SUPPORTING INFORMATION

Dual Delivery of siRNA and Plasmid DNA using Mesoporous Silica Nanoparticles to Differentiate Induced Pluripotent Stem Cells into Dopaminergic Neurons

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List of Table and Figures.

Table S1. The time-(0.5, 1, 2, 4 hr) flow cytometry profiles of iPSCs after incubation with FMSN(+). Control is defined as incubation with no (0 μg/mL) FMSN(+), pNurr1-FMSN(+) and pNurr1-siRex1-FMSN(+). (64 μg/mL)

	CTL	**pNurr1-siRex1-FMSN(+)**	**pNurr1-FMSN(+)**	**FMSN(+)**
30 min	0. 8% ± 0.1%	2.1% ± 0.4%	2.4% ± 0.6%	2.1% ± 0.1%
1 hr	0.7% ± 0.0%	5.0% ± 0.8%	4.6% ± 0.5%	3.4% ± 0.0%
2 hr	0.6% ± 0.1%	12.9% ± 0.1%	13.4% ± 0.3%	27.1% ± 2.0%
4 hr	0.5% ± 0.0%	77.5% ± 3.0%	67.3% ± 2.5%	88.6% ± 0.4%

Table S2. The flow cytometry analysis of quantification of the percentage of dopaminergic neurons expressing neural markers: tyrosine hydroxylase (Th) and dopaminergic transporter (Dat). Data are means ± standard deviation of three independent experiments.

Day7	**CTL**	**pNurr1-siRex1-FMSN(+)**	**pNurr1-FMSN(+)**	**pNurr1-siRex1-Lipo**	**pNurr1-Lipo**
Th	52.2% ± 1.3%	83.0% ± 0.4%	58.8% ± 0.2%	76.1% ± 0.5%	60.8% ± 0.4%
Dat	43.6% ± 0.0%	65.2% ± 2.9%	42.9% ± 1.4%	59.6% ± 6.8%	50.7% ± 2.6%

Day14	**CTL**	**pNurr1-siRex1-FMSN(+)**	**pNurr1-FMSN(+)**	**pNurr1-siRex1-Lipo**	**pNurr1-Lipo**
Th	51.4% ± 1.2%	89.9% ± 0.5%	72.0% ± 1.8%	95.3% ± 0.4%	71.4% ± 7.1%
Dat	49.3% ± 0.0%	88.5% ± 2.0%	71.7% ± 3.8%	72.1% ± 0.1%	67.1% ± 1.9%

