SUPPLEMENTAL MATERIAL

Cleavage of E-cadherin and β -catenin by calpain affects Wnt signaling and spheroid formation in suspension cultures of human pluripotent stem cells

Sarah A. Konze^{1,2}, Laura van Diepen³, Anke Schröder⁴, Ruth Olmer^{2,5}, Hanna Möller^{1,2}, Andreas Pich⁴, Robert Weißmann³, Andreas W. Kuss³, Robert Zweigerdt^{2,5} & Falk F.R. Buettner^{1,2,*}

¹Institute for Cellular Chemistry, Hannover Medical School, 30625 Hannover, Germany

²REBIRTH Cluster of Excellence, Hannover Medical School, 30625 Hannover, Germany

³Institute for Human Genetics & Institute for Genetics & Functional Genomics, Ernst-Moritz-Arndt University,

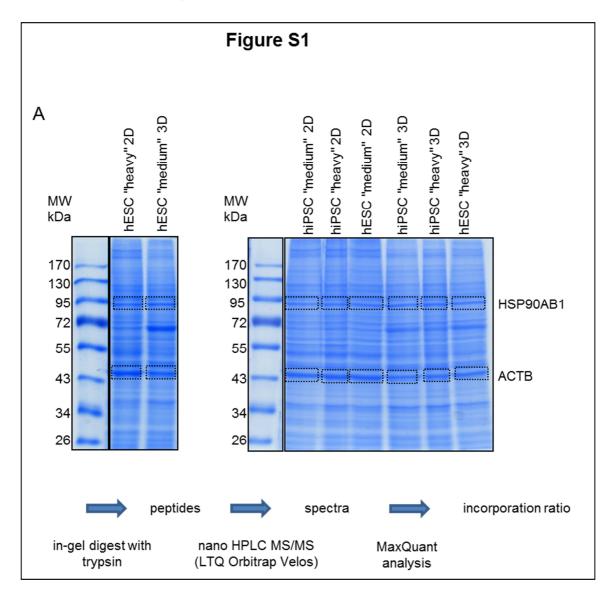
17475 Greifswald, Germany

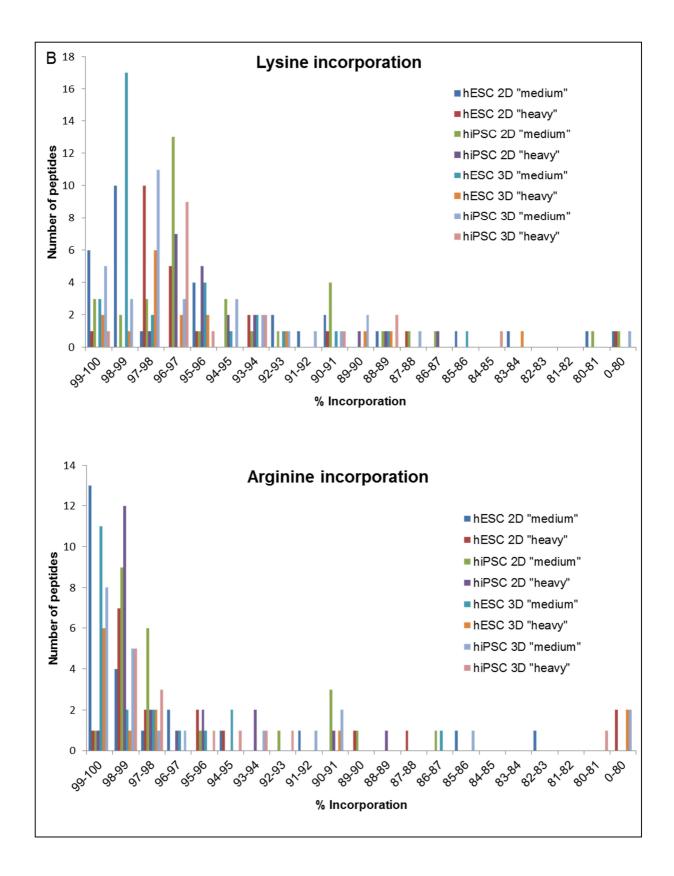
⁴Institute of Toxicology, Hannover Medical School, 30625 Hannover, Germany

⁵Leibniz Research Laboratories for Biotechnology and Artificial Organs, Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover Medical School, 30625 Hannover, Germany

