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Supplemental Information

Mineral-Coated Microparticles Enhance mRNA-Based Transfection of Human Bone Marrow Cells

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Supplemental information

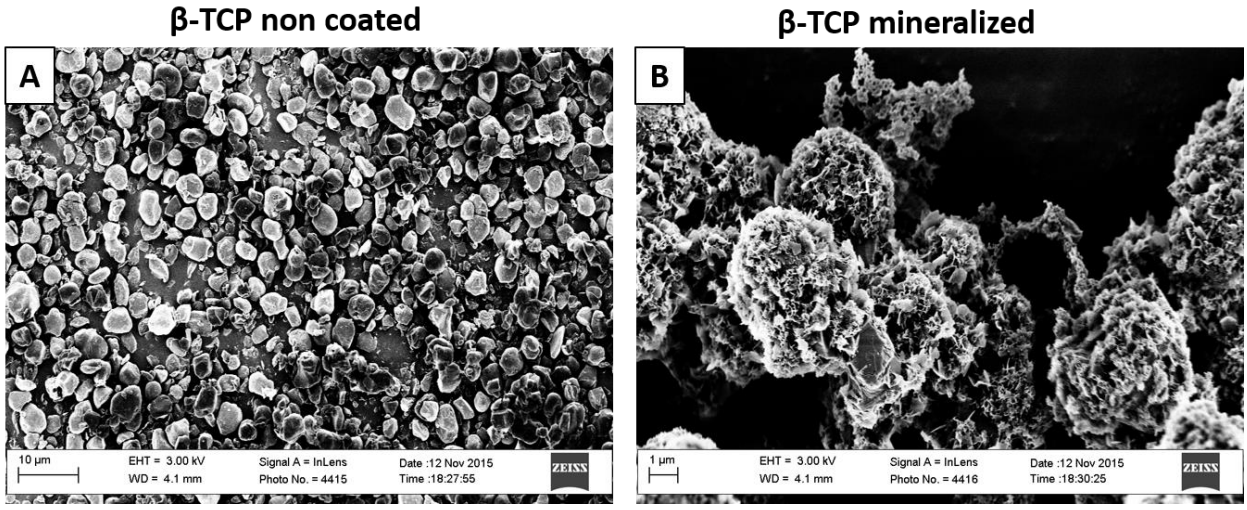

Figure S1: SEM micrographs of β-TCP core particles before (A) and after the mineral coating with regular SBF (B).

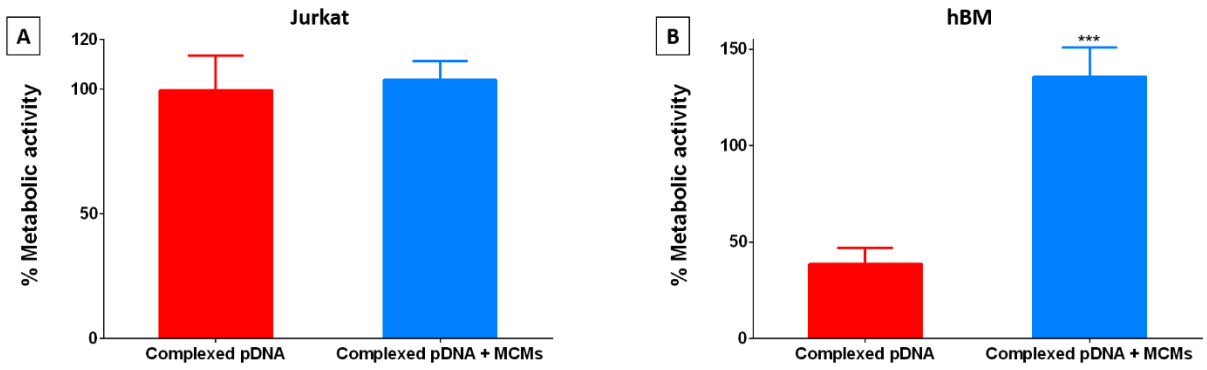

Figure S2: Characterization of cell metabolic activity of Jurkat (A) or hBM cells (B) after incubation for 2 days with complexed pDNA. The delivery of complexed pDNA with or without MCM did not seem to affect the metabolic activity of Jurkat cells but the particles were found to exert a protective role on hBM. (*) represents statistically significant differences using paired Student's t-test n = 4, $p < 0.05$.