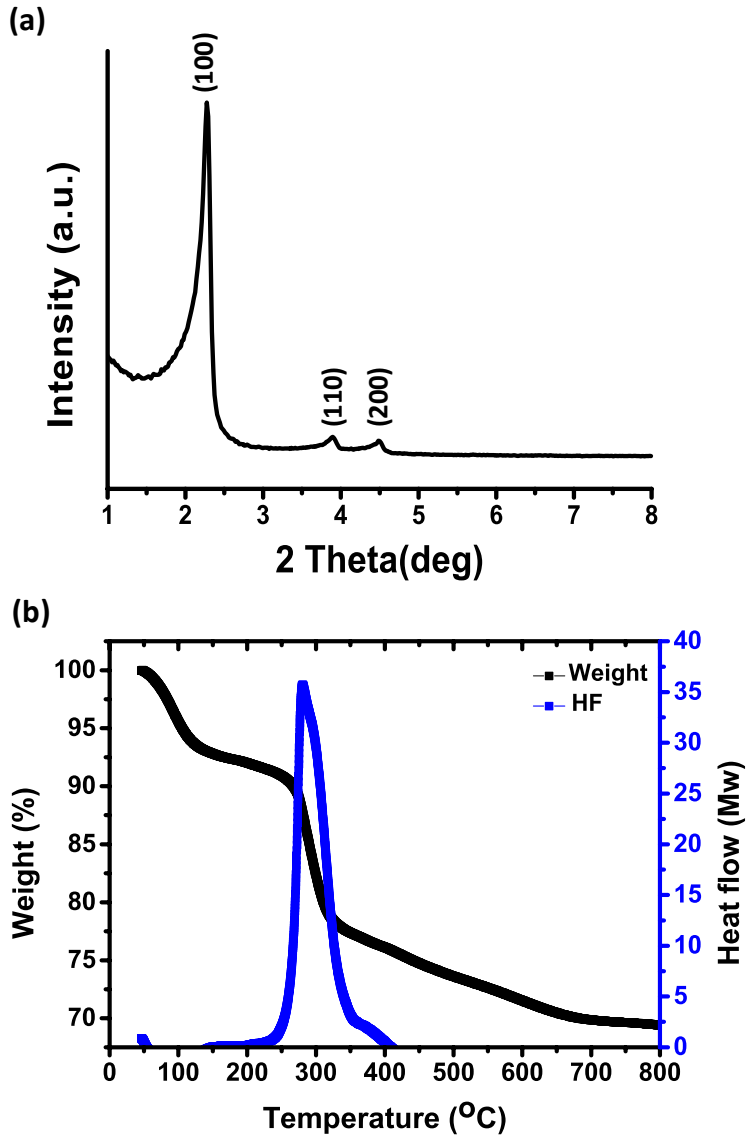

Figure S1. (a): The X-ray diffraction patterns of FMSN(+). The (100), (110) and (200) diffraction peaks are characteristic 2D hexagonal structure. (b): Thermogravimetric analysis of FMSN(+). The decrease of weight below 150 ^{o}C is due to physically adsorbed water. The weight loss (10.5%) at near 300 ^{o}C is due to the decomposition of N-triaminethoxysilylpropyl-N, N, N-trimethylammonium (TMA) surface group. The slow weight loss from 320 ^{o}C to 600 ^{o}C (8.0%) is due to FITC-APTS.