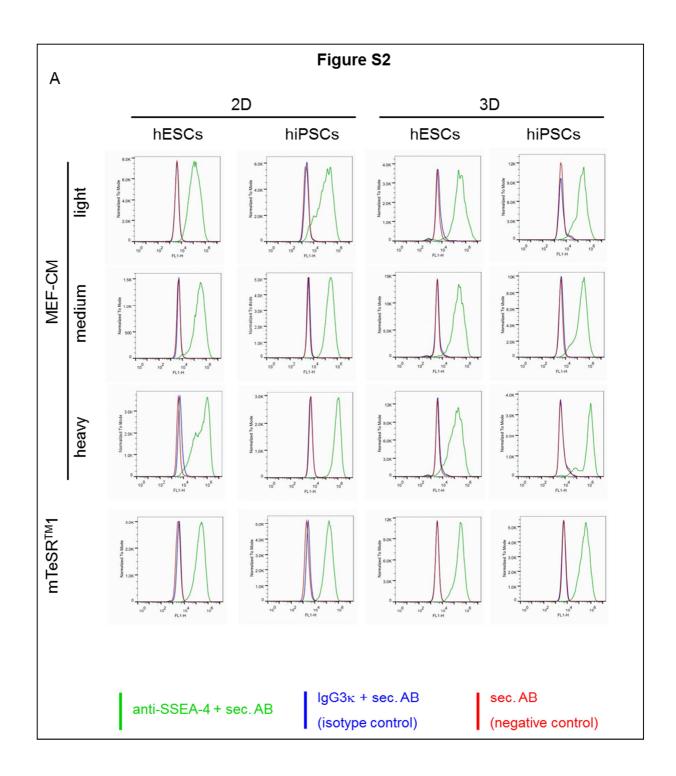
*To whom correspondence should be addressed: Institute for Cellular Chemistry, Hannover Medical School, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany. Tel.: +49(0)511/532-8245; Fax: +49(0)511/532-8801; E-mail: buettner.falk@mhhannover.de

