

Figure S1. The effect of prolonged and 3D culture onto pluripotent (A), chondrogenic signalling pathways (B, C) and mesenchymal condensation (D, E) gene markers. RT-qPCR represents gene expression of markers specific to chondrogenesis during of chondrogenic differentiation. As a control the HC-402-05a was used and non-differentiated pluripotent stem cells. The error bars represent SD from three experiments. The statistical analysis was performed using ANOVA with Tukey's post-hoc multicomparison test (* - p<0,05). ChD – Chondrogenic differentiation; 2D – two-dimensional culture; 3D – three-dimensional culture.

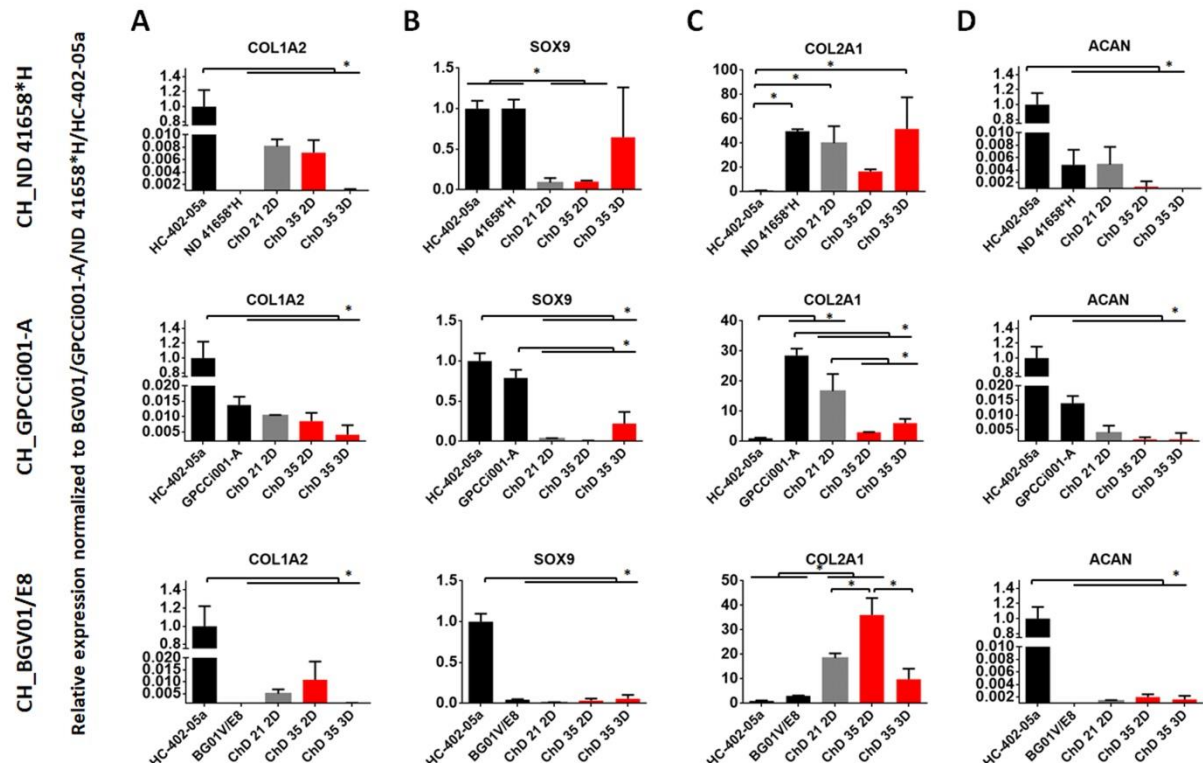


Figure S2. The effect of prolonged and 3D culture onto expression of chondrogenic markers. RT-qPCR represents gene expression of markers specific to chondrogenesis during chondrogenic differentiation (A, B, C & D). As a control the HC-402-05a was used and non-differentiated pluripotent stem cells. The error bars represent SD from three experiments. The statistical analysis was performed using ANOVA with Tukey's post-hoc multicomparison test (* - $p < 0,05$). ChD – Chondrogenic differentiation; 2D – two dimensional culture; 3D – three-dimensional culture.