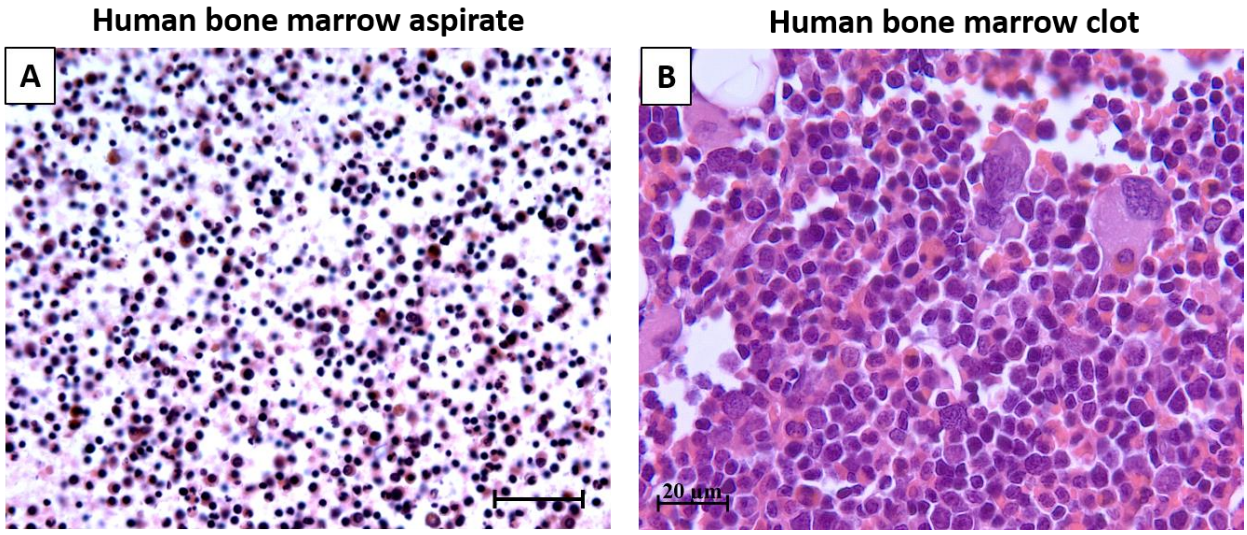

Figure S3: H&E stain of human bone marrow aspirates culture in suspension (A) or allowed to clot (B). Scalebar in image (A) is 50 µm, in image (B) is 20 µm.