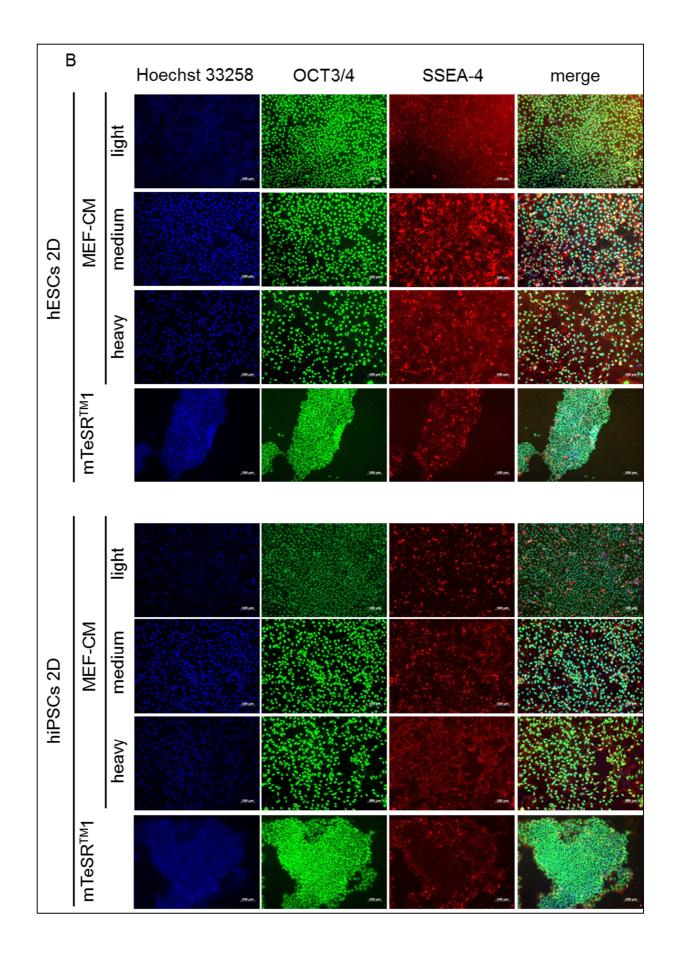
Supplementary Figures

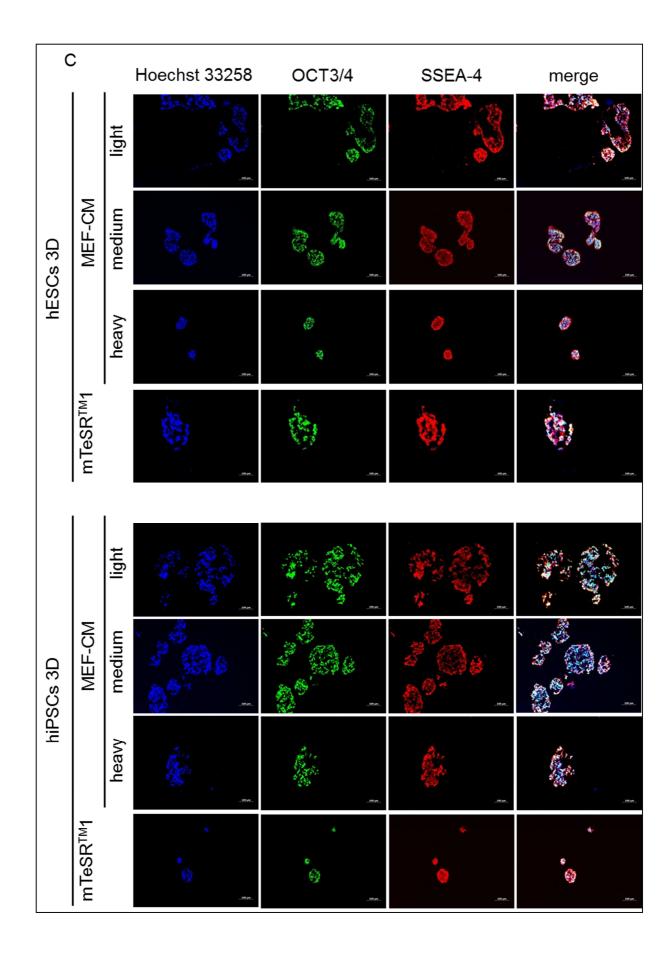




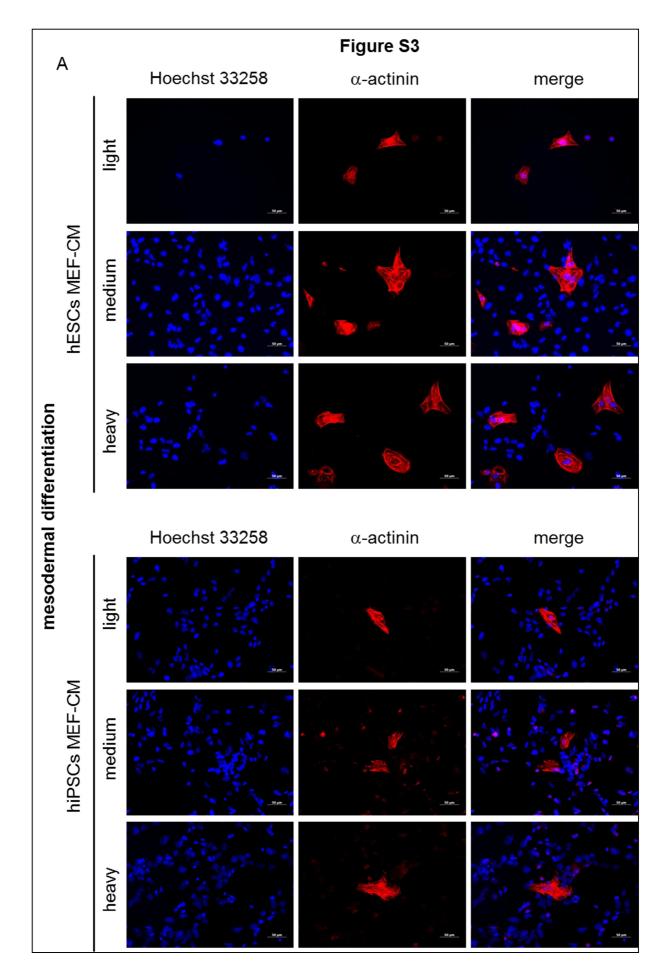
SUPPL. FIG. S1, related to Fig. 2. **Determination of SILAC incorporation efficiencies in hPSCs.** *A*, 10 µg of each "medium" or "heavy" SILAC labeled protein preparation of hESCs and hiPSCs grown under 2D or 3D conditions were separated by SDS-PAGE on 10% gels. Two distinct bands were cut out per lane, pooled and measured by LC-MS/MS. MaxQuant based analysis led to the identification of β -actin (ACTB) and heat-shock protein HSP 90- β (HSP90AB1). *B*, peptide lists were extracted for ACTB and HSP90AB1 from MaxQuant and incorporation efficiencies were calculated from the MaxQuant-derived ratio values for lysine and arginine terminated peptides, respectively. Incorporation efficiency = (1-1/(ratio+1)) x 100%. Peptides were assigned to categories representing the percentage of label incorporation.