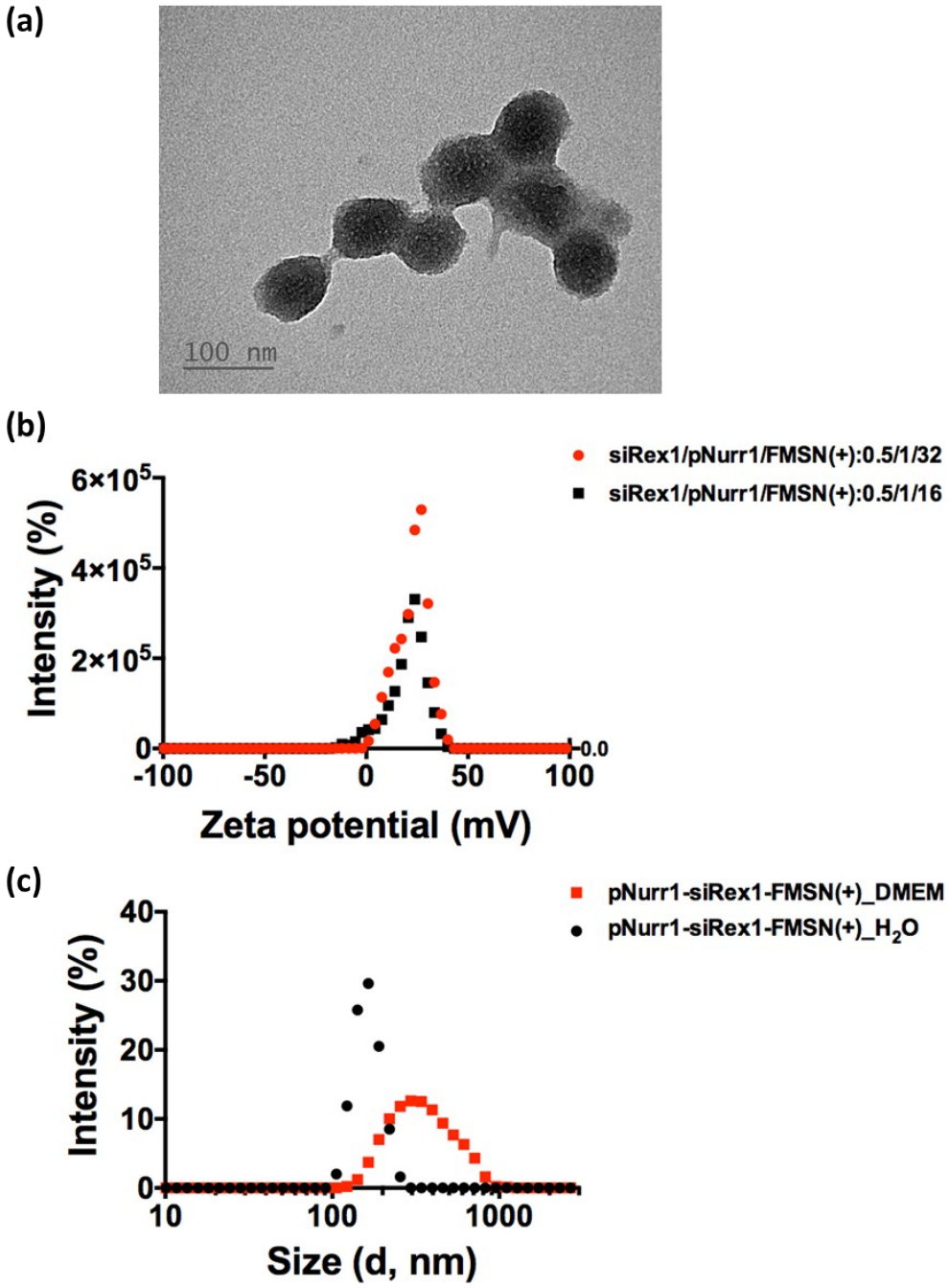

Figure S2. (a): The TEM image of pNurr1-siRex1-FMSN(+), (b): The zeta potential studies of pNurr1-siRex1-FMSN(+), which the ratio between siRNA/DNA and FMSN(+), 0.25/1/16 and 0.25/1/32 respectively, (c): The DLS sizes of pNurr1-siRex1-FMSN(+) in DMEM solution and H_2O.

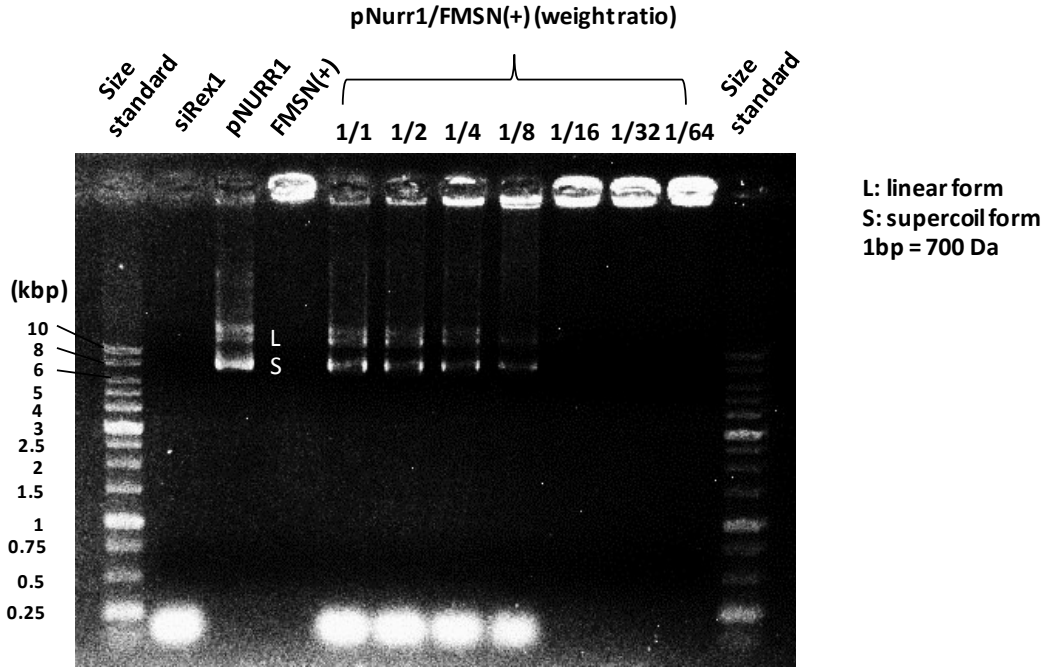

Figure S3. The gel electrophoresis examination of DNA-siRNA-FMSN(+) (pNurr1-siRex1-FMSN(+)) complex in 1% agarose gel. DNA ladder, siRex1, pNurr1, and FMSN(+) were references. (110V for 30 minutes)

