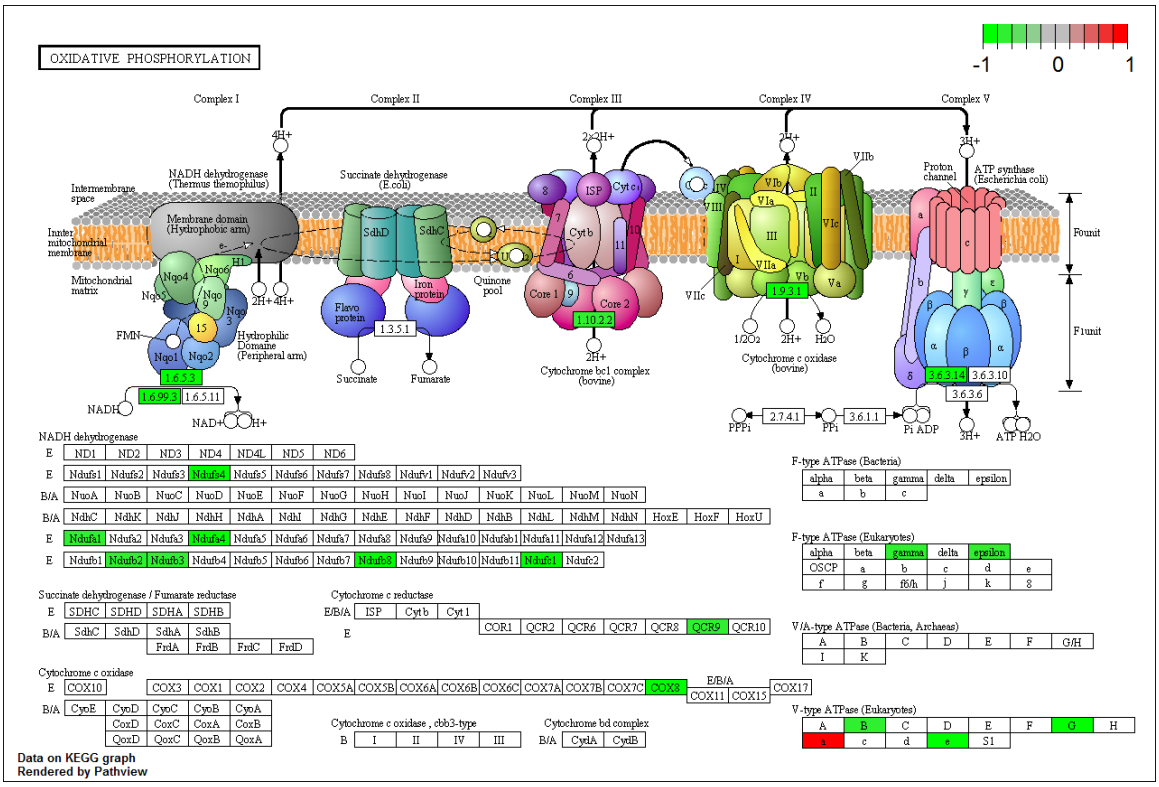
Supplementary Information

Biochemical re-programming of human dermal stem cells to neurons by increasing mitochondrial membrane potential

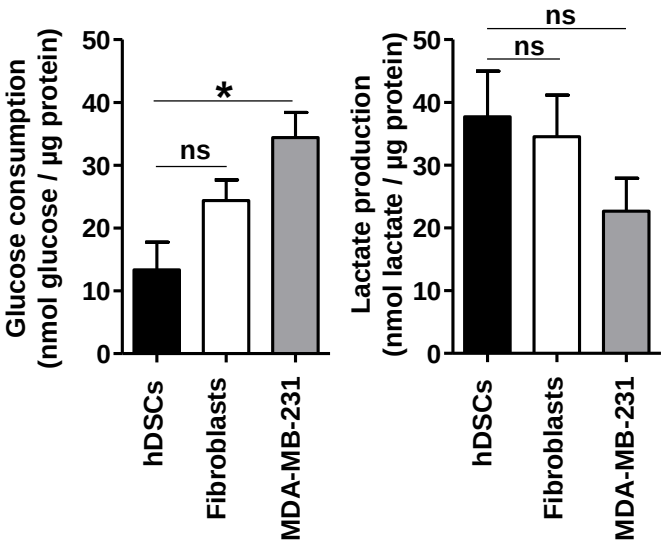
He Liu, Zhaoyue He, Simon Leonhard April, Marcel Philipp Trefny, Jean-Sébastien Rougier, Souzan Salemi, Radu Olariu, Hans Rudolf Widmer and Hans-Uwe Simon