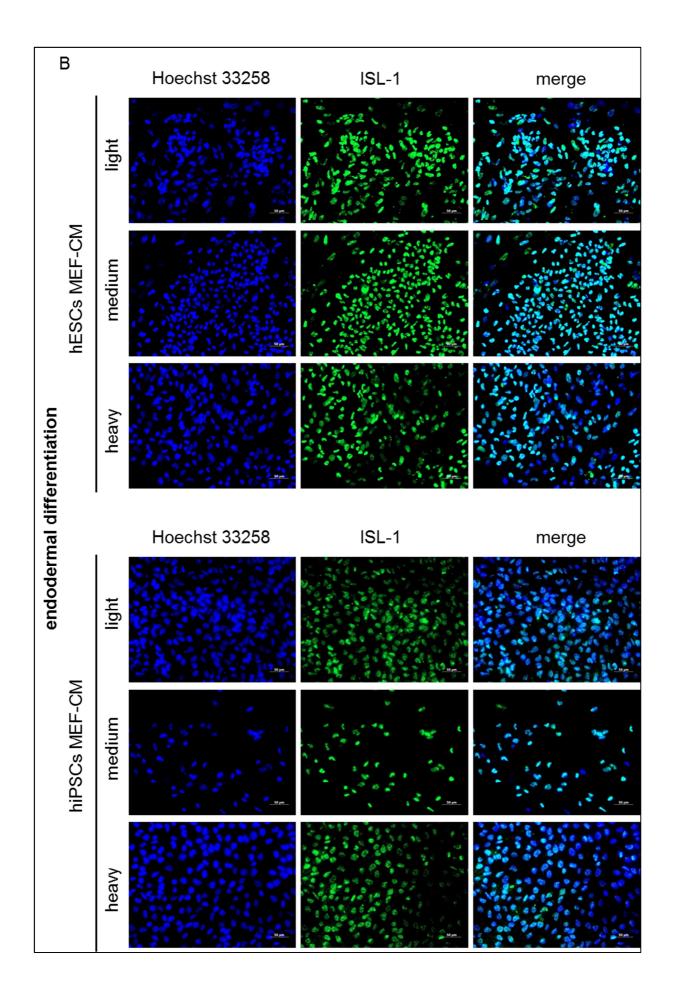


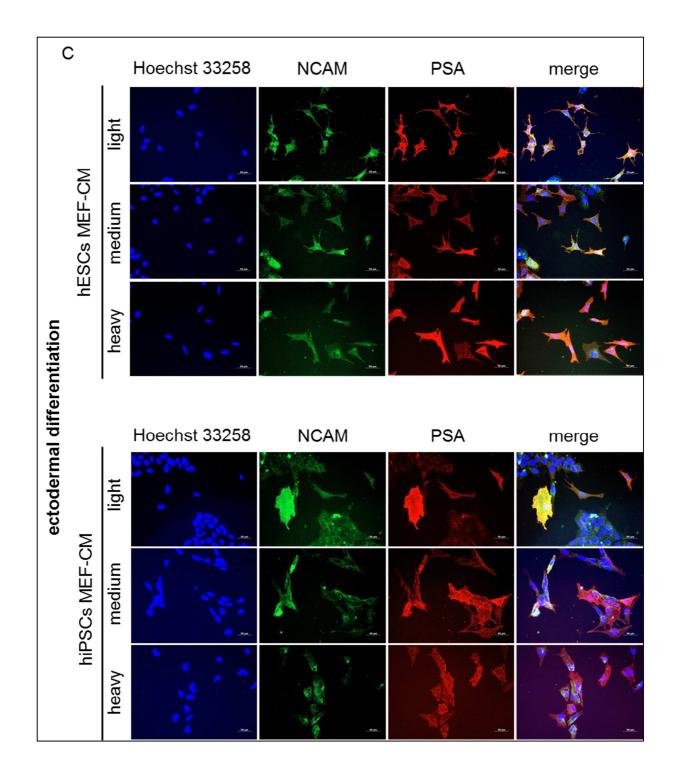


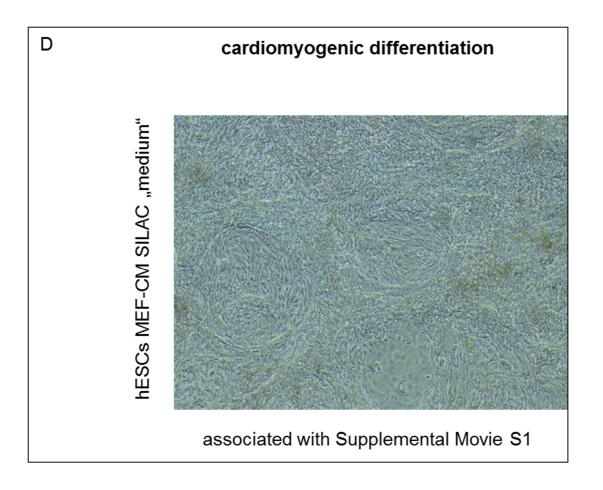


SUPPL. FIG S2 related to Fig. 2. **Analysis of pluripotency markers of hESCs and hiPSCs grown in 2D or 3D by flow cytometry and immunofluorescence microscopy.** *A*, analysis of SSEA-4 expression for hESCs and hiPSCs grown under 2D or 3D conditions by flow cytometry. The analysis was performed for each SILAC labeling condition (MEF-CM "light", "medium", and "heavy") and mTeSR[™]1 as a positive control. The green line represents the experimental analysis. The isotype control and