Supplementary Information

Innovative strategy for 3D transfection of primary human stem cells with BMP-2 expressing plasmid DNA: A clinically translatable strategy for bone regeneration

Maruthibabu Paidikondala[1], Sandeep Kadekar[1], Oommen P. Varghese[1]*

***Corresponding author**

1 Translational Chemical Biology Laboratory, Department of Chemistry, Ångström Laboratory, Uppsala University, 751 21, Uppsala, Sweden.

E-mail: oommen.varghese@kemi.uu.se

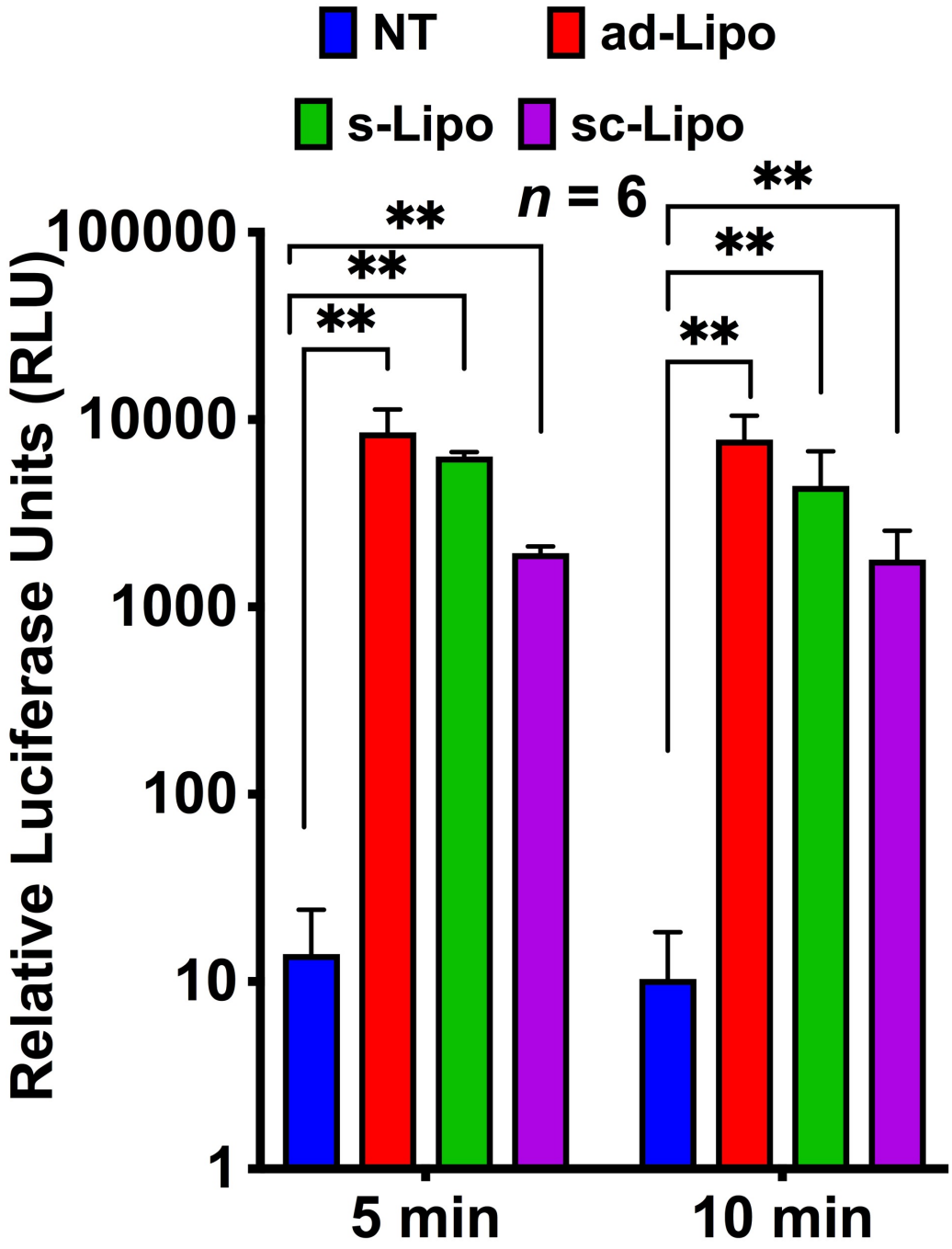

Fig. S1 Luciferase plasmid expression analysis using ONE-Glo™ Luciferase Assay System in hMSCs transfected with the assistance of Lipofectamine 2000 reagent in 2D. Mean values ± SD of 2 independent experiments done in triplicates ($n = 6$) are shown. Statistical analysis was performed using one-way ANOVA with Bonferroni's multiple comparison corrections (*$p < 0.0001$).

Transfection *condition*	**Number of cells Per well/gel**	**Volume of Lipofectamine Per well/gel**	**Total amount of Plasmid DNA transferred**
Adherent (2D)	**35000 cells per well in a 24-well plate**	**1.5 µL**	**100 ng**
Suspension in 2D	**35000 cells per well in a 24-well plate**	**1.5 µL**	**100 ng**
Suspension followed by centrifugation in 2D	**35000 cells per well in a 24-well plate**	**1.5 µL**	**100 ng**
Suspension/direct addition in 3D (gene expression analysis)	**200000 cells per 200 µL gel in a well of a 24-well plate**	**6 µL**	**500 ng**
Suspension followed by centrifugation in 3D (gene expression analysis)	**200000 cells per 200 µL gel in a well of a 24-well plate**	**6 µL**	**500 ng**
Suspension/direct addition in 3D (cell viability)	**100000 cells per 100 µL gel in a well of a 96-well plate**	**3 µL**	**250 ng**
Suspension followed by centrifugation in 3D (cell viability)	**100000 cells per 100 µL gel in a well of a 96-well plate**	**3 µL**	**250 ng**

Table S1. Table showing different volumes of Lipofectamine 2000 reagent, and concentrations of plasmid DNA used to transfect cells in different experimental conditions in 2D and 3D.