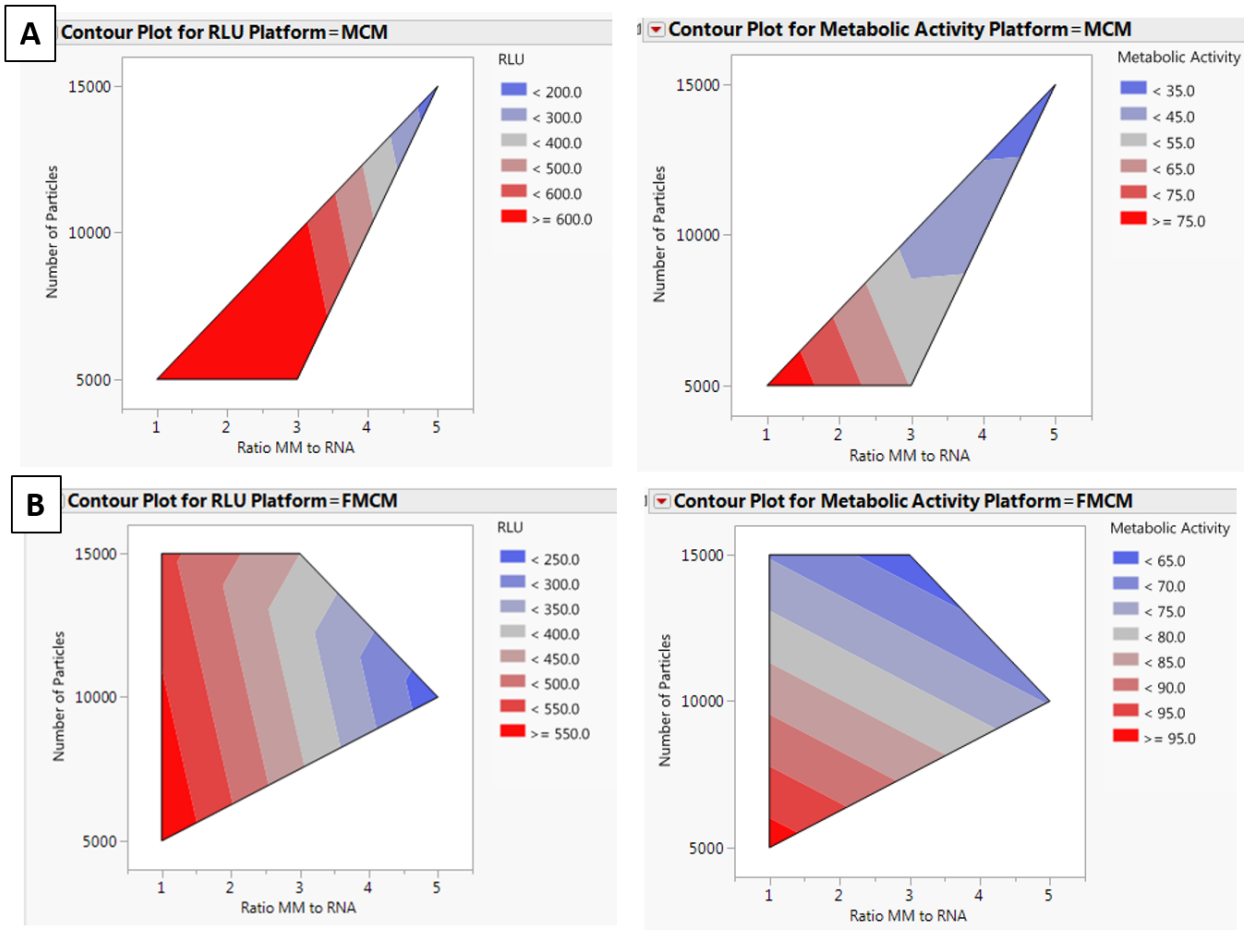

Figure S4: Contour plots displaying the results of the screening design performed on Jurkat cells for the delivery of complexed mRNA encoding for Gaussia Luciferase via MCM (A) or FMCM (B). The plots display the effects of the ratio MessengerMax to mRNA (v/v) and the total number of particles (which correlates to the total amount of complexed mRNA delivered). The conditions with the highest results are highlighted red while the lowest results are blue. The transfection efficiency was assessed by measuring the luciferase activity and the metabolic activity was assessed by performing a Cell Titer Blue® assay. The lower ratio MessengerMax to mRNA enable higher cell metabolic activity and transfection, but when the complexes where delivered via MCM, the higher ratio was found to be effective in transfecting Jurkat cells.