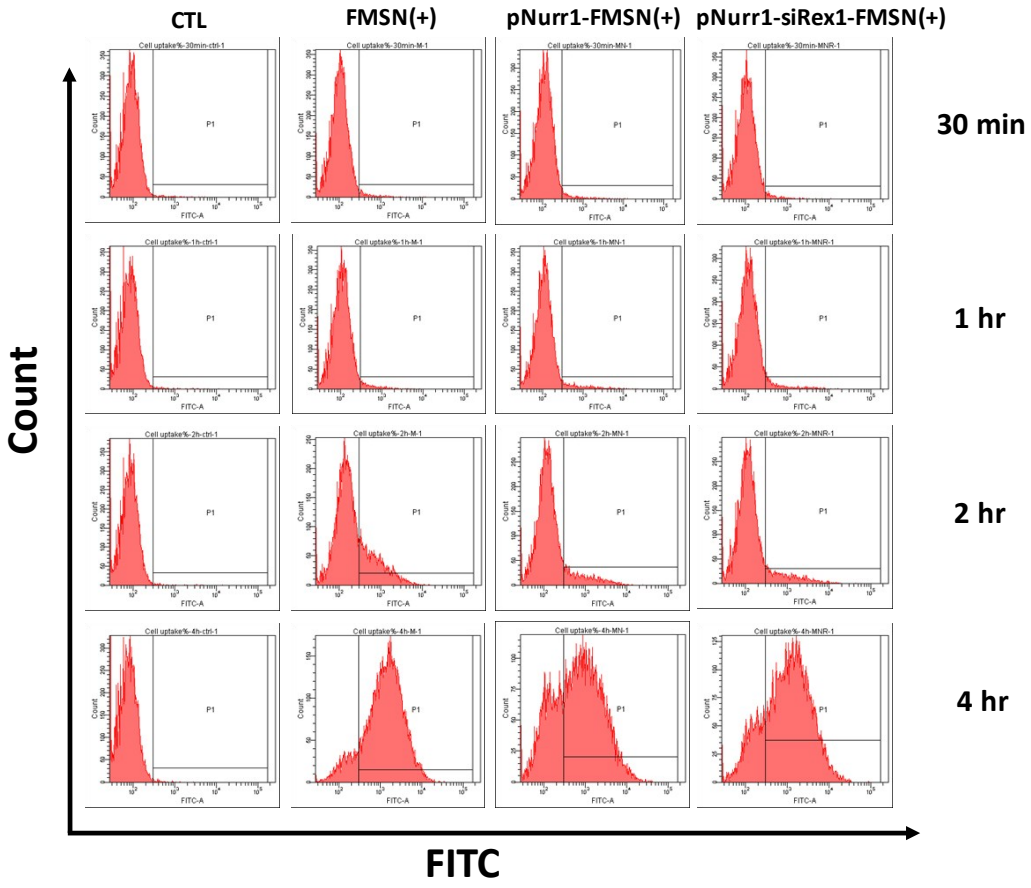

Figure S4. The time-(0.5, 1, 2, 4 hr) flow cytometry profiles of iPSCs after incubation with FMSN(+), pNurr1-FMSN(+) and pNurr1-siRex1-FMSN(+). (64μg/mL) Control is defined as the non-treatment condition. The numbers of positively labeled cells (defined as the P2 region) were presented as the percentage of total counting cells in each condition.

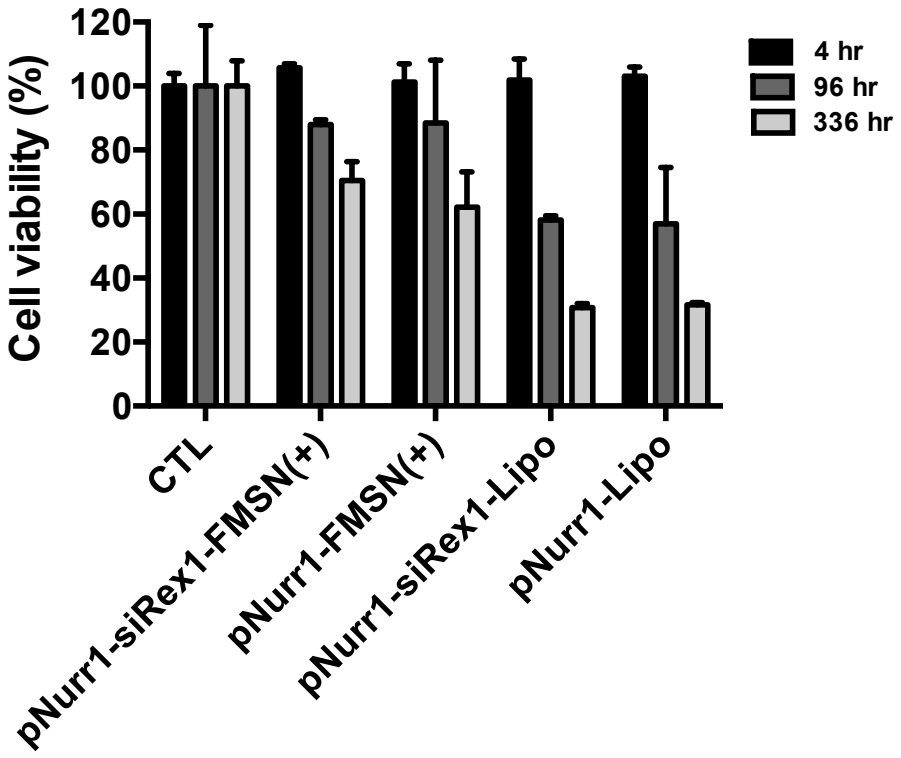

Figure S5. The cell viability assay of FMSN(+) and Lipofectamine 2000. Delivery of pNurr1-FMSN(+), pNurr1-siRex1-FMSN(+), pNurr1-Lipo and pNurr1-siRex1-Lipo into iPSCs to evaluate the in vitro cell proliferation (96 and 336 hour).The cell viability was examined by calculating the cell number after the cells were trypsinized. (amount of pNurr1: 2 μg/well; amount of Lipofectamine 2000: 6 μL/well; amount of FMSN(+): 32 μg/well, the cell number: 2×10^5cells/well)

