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Supplementary Information

Enhanced Non-Viral Gene Delivery to Human Embryonic Stem Cells via Small Molecule-Mediated Transient Alteration of Cell Structure

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1. Cellular spreading analysis through imaging

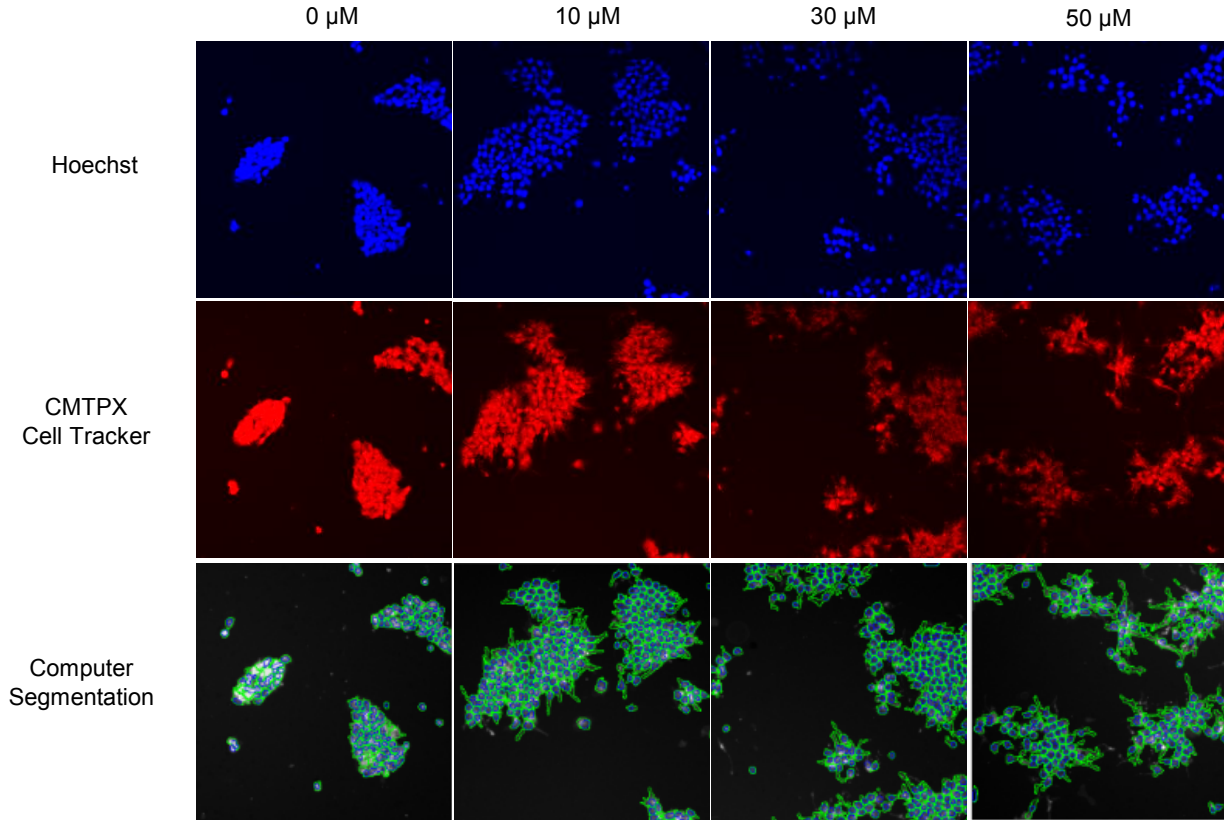

Fig. S1 Representative images of the alteration of the hESC cytoplasmic area following treatment with Y-27632 of various concentrations (0, 10, 30 and 50 μM). First row represents the Hoechst channel and the second row represents CMTPX cell tracking channel. The third row represent the software generated segmentation used for analysis. The blue outline represents the identified nuclei and the green outline is the identified CMTPX stain of the cellular cytoplasm from which the cell area was calculated.

hESCs were seeded on Matrigel-coated 96-well plates and cultured in mTeSR medium for 24 h. Y-27632 was added into the culture medium at a final concentration of 0, 10, 30 or 50 μM, and incubated at 37°C for 4 h. After 4 h of treatment, the cells were stained with CMTPX Cell Tracker dye and Hoechst according to manufactures instructions. Five wells were imaged with 9 fields each using the GE InCell Analyzer 2000. The images were then analyzed using the GE InCell Analyzer workstation. The nuclei were identified using top-hat segmentation in the Hoechst channel, and the cell area was analyzed and calculated through the analysis in the CMPTX channel using multiscale top-hat. The cytoplasmic area was then normalized to the number of nucleus counted.

