Supplemental Material

BDNF and NT3 Reprogram Human Ectomesenchymal Dental Pulp Stem Cells to Neurogenic and Gliogenic Neural Crest Progenitors Cultured in Serum-Free Medium

Jon Luzuriaga^a Jose Ramon Pineda^{a,b} Igor Irastorza^a Veronica Uribe-Etxebarria^a Patricia García-Gallastegui^a Juan Manuel Encinas^{b,d} Pablo Chamero^c Fernando Unda^a Gaskon Ibarretxe^a

^aDepartment of Cell Biology and Histology, Faculty of Medicine and Nursing, University of the Basque Country, UPV/EHU, Leioa, Spain, ^bAchucarro Basque Center for Neuroscience, UPV/EHU Scientific Park, Leioa, Spain, ^cLaboratoire de Physiologie de la Reproduction et des Comportements UMR 0085 INRA/CNRS/IFCE/Université de Tours, Nouzilly, France, ^dIkerbasque, The Basque Foundation for Science, Bilbao, Spain



Supplementary Figure 1: Alizarin Red analysis of differentiation to osteogenic lineage cells of hDPSCs grown in DMEM+10% FBS, STP and STP/NTF2 media

After a preconditioning growth phase of hDPSCs in different culture media, cells were cultured in parallel and were subjected to osteogenic induction by adding dexamethasone, β glycerolphosphate and ascorbate in the presence of DMEM+10 % FBS for 14 days. Alizarin Red staining was performed to detect calcified matrix (red coloration). hDPSCs showed the capacity to generate a bone matrix only when they had previously been cultured with DMEM+10% FBS. In contrast, hDPSCs pre-cultured either with raw STP or BDNF/NT-3-supplemented STP did not generate calcified spots. **b)** Quantification of Alizarin Red absorbance for the different culture media conditions. Statistics were analyzed by ANOVA following by Scheffe post-hoc analysis *=p<0.05.



Supplementary Figure 2: Neural lineage characterization of hDPSCs after 3 weeks in Neurocult being preconditioned with BDNF/NT-3. a) Co-immunostaining for mature neuronal marker MAP-2 (left) and tyrosine hydroxylase (TH -middle) marker of dopaminergic neurons. b) GABA staining for GABAergic neuronal fate. Quantification showing the percentage of positive cells for (c) Map2, (d) TH and (e) GABA. Statistical analysis by one-way ANOVA followed by Scheffe post-hoc test. *=p<0.05, **=p<0.01 and ***=p<0.001. (n=575 for DMEM, 113 for STP and 136 cells for MAP-2/TH staining and n=988, n=148 and n=89 for GABA immunostaining). Experiments were performed in triplicate.

Supplementary Movie 1: Video of recording of Ca^{+2} influx after 30 s of 50 μ M KA. Arrows mark responding cells.

To see the video, please use the following link: <u>https://www.cellphysiolbiochem.com/Articles/000096/SM/Supplementary movie 1.avi</u>

Supplementary Movie 2: Video of recording of Ca⁺² influx after 30 s of 100 μ M ATPyS. Arrows mark responding cells.

To see the video, please use the following link: <u>https://www.cellphysiolbiochem.com/Articles/000096/SM/Supplementary movie 2.avi</u>

Supplementary Movie 3: Video of recording of Ca^{+2} influx after 30 s of specific L-type VDCCs agonist 10 μ M Bay-K 8644. Arrows mark responding cells.

To see the video, please use the following link: <u>https://www.cellphysiolbiochem.com/Articles/000096/SM/Supplementary movie 3.avi</u>

Supplementary Movie 4: Video of recording of Ca⁺² influx after 30 s of vehicle addition. Arrows label cells with spontaneous Ca+2 diffusion through GAP junctions.

To see the video, please use the following link: <u>https://www.cellphysiolbiochem.com/Articles/000096/SM/Supplementary movie 4.avi</u>

Supplementary Movie 5: Video recording of both hDPSCs cultures DMEM+10% FBS and STP, showing Ca²⁺ waves triggered by application of kainate, ATPγS and Bay-K 8644 to confluent hDPSC cultures in both DMEM+10% FBS and STP conditions.

To see the video, please use the following link: <u>https://www.cellphysiolbiochem.com/Articles/000096/SM/Supplementary movie 5.avi</u>

Supplementary Movie 6: Video Video recording of hDPSCs cultures with STP+NTP, showing Ca2+ waves triggered by application of kainate, Bay-K 8644 and ATPγS to hDPSC cultures.

To see the video, please use the following link: <u>https://www.cellphysiolbiochem.com/Articles/000096/SM/Supplementary movie 6.avi</u>