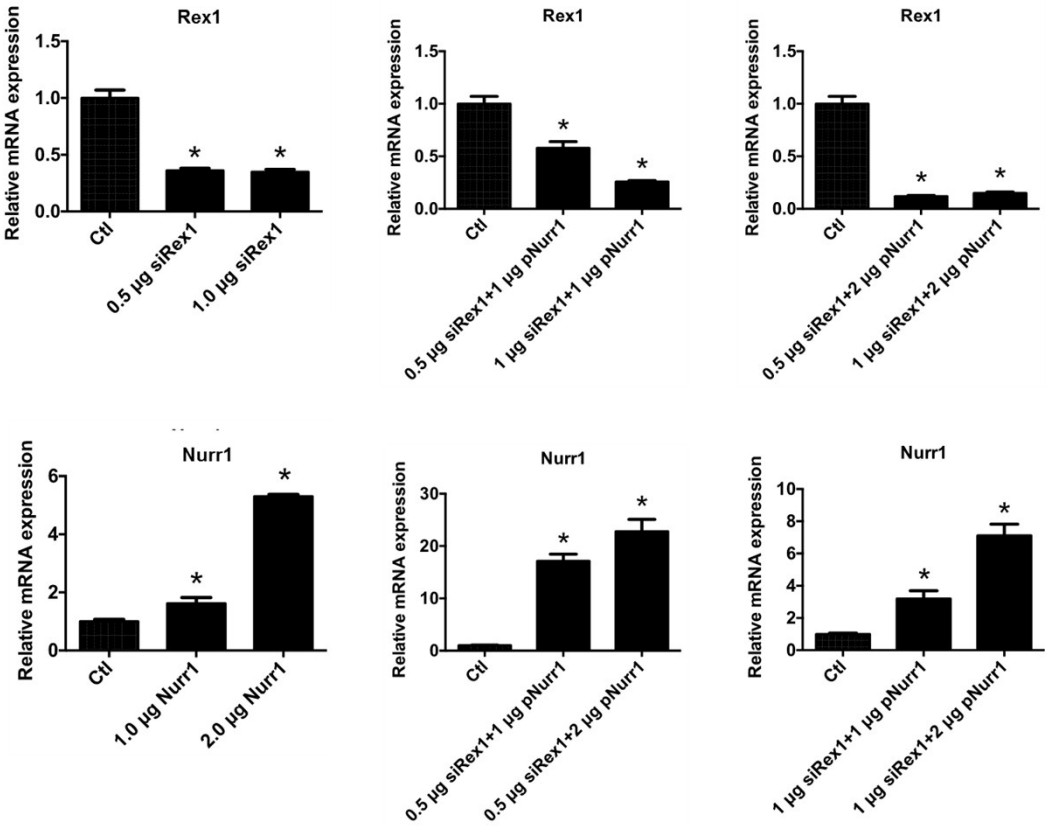

Figure S6. The exogeneous messenger ribonucleic acid (mRNA) levels of iPSCs that were determined after transfection by different weight ratio of siRex1:pNurr1 using FMSN(+) as carriers (64 μg/mL, transfection for 4 hr).