2. GFP transfection analysis through imaging

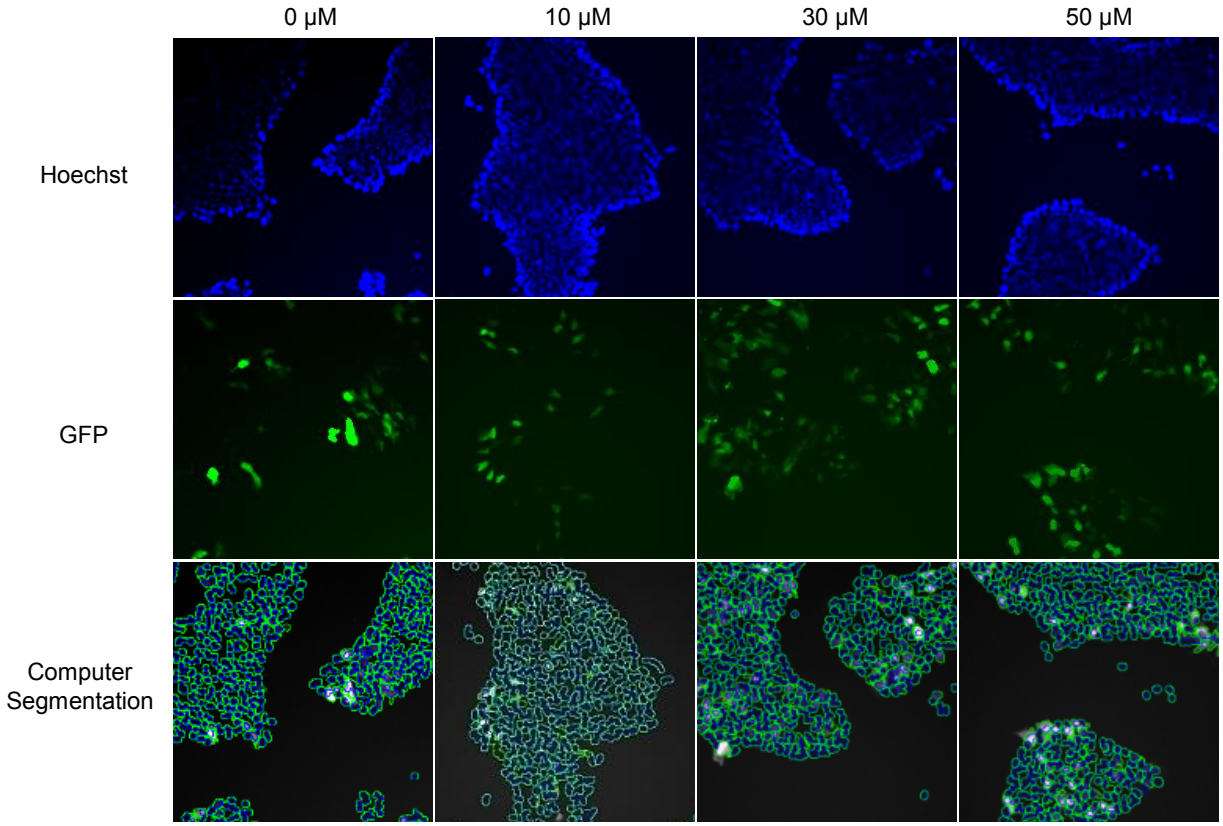

Fig. S2 Representative images of the transfection of hESCs after alteration of the cytoplasm area with the treatment of Y-27632 at various concentrations (0, 10, 30 and 50 µM). First row represents the Hoechst channel and the second row represents the GFP channel. The third row represent the software generated segmentation used for analysis. Blue outline represents the identified nuclei and green outline is the determined cell cytoplasm as extrapolated by the nuclei and GFP channel.