The supplementary information contains 5 figures and 1 table.

a


OXIDATIVE PHOSPHORYLATION
-1
0
1
Complex I
Complex II
Complex III
Complex IV
Complex V
NADH dehydrogenase (Thermus thermophilus)
Succinate dehydrogenase (E.coli)
Cytochrome bc1 complex (bovine)
Cytochrome c oxidase (bovine)
ATP synthase (Escherichia coli)
Intermembrane space
Inner mitochondrial membrane
Mitochondrial matrix
Membrane domain (Hydrophobic arm)
Hydrophilic Domaine (Peripheral arm)
Quinone pool
Flavo protein
Iron protein
Succinate
Fumarate
NADH
NAD+
FMN
Proton channel
F1unit
Founit
NADH dehydrogenase
Succinate dehydrogenase / Fumarate reductase
Cytochrome c reductase
Cytochrome c oxidase
Cytochrome c oxidase , cbb3-type
Cytochrome bd complex
F-type ATPase (Bacteria)
F-type ATPase (Eukaryotes)
V/A-type ATPase (Bacteria, Archaeas)
V-type ATPase (Eukaryotes)
Data on KEGG graph
Rendered by Pathview

b


Glucose consumption (nmol glucose / µg protein)
Lactate production (nmol lactate / µg protein)
*
ns
ns
ns
0
10
20
30
40
50
hDSCs
Fibroblasts
MDA-MB-231

c

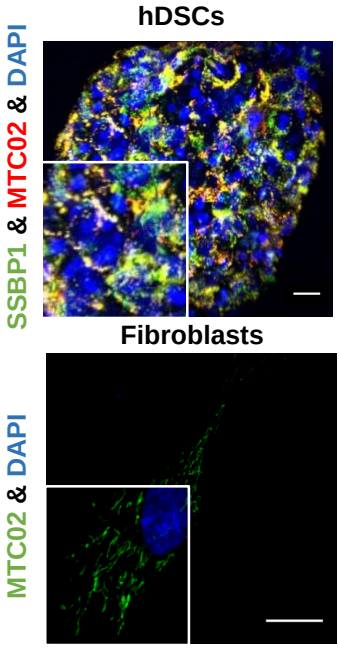

hDSCs
SSBP1 & MTC02 & DAPI
Fibroblasts
MTC02 & DAPI

Fig. S1: hDSCs exhibit an impaired ETC, are glycolytic and show rounded, condensed mitochondria in contrast to autologous fibroblasts with long, tubular mitochondria

(**a**) RNA was isolated from hDSCs and from autologous fibroblasts obtained from 3 different donors and assessed with next generation sequencing. The genes engaged in ETC are presented as a KEGG signaling pathway using the Pathview package. Color code: red and green indicate genes which were more highly or less expressed in hDSCs, respectively. (**b**) Glucose consumption and lactate production were measured in hDSCs and in human fibroblasts as described in the Materials and Methods. The MDA-MB-231 breast cancer cell line, known to rely on glycolysis, serves here as a control. Data shown are means ± SDs of 3 independent experiments with cells from 3 different donors. An unpaired one-way ANOVA with Tukey's multiple comparisons test was performed. (**c**) Representative images of the mitochondria of hDSCs and fibroblasts were obtained by confocal imaging after staining the cells with mitochondrial marker proteins as indicated. The hDSC image shows the mitochondria in cells of an entire spheroid and was rendered with Imaris in 3D from a z-stack of 146 confocal images. The fibroblast mitochondria are illustrated for a single cell. Scale bars: 20 µm.