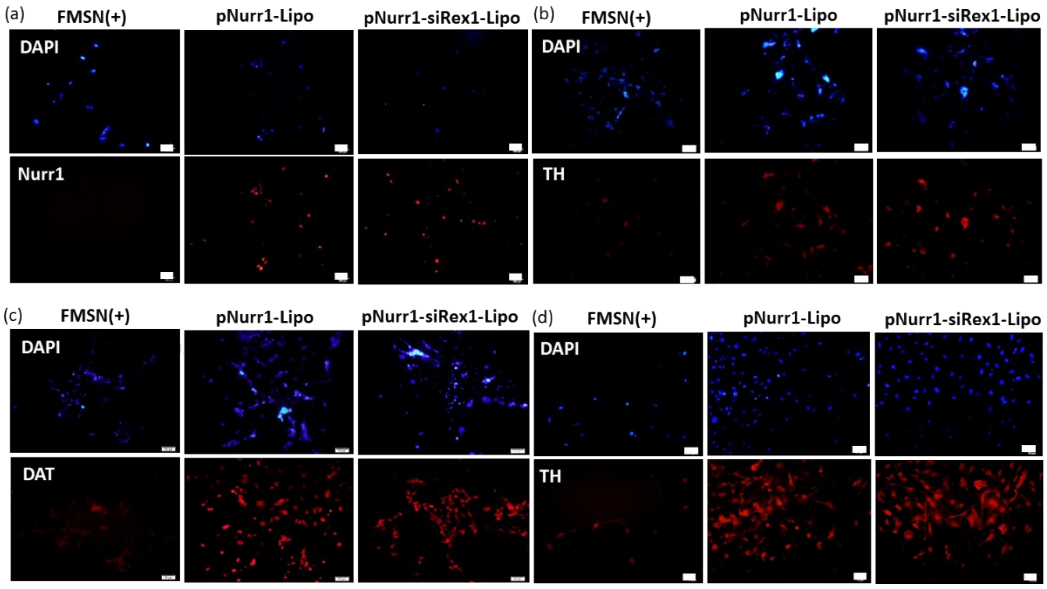

Figure S7. Immunofluorescence staining for the expression of the nuclear receptor related 1 protein (Nurr1, red), tyrosine hydroxylase (Th) and dopamine transporter (Dat) in pNurr1-Lipo and pNurr1-siRex1-Lipo-treated iPSCs as positive controls and FMSN(+) (64 μg/mL, transfection for 4 hr). (a), (b): iPSCs were differentiated for 7 days. (c), (d): iPSCs were differentiated for 14 days. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI, blue). Scale bars: (a), (c) are 50 μm; (b), (d) are 100 μm.