the negative control are represented by the blue line and the red line, respectively. *B* and *C*, immunofluorescence microscopy for OCT3/4 and SSEA-4 expression in hESCs and hiPSCs grown under 2D ("*B"*) or 3D ("*C"*) conditions for each SILAC labeling condition (MEF-CM "light", "medium", and "heavy") and mTeSR[™]1 as a positive control. The scale bar represents 100 µm. hESCs and hiPSCs in SILAC MEF-CM including the Rho kinase inhibitor Y27632 (RI) grew as a monolayer under 2D conditions whereas hPSCs in mTeSR[™]1 without RI showed typical colony morphology.

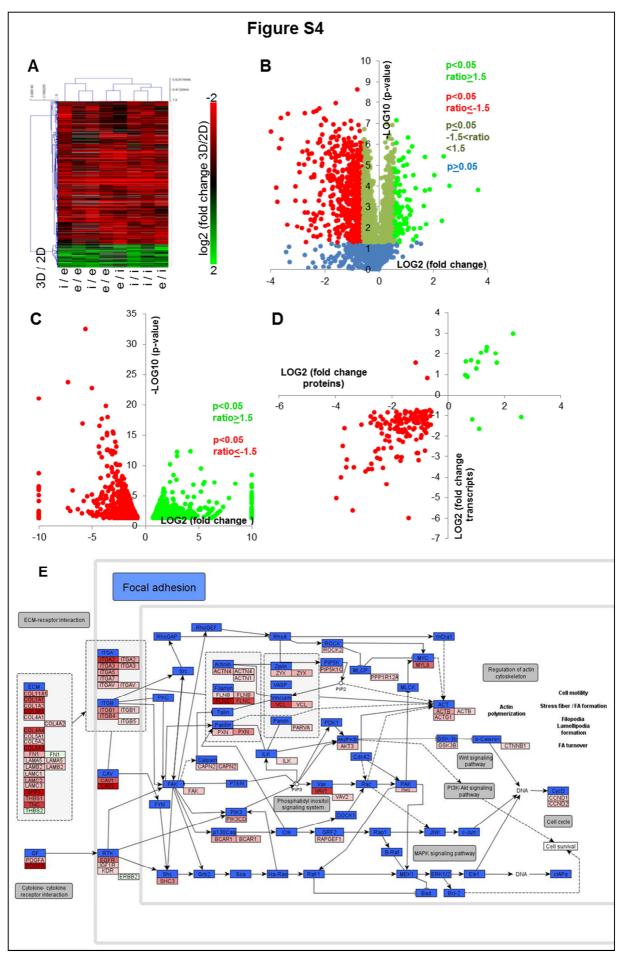