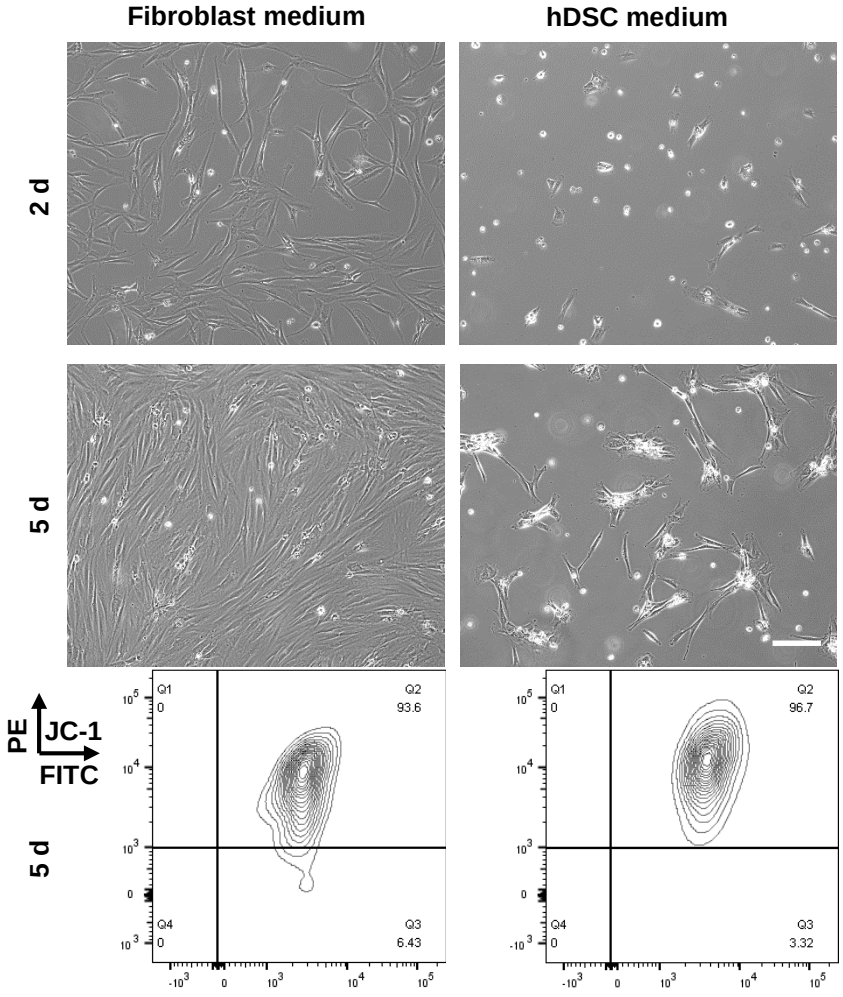

Fibroblast medium
hDSC medium
2 d
5 d
5 d
PE
JC-1
FITC
Q1
0
Q2
93.6
Q4
0
Q3
6.43
Q1
0
Q2
96.7
Q4
0
Q3
3.32

Fig. S2: Human fibroblasts show a homogeneous cell population with high $_{\Delta}\psi_m$ in fibroblast culture media as well as in media used for hDSC culture

Human fibroblasts were cultured in fibroblast media or in the cell culture media used for hDSCs. Cell morphology was observed and JC-1 staining followed by FACS analysis was performed after 5 days in culture. Human fibroblasts show a homogeneous cell population with high $_{\Delta}\psi_m$ under both culture conditions. Scale bar: 100 µm.