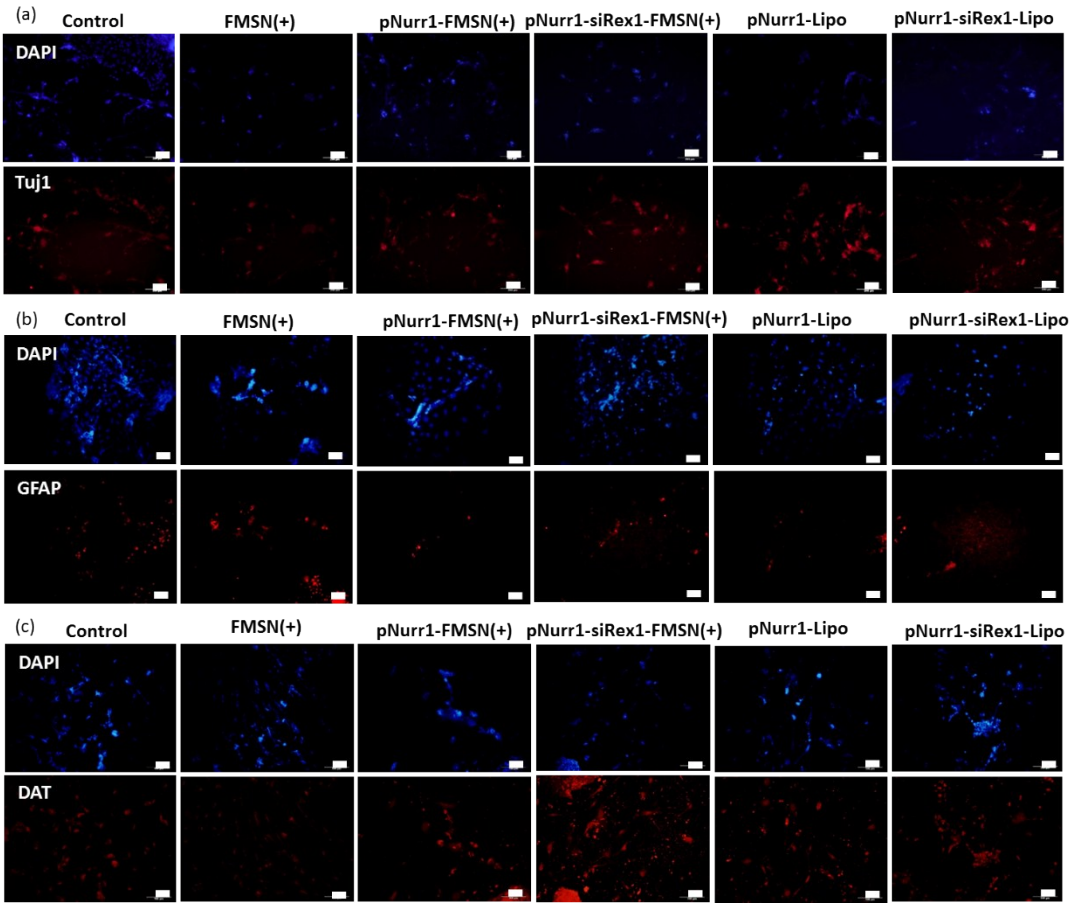

Figure S8. Immunofluorescence staining for the expression of the (a): Neuron-specific class III beta-tubulin (Tuj1, red), (b): Glial fibrilliary acidic protein (Gfap, red), (c): Dopamine transporter (Dat, red) in 64 μg/mL pNurr1-FMSN(+) and pNurr1-siRex1-FMSN(+)-treated iPSCs and controls. iPSCs were transfected for 4 hr and differentiated for 7 days. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI, blue). pNurr1-Lipo and pNurr1-siRex1-Lipo: liposomal transfection; positive control for transfection. Scale bars are 50 μm.

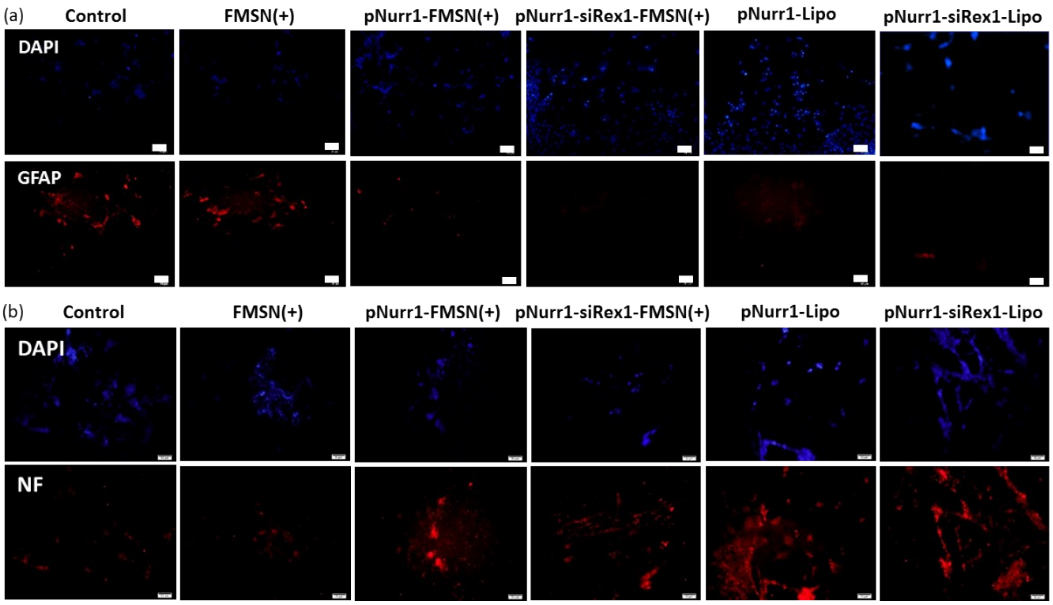

Figure S9. Immunofluorescence staining for the expression of (a): Glial fibrilliary acidic protein (Gfap, red), and (b): Neurofilament (Nf, red) under pNurr1-FMSN(+) and pNurr1-siRex1-FMSN(+) treatment for 14 days. (64 μg/mL, transfection for 4 hr) Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI, blue). pNurr1-Lipo and pNurr1-siRex1-Lipo: liposomal transfection; positive control for transfection. Scale bars are 50 μm.

