

## **Supplementary Information**

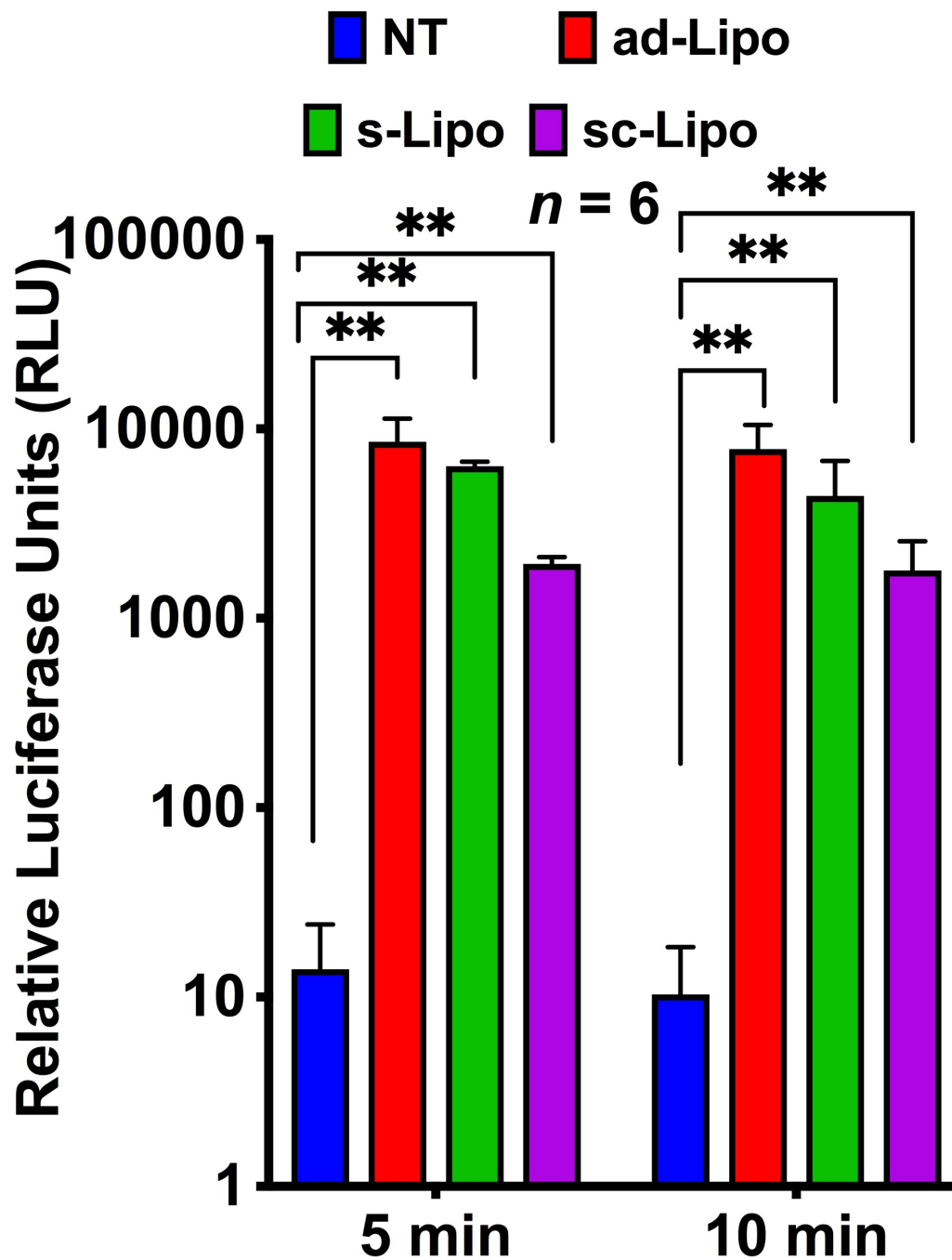
**Innovative strategy for 3D transfection of primary human stem cells with BMP-2 expressing plasmid DNA: A clinically translatable strategy for bone regeneration**

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**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  $\pm$  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 ( $*p < 0.0001$ ).

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

**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.