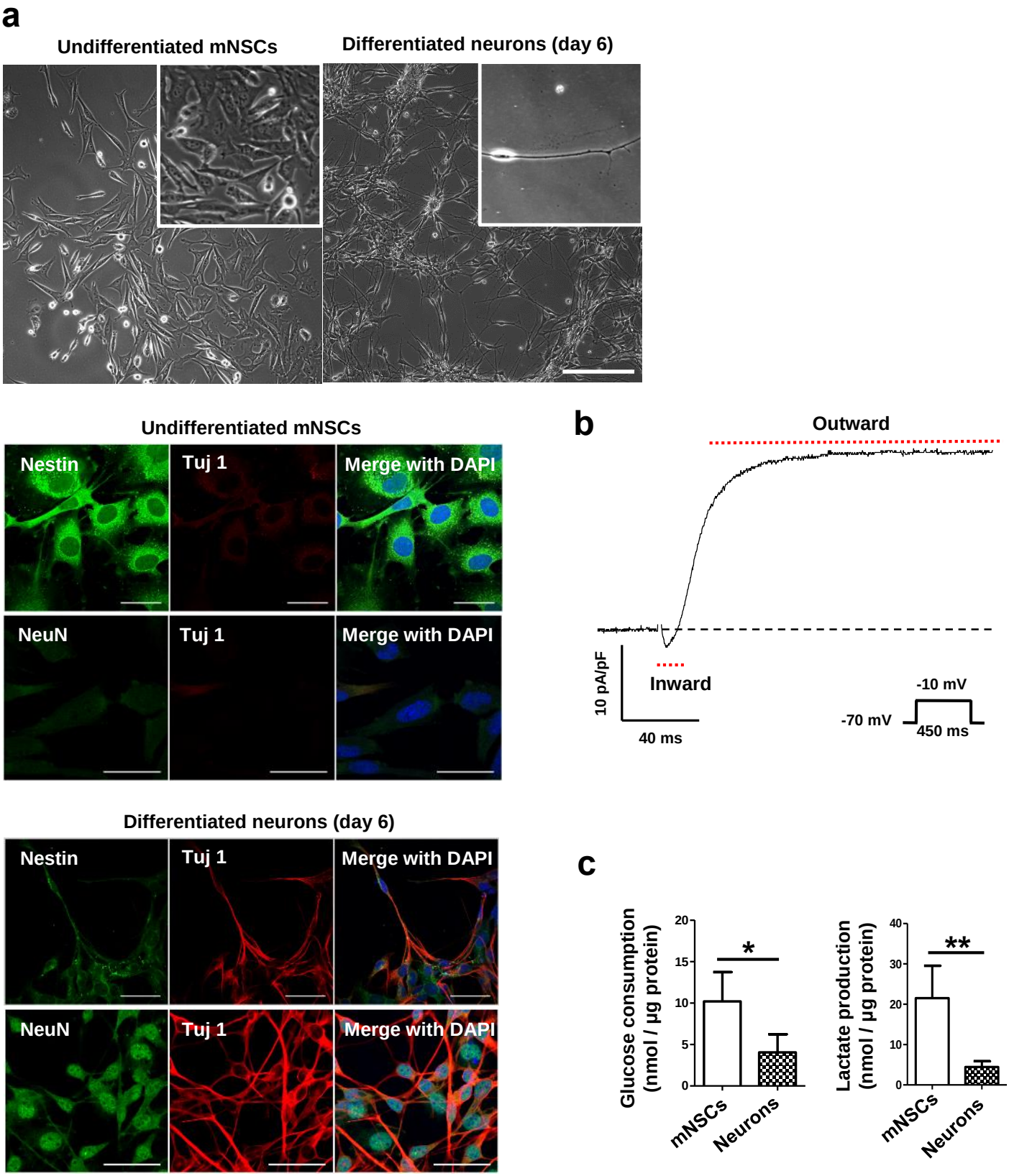
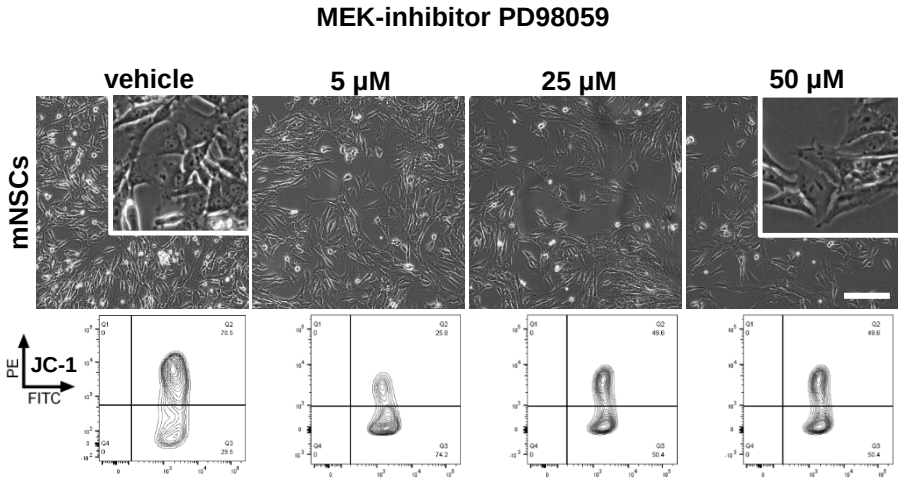

a
Undifferentiated mNSCs
Differentiated neurons (day 6)
Undifferentiated mNSCs
Nestin
Tuj 1
Merge with DAPI
NeuN
Tuj 1
Merge with DAPI
Differentiated neurons (day 6)
Nestin
Tuj 1
Merge with DAPI
NeuN
Tuj 1
Merge with DAPI
b
Outward
Inward
10 pA/pF
40 ms
-10 mV
-70 mV
450 ms
c
Glucose consumption (nmol / µg protein)
20
15
10
5
0
*
mNSCs
Neurons
Lactate production (nmol / µg protein)
40
30
20
10
0
**
mNSCs
Neurons