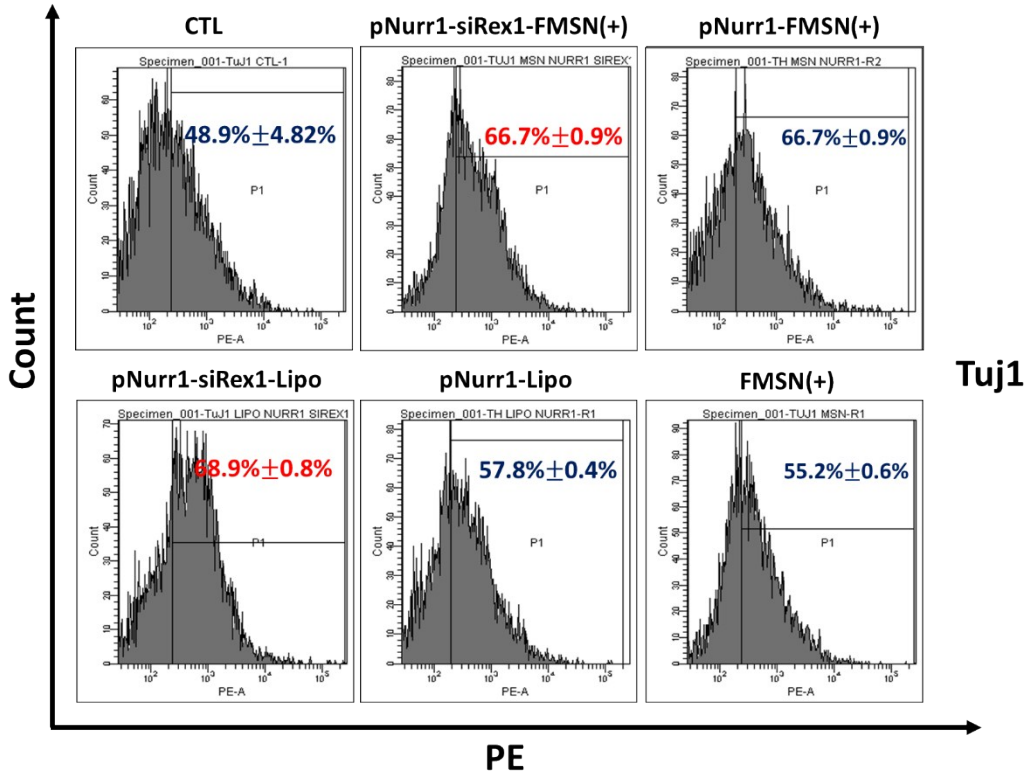

Figure S10. Quantification of the percentage of neurons progenitor marker, Tuj1, identified by flow cytometry. iPSCs were transfected for 4 hr and differentiated for 7 days under 64 μg/mL pNurr1-siRex1-FMSN(+), pNurr1-FMSN(+) and positive control condition. Control is under non-treated condition.

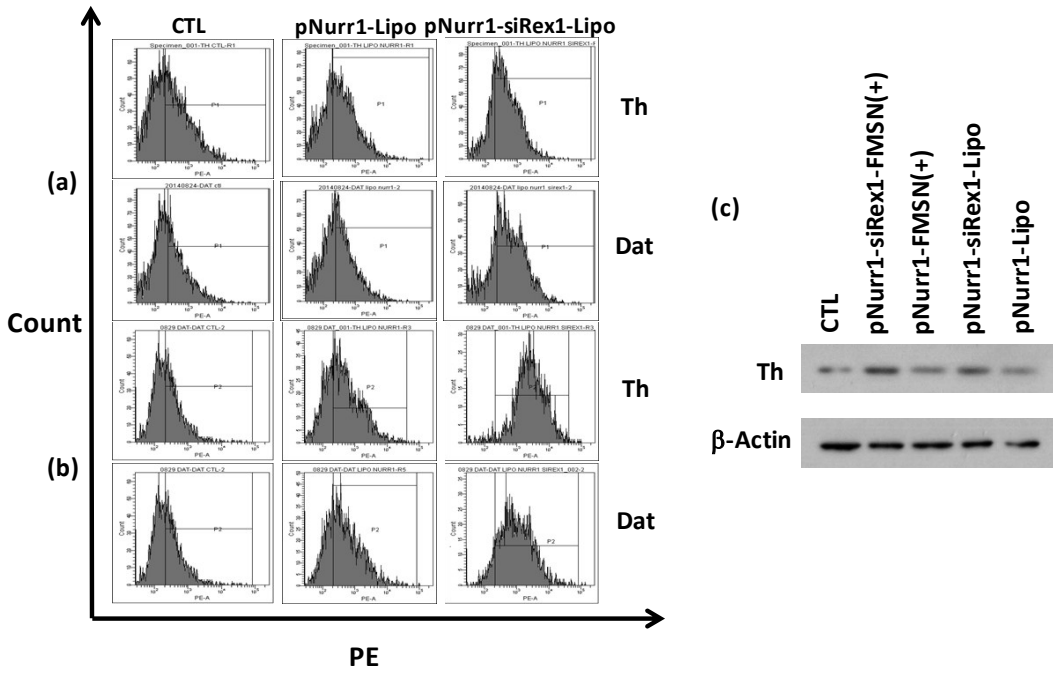

Figure S11. Quantification of the percentage of dopaminergic neurons expressing neuron markers: tyrosine hydroxylase (Th) and dopaminergic transporter (Dat) by flow cytometry. iPSCs were transfected for 4 hr and differentiated for (a): 7 days, (b): 14 days under the treatment of pNurr1-siRex1-Lipo, (c) the western blotting analysis. Control is under non-treated condition.