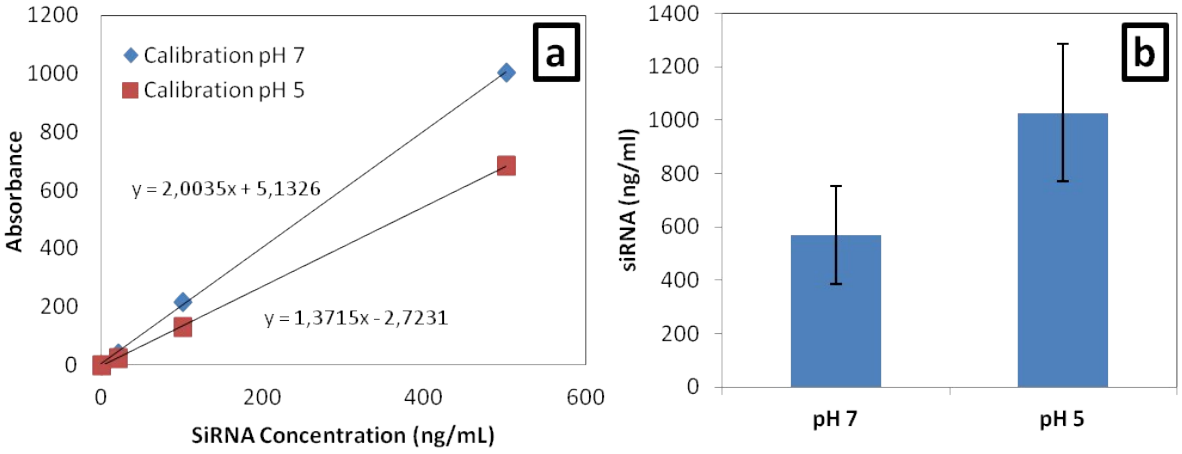

Figure S2. siRNA titrated by spectrofluorimetry with the RiboGreen® fluorescent dye at pH 7 and pH 5. (a) Calibration curves at pH 5 and pH 7. **(b)** Determination of the concentration of siRNA titrated by RiboGreen® in the presence of PIC micelles in neutral (pH 7) and acidic (pH 5) aqueous media.

S3: Determination of non-cytotoxic concentrations of chemical endocytosis inhibitors.

Protocol: Five thousands (5,000) cells were seeded into a 96-well assay plate and allowed to adhere for 24h. The medium was then replaced by fresh optiMEM containing a range of concentrations of each inhibitor, and cells were incubated during 4 hours before the MTT assay. The concentrations tested and validated for each inhibitor are detailed in Table 2, as well as the targeted endocytosis pathways.

A

Inhibitor	Targeted pathway	Endocytosis inhibition concentration
Methyl-β-cyclodextrine (MβCD)	Cholesterol/Lipid raft-dependent endocytosis	500 µg/mL
Genistein (Gen)	Caveolae-dependent endocytosis	54 µg/mL
Chlorpromazine (CP)	Clathrin-dependent endocytosis	10 µg/mL