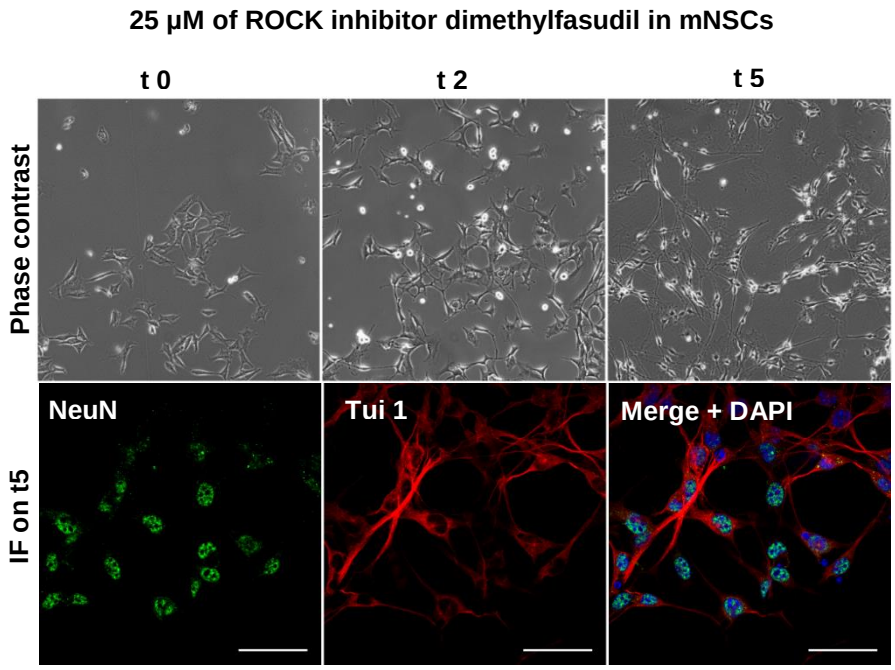
Fig. S3: mNSCs have a larger mitochondrial content and are more glycolytic than differentiated neurons

(**a**) mNSCs were cultured in stem cell medium or neuronal differentiation medium for 6 days before immunofluorescence staining for the stem cell marker Nestin in mNSCs together with the neuronal markers, Tuj1 and NeuN for differentiated neurons. Scale bars: 200 µm (phase contrast) and 50 µm (confocal images). (**b**) Electrophysiological recording of outward and inward currents in differentiated neurons using whole cell patch clamp recording in voltage-clamp mode. (**c**) Glucose consumption and lactate production in mNSCs and differentiated neurons were measured. Data shown are means ± SDs of 3 independent experiments. An unpaired, two-tailed student t test was performed.

a


MEK-inhibitor PD98059
vehicle
5 µM
25 µM
50 µM
mNSCs
PE
JC-1
FITC

b


25 µM of ROCK inhibitor dimethylfasudil in mNSCs
t 0
t 2
t 5
Phase contrast
IF on t5
NeuN
Tui 1
Merge + DAPI

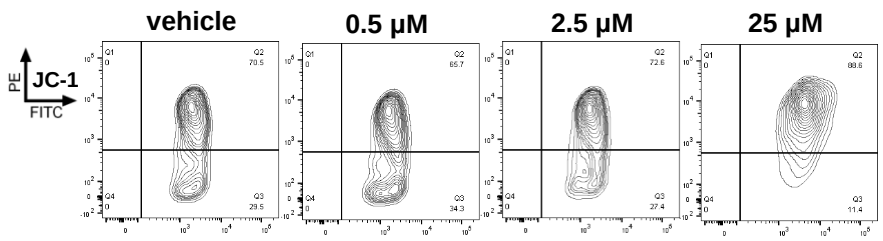

ROCK inhibitor dimethylfasudil in mNSCs
vehicle
0.5 µM
2.5 µM
25 µM
PE
JC-1
FITC

Fig. S4: The MEK inhibitor does not induce neuronal differentiation of mNSCs, nor in contrast to the ROCK inhibitor, does it increase $\Delta\psi_m$

(**a**) mNSCs were treated with different concentrations of the MEK inhibitor for 4 days; cell morphology was observed under the light microscope and mitochondrial polarization was analyzed by JC-1 staining. (**b**) mNSCs were treated with different concentrations of the ROCK inhibitor, dimethylfasudil for 4 days. Cell morphology and the neuron markers, Tuj1 and NeuN were observed after staining. $\Delta\psi_m$ was assessed by JC-1 staining. Tuj1 and NeuN double postive neurons were observed when 25 μM of dimethylfasudil were applied to mNSCs for 4 days accompanied with increased $\Delta\psi_m$.