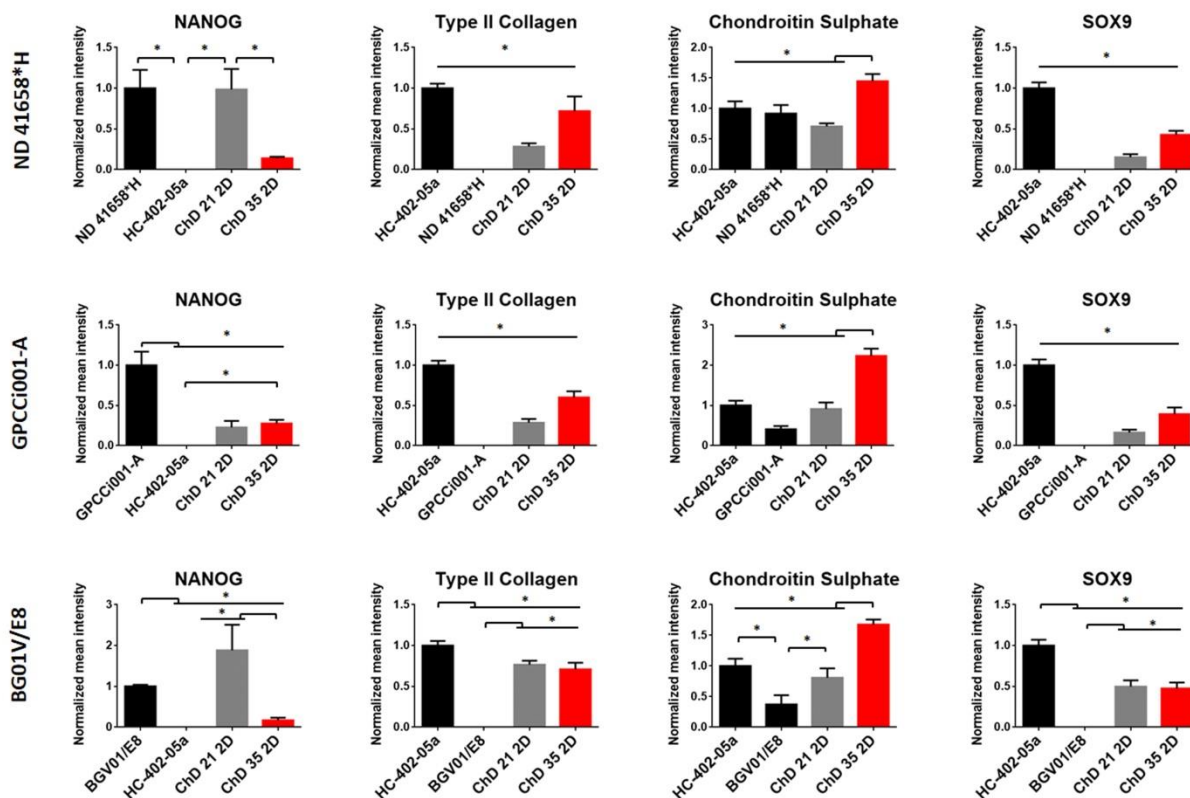


Figure S3. The semi-quantification of immunofluorescence staining for pluripotent and chondrogenic markers of differentiated and controls cell populations. Decreased mean intensity of NANOG and type II collagen and SOX9 of differentiated PSC populations was observed in comparison with controls. The chondroitin sulphate intensity was the highest at ChD 35 of PSC chondrogenic differentiation. As a control the HC-402-05a was used and non-differentiated pluripotent stem cells. The error bars represents SD from three experiments. The statistical analysis was performed using ANOVA with Tukey's post-hoc multicomparison test (* - $p < 0,05$). ChD – Chondrogenic differentiation; 2D – two dimensional culture; 3D – three-dimensional culture.

Table S1. List of primers

Target mRNA	Forward Sequence	Reverse Sequence	Reference sequence	Probe no.
ACAN	cgatgaatcagaatcaactgctg	gtgtccctctgtctccttgc	NM_013227.3	31
CDH1	aagttttccacaaagtcacg	tgcttgattccagaaacg	NM_004360.3	77
CDH2	atccgacgaatggtgaaag	ctgtgggggtcattgtcagc	NM_001792.3	45
COL1A2	ggcagtgatggaagtgtgg	ccaacagctccaatttcacc	NM_000089.3	45
COL2A1	ttctggagaccaaggtgctt	ttcattagcaccatctttgc	NM_001844.4	67
FGFR3	agaggccacacttcaagc	cgacaggtccagggtactcgt	NM_000142.4	22

<i>NANOG</i>	atgcctcacacggagactgt	aagtgggtgtttgccttg	NM_024865.2	31
<i>NCAM1</i>	cgaccatccacctcaaagtc	cggaggcttcacaggttaaga	NM_000615.6	68
<i>OCT4</i>	cttcggatttcgccttctc	cttagccaggtccgaggat	NM_002701.4	77
<i>SMAD3</i>	caccacgcagaacgtcaa	gatgggacacctgcaacc	NM_005902.3	9
<i>SOX2</i>	tgcctctttaagactaggactgaga	gccgccgatgattgttatta	NM_003106.3	45
<i>SOX9</i>	ctcgccacactcctctc	cgcttcaggtcagccttg	NM_000346.3	77

Aggrecan ACAN; E-cadherin – CDH1; N-cadherin – CDH2; Type 1 collagen - COL1A2; Type II collagen - COL2A1; Fibroblast Growth Factor Receptor 3 - FGFR3; Neural Cell Adhesion Molecule 1 - NCAM1; Octamer-binding transcription factor 4 – OCT4; SMAD Family Member 3 - SMAD3; SRY (sex determining region Y)-box 2 – SOX2; SRY (sex determining region Y)-box 9 - SOX9.

Table S2. List of antibodies

Primary antibodies	Host / Isotype	Vendor	Dilution
NANOG	Mouse / IgG1	BD Pharmigen, CA, USA	1:50
SOX9	Rabbit / IgG	Merck-Millipore, Darmstadt Germany	1:125
Type II collagen	Rabbit / IgG	Abcam, Cambridge, UK	1:100
Chondroitin Sulphate	Mouse / IgM	Abcam, Cambridge, UK	1:100
Secondary antibodies	Host / Isotype	Vendor	Dilution
Anti-Mouse IgG conjugated with Alexa Fluor 488	Donkey/ IgG	Jackson ImmunoResearch, PA, USA	1:500
Anti-Rabbit IgG conjugated with Alexa Fluor 488	Donkey/ IgG	Jackson ImmunoResearch, PA, USA	1:500
Anti-Mouse IgM conjugated with Alexa Fluor 488	Donkey/ IgG	Jackson ImmunoResearch, PA, USA	1:500