B

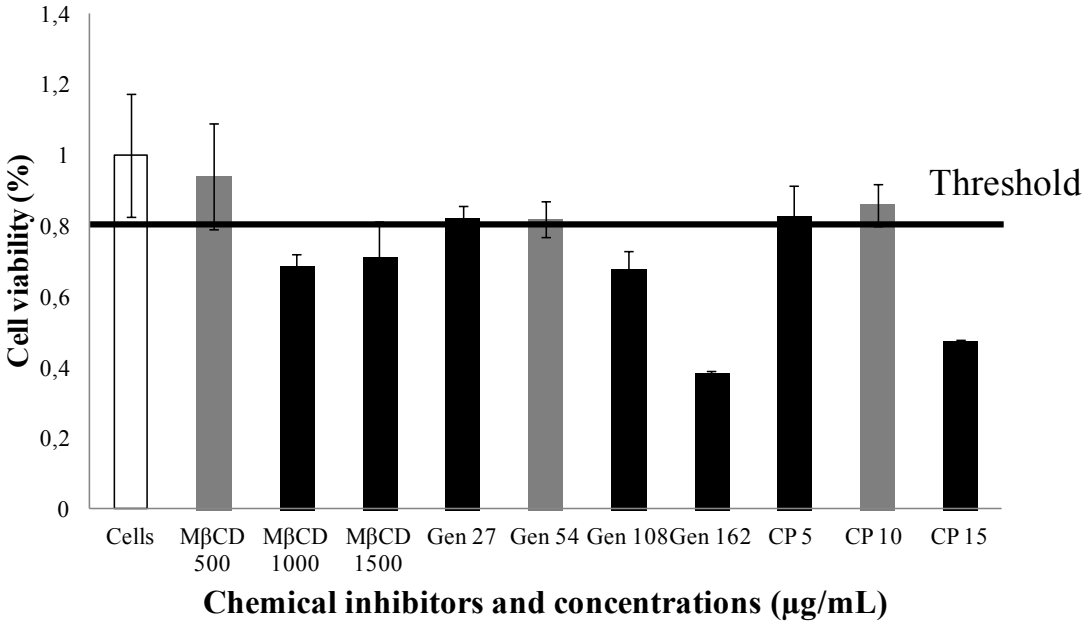

Figure S3. A/ Endocytosis chemical inhibitors: targeted trafficking pathways and non-cytotoxic concentrations used for the endocytosis inhibition study. B/ Determination of non-cytotoxic concentrations of chemical endocytosis inhibitors. Threshold was set at a minimum of 80% cell survival. Validated concentrations for endocytosis inhibition without significant cytotoxicity are shown in light grey. MβCD: methyl- β-cyclodextrin, Gen: genistein, CP: chlorpromazine, Cytoch D: cytochalasin D.

S4 : Kinetics of siRNA-TAMRA internalization in MSC studied by FACS.

Protocol: 150,000 mMSC were plated in each well of a 6-well assay plate and reached 70 % confluence after 24 h. The growth medium was then replaced by fresh medium and cells were treated with 250 pmol of siRNA, corresponding to 165 µL of micelles formulated at 1.5 µM in siRNA per well to reach a final volume of 2 mL per well. This corresponds to a dilution of $1/12^{e}$ of micelles. 165 µL of blank micelles were also used for negative control. Cells without treatment were defined as the positive control and data were normalized to this condition. Lipofectamine2000® was used as a commercial standard at a concentration 2.5 µg/mL. Each condition was repeated in duplicate. Cells were then incubated at 37 °C and 5 % CO_2 in a humidified atmosphere. At desired time points (2, 4, 6, 24 and 48 h), cells were trypsinized after being washed twice with Hank's Balanced Salt Solution without Ca^{2+} (HBSS, Life Technologies). Trypsin was neutralized in complete DMEM and cells were washed twice again (by centrifugation at 1800 rpm, 4°C, 5 min and re-suspension in cold HBSS cycles into Eppendorf tubes). Finally, cells were centrifugated at 4 °C, 1800 rpm during 5 min and re-suspended into a fixation solution (0.02 % Azide and 2 % of Formaldehyde in HBSS). Samples were analyzed with a BD LSRFortessa™ flow cytometer equipped with laser of 561 nm for TAMRA excitation. For quantification, 20,000 events per sample were collected. WinMDI Software was used for analysis.

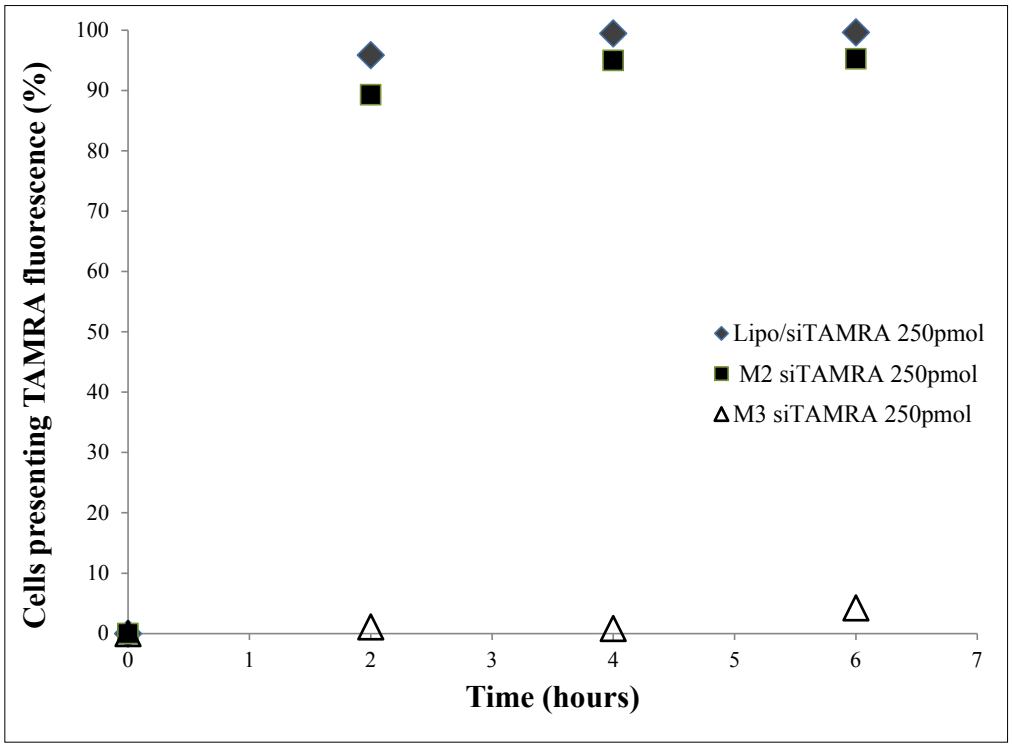

Figure S4. Kinetics of siRNA-TAMRA internalization in MSC determined by FACS analysis