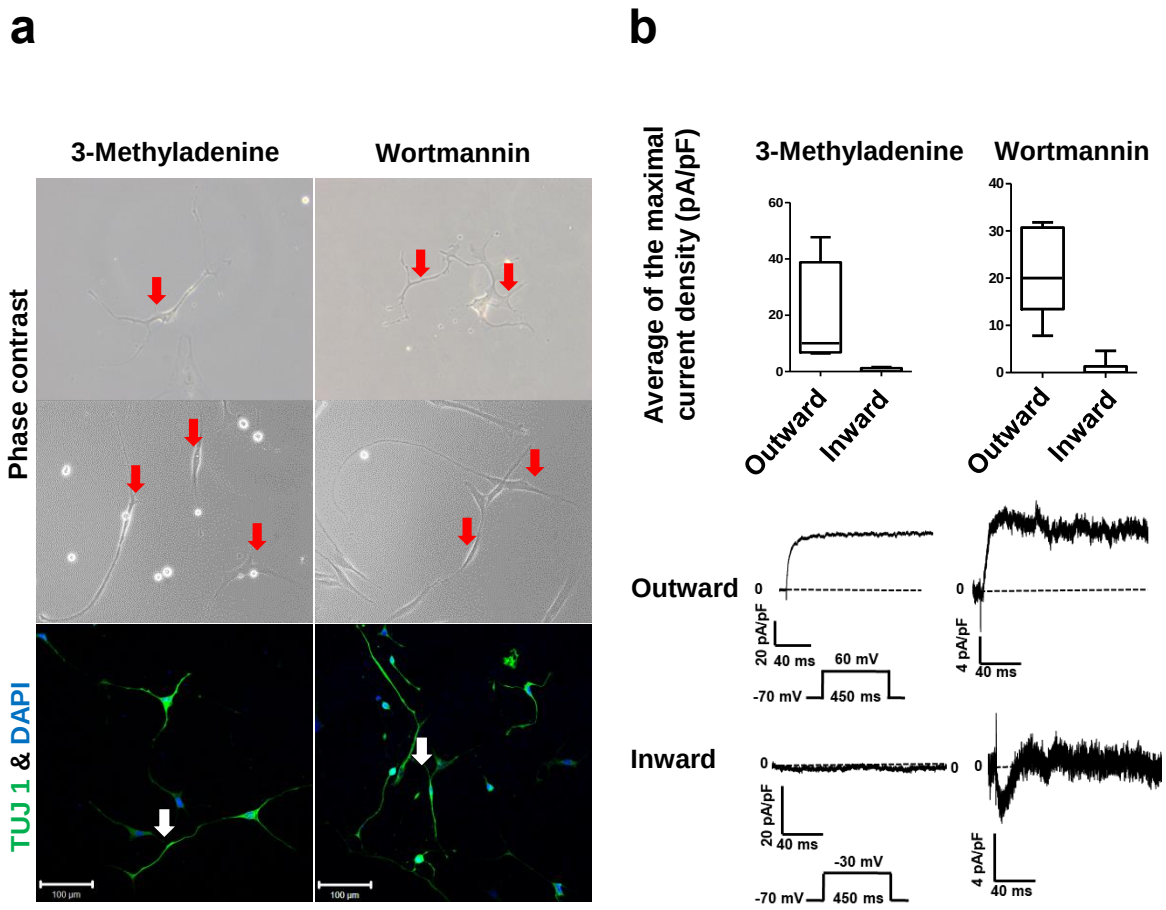

a
3-Methyladenine
Wortmannin
Phase contrast
TUJ 1 & DAPI
100 µm
100 µm
b
Average of the maximal current density (pA/pF)
3-Methyladenine
Wortmannin
60
40
20
0
40
30
20
10
0
Outward
Inward
Outward
Inward
Outward
Inward
0
20 pA/pF
40 ms
60 mV
-70 mV
450 ms
4 pA/pF
40 ms
-30 mV

Fig. S5: Both 3-methyladenine and wortmannin induce neuronal differentiation of hDSCs

(**a**) 10 mM of 3-methyladenine or 50 µM of wortmannin were applied to hDSCs in DMEM/10%FCS for 7 days to evaluate morphological changes in the cells as well as to detect TUJ1 expression. Neuron-like cells are indicated with red arrows and cell-cell contacts are indicated with white arrows. Scale bars: 100 µm. (**b**) Treated cells were examined in patch clamp experiments. Averages for maximal current density (pA/pF) were obtained with whole-cell currents in voltage-clamp mode; cells were held at -70 mV. Thereafter, step depolarization from -90 mV to +60 mV at 10-mV intervals was performed. Representative traces of outward and inward currents are shown. Data were obtained from 3 independent experiments with cells from 3 different donors.

Table S1. The list of 37 mitochondrion-encoding genes that were differentially expressed in hDSCs as compared with fibroblasts

Ensembl gene_ID	HGNC symbol	Start position	End position	Mean TPM (hDSCs)	Mean TPM (fibroblasts)
ENSG00000210049	MT-TF	577	647	6.546253437	4.453279255
ENSG00000211459	MT-RNR1	648	1601	3540.727938	2232.500724
ENSG00000210077	MT-TV	1602	1670	15.65646836	15.5117618
ENSG00000210082	MT-RNR2	1671	3229	16438.36956	15148.46827
ENSG00000209082	MT-TL1	3230	3304	236.8893434	174.1251035
ENSG00000198888	MT-ND1	3307	4262	4959.599944	3705.037042
ENSG00000210100	MT-TI	4263	4331	57.92035175	22.6255555
ENSG00000210107	MT-TQ	4329	4400	9.879680449	3.493799614
ENSG00000210112	MT-TM	4402	4469	26.58710765	16.97342013
ENSG00000198763	MT-ND2	4470	5511	4191.42372	2152.184832
ENSG00000210117	MT-TW	5512	5579	5.723560479	4.792031676
ENSG00000210127	MT-TA	5587	5655	15.47672445	8.811264652
ENSG00000210135	MT-TN	5657	5729	6974.479242	8954.513716
ENSG00000210140	MT-TC	5761	5826	3263.641389	4109.656271
ENSG00000210144	MT-TY	5826	5891	154.3081472	217.764941
ENSG00000198804	MT-CO1	5904	7445	26078.84259	19894.04387
ENSG00000210151	MT-TS1	7446	7514	6.632042157	8.290634983
ENSG00000210154	MT-TD	7518	7585	27.06963649	29.13355298
ENSG00000198712	MT-CO2	7586	8269	19135.83081	16120.31244
ENSG00000210156	MT-TK	8295	8364	20.17834409	16.72521946
ENSG00000228253	MT-ATP8	8366	8572	9145.660901	5720.899929
ENSG00000198899	MT-ATP6	8527	9207	9449.575612	6130.773683
ENSG00000198938	MT-CO3	9207	9990	16895.57068	11414.60903
ENSG00000210164	MT-TG	9991	10058	111.9310765	61.17073927
ENSG00000198840	MT-ND3	10059	10404	6570.507313	4890.69957
ENSG00000210174	MT-TR	10405	10469	372.7425099	218.2913479
ENSG00000212907	MT-ND4L	10470	10766	20368.66284	16249.82546
ENSG00000198886	MT-ND4	10760	12137	7609.246856	6486.99096
ENSG00000210176	MT-TH	12138	12206	551.805587	210.3987301
ENSG00000210184	MT-TS2	12207	12265	240.6736373	111.8878472
ENSG00000210191	MT-TL2	12266	12336	112.545851	34.33214645
ENSG00000198786	MT-ND5	12337	14148	6393.090686	4570.713491
ENSG00000198695	MT-ND6	14149	14673	4562.521656	2703.935053
ENSG00000210194	MT-TE	14674	14742	69.92235622	40.43757188
ENSG00000198727	MT-CYB	14747	15887	9587.458971	6050.60138
ENSG00000210195	MT-TT	15888	15953	30.7348021	27.54944182
ENSG00000210196	MT-TP	15956	16023	4494.546722	5357.410913

HGNC: HUGO Gene Nomenclature Committee