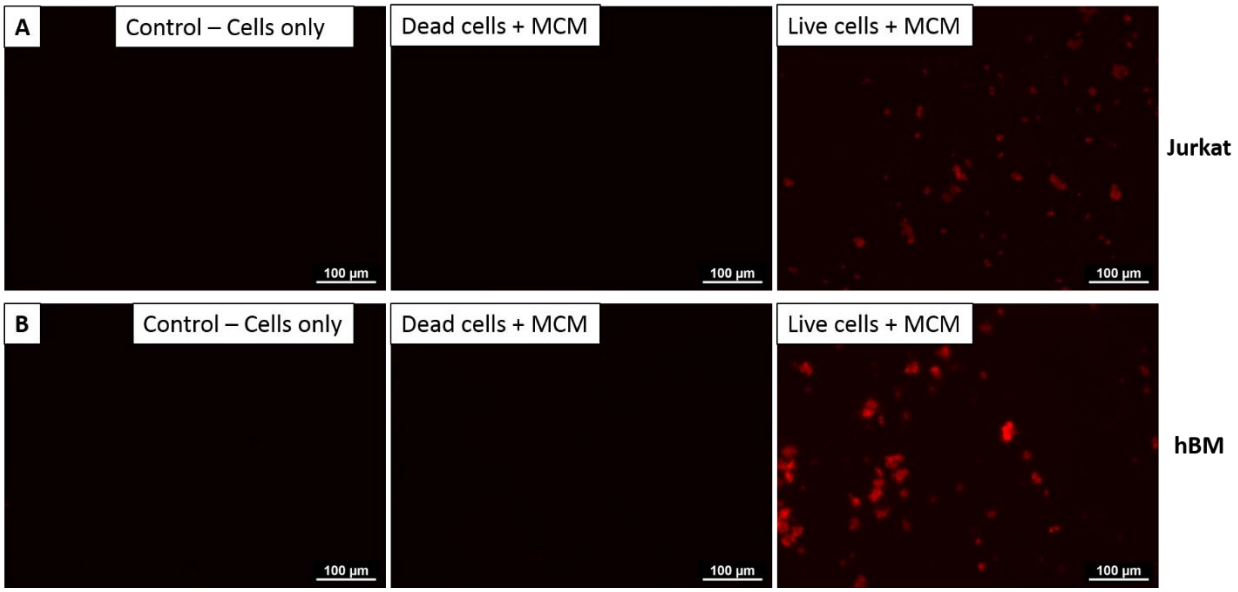

Figure S5: Exposure of Jurkat cells to MCM causes an increase of endosomal activity as shown by an increased uptake of labelled dextran. The images show Jurkat (A) or hBM cells (B) co-cultured with labelled dextran alone or in presence of MCM respectively. Before imaging the cells were fixed and the MCM dissolved using HCl. To exclude the effects of possible residues of MCM we have also included a control group of cells that were fixed before exposure to MCM. Only live cells interacting with MCM internalized the labelled dextran.

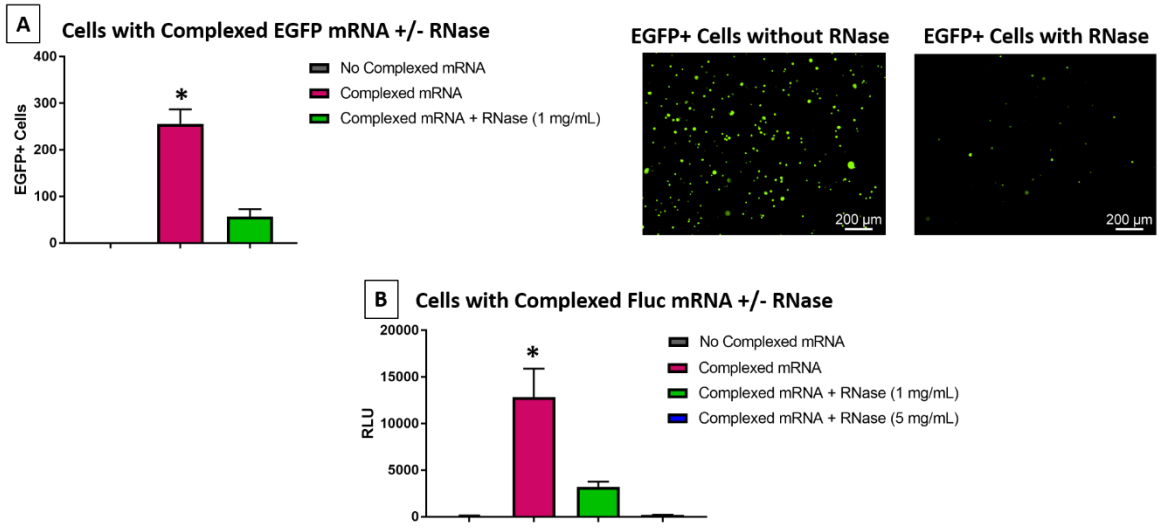

Figure S6: Transfection of Jurkat cells using complexed mRNA encoding for EGFP (A) or Firefly luciferase (B) with or without RNAse A. The complexes partially shield the mRNA from degradation but the presence of RNAses drastically decrease the efficacy of mRNA delivery. (*) represents statistically significant differences using paired Student's t-test $n = 4$, $p < 0.05$.

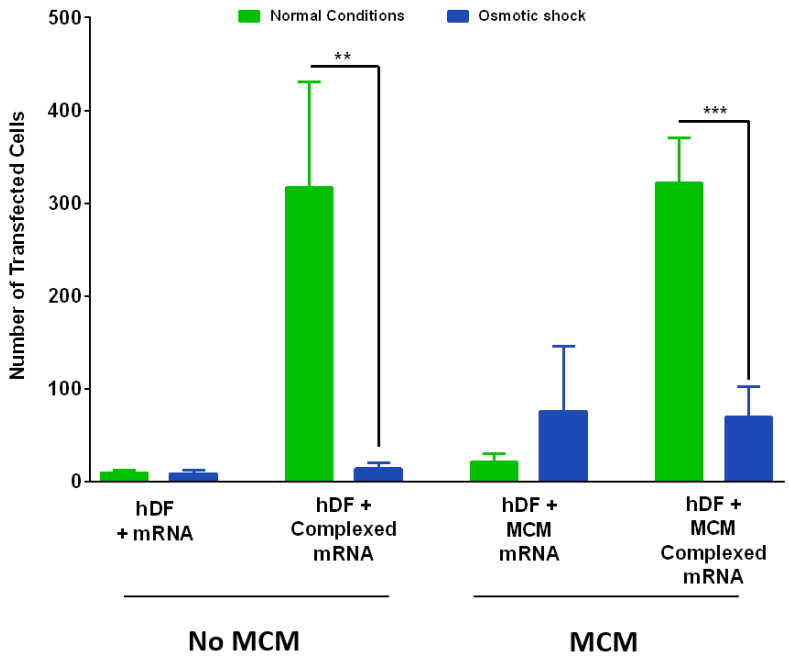

Figure S7: Transfection of human dermal fibroblasts (hDF) using complexed mRNA encoding for EGFP. To test the stability of the complexes, samples were exposed to an osmotic shock which consisted in a sequential exposure to hypotonic and hypertonic buffers. Complexes were probably destabilized by osmotic shocks and lost efficacy. (*) represents statistically significant differences using paired Student's t-test $n = 4$, $p < 0.05$.

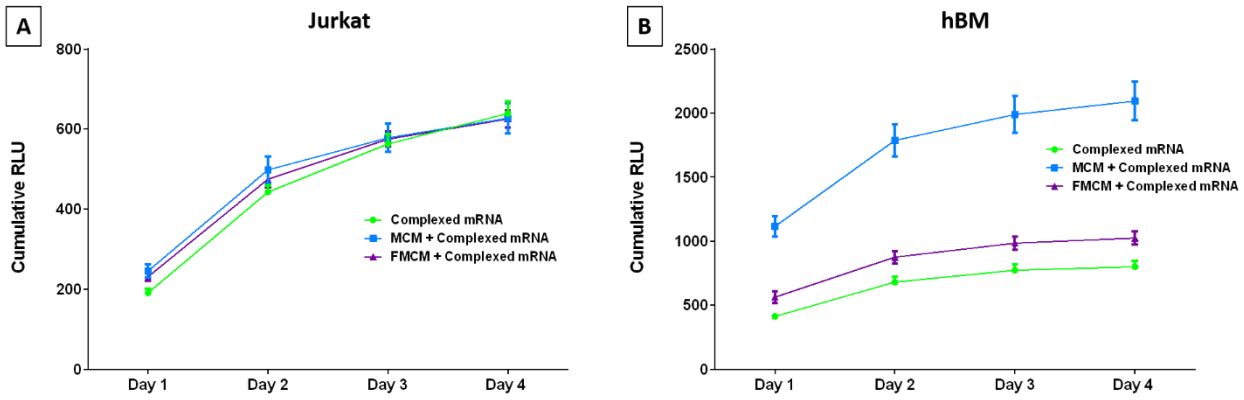

Figure S8: Transfection of Jurkat (A) or hBM cells (B) using complexed mRNA encoding for Gaussia Luciferase (G-Luc). Cells were found to express the G-Luc mRNA for 4 days and the luciferase activity was higher in hBM when the complexes were delivered via MCM.

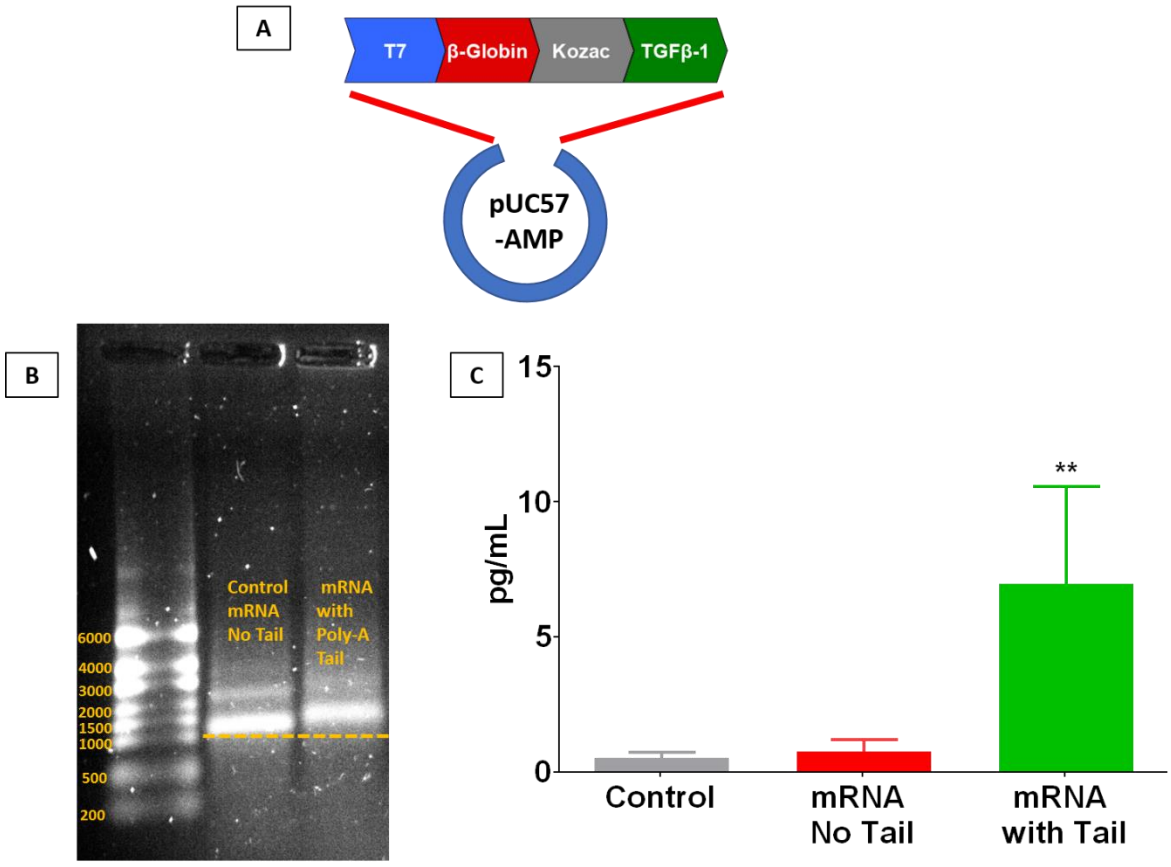

Figure S9: (A) Schematic representation of the custom DNA template designed for the synthesis of TGF-β1 mRNA. The custom sequence was inserted in a pUC57 backbone plasmid. (B) mRNA gel electrophoresis showing that tailed mRNA is longer than the non-tailed control. (C) Quantification of the amount of active TGF-β1 secreted in the supernatant by rat bone marrow cells transfected with complexed therapeutic mRNA. The cells transfected with the full mRNA sequence, containing the poly-A tail, secreted the highest amount of human TGF-β1. (*) represents statistically significant differences using One-way ANOVA followed by Tukey's multiple comparisons test n=4, $p<0.05$.

Reagent	Concentration (mM)
NaCl	141
KCl	4
$MgSO_4$	0.5
$MgCl_2$	1
$NaHCO_3$	4.2
Hepes	20
$CaCl_2$	5
KH_2PO_4	2
NaF*	1

*Added only to synthesized FMCM

Table S1: List of reagents used for the biomineralization of β-TCP microparticles

Type of coating	Number of particles	Mass
Regular SBF (MCM)	10,000	164 µg
Fluoride-doped SBF (FMCM)	10,000	57 µg

Table S2: Table indicating the mass of particles needed to obtain 10,000 particles.

Supplemental methods:

mRNA synthesis.

We synthesized a custom mRNA sequence encoding for human TGF-β1 (NCBI Reference Sequence: NP_000651.3). Briefly, we designed a synthetic gene containing a T7 promoter, a 5' β-globin sequence, a kozac sequence and the gene encoding for human TGF-β1. A schematic of the custom mRNA is provided in Supplementary Figure S9. The synthetic gene was manufactured by Genewiz® and inserted in a pUC57-Amp plasmid that served as a template for the synthesis of mRNA. The mRNA was then synthesized using the HiScribe T7 ARCA mRNA kit (NEB E2060S) following the manufacturer's instructions.