hESCs were seeded on Matrigel-coated 24-well plates and cultured in mTeSR medium for 24 h. Before transfection, Y-27632 was added into the culture medium at a final concentration of 0, 10, 30 or 50 µM, and incubated at 37 °C for 4 h. Nanoparticles from pEGFP-N1 and FHD were added dropwise into the culture medium and cells were further incubated at 37°C for 4 h. The medium was then replaced with fresh mTeSR medium, and cells were further cultured for 48 h. The hESCs were then stained with Hoechst and imaged using the GE InCell Analyzer. Using the InCell Analyzer Workstation, the nuclei were identified using top-hat segmentation in the Hoechst channel and the numbers of cells were then

calculated. The identified nuclei were then further segmented by multiscale top-hat in the GFP signal to determine the number of cells with positive GFP signals. The transfection efficiency was then determined by dividing the number of GFP positive cells by the total number of cells.

3. Cell Cytotoxicity of Y-27632 Treatment with FHD Transfection

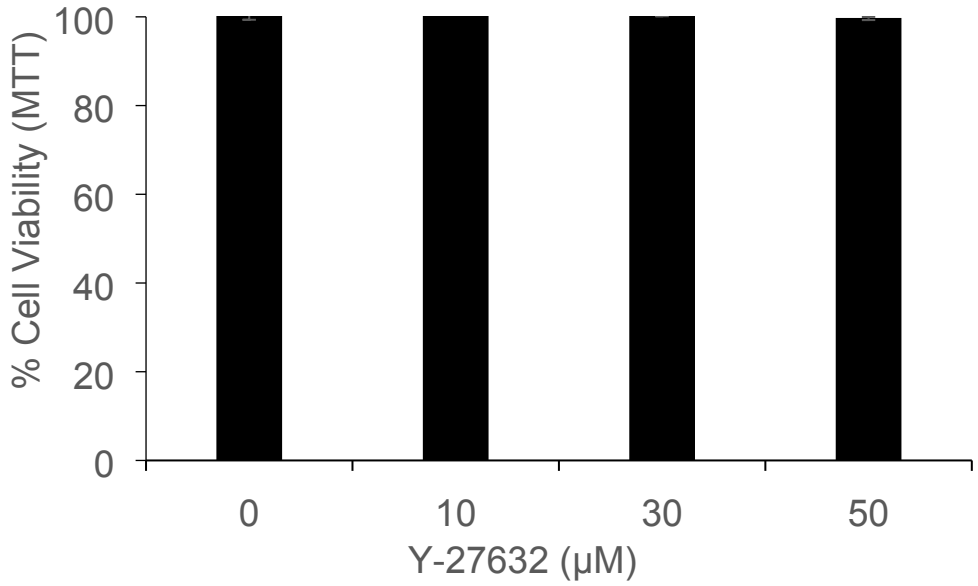

Fig. S3 Cytotoxicity of H1 hESCs 48 h after transfection with FHD at various concentrations MTT cell viability assay of the FHD transfection with varying concentrations (0-50 μM) of Y-27632 treatment in H1 hESCs after 48 h.

For the MTT assays cytotoxicity assessment, 1 6-well well of confluent hESCs was were plated into on matrige-coated 24 wells of a matrigel coated 48-well plate one day before transfection. The cells were then pre-treated for 4 h with 0, 10, 30, and 50 μM of Y-27632 for 4 h and transfected with FHD as described previously above with FHD. The cells were further incubated for 4 h at 37°C in the transfection mix before removal of the transfection reagent and replacement of fresh media. being returned to fresh growth media . After 48 h, the cell viability was monitored by the MTT assay and was represented as percentage viability of control cells that did not receive any transfection treatment. For the MTT assay, the cells were washed with PBS and MTT solution was added. Following 4-h incubation at 37°C, MTT solubilization solution (10% Triton X-100 in acidic (0.1M HCl) isopropanol) was added to the cells and the absorbance of 570 nm light was quantified on a Perkins Elmer plate reader (Waltham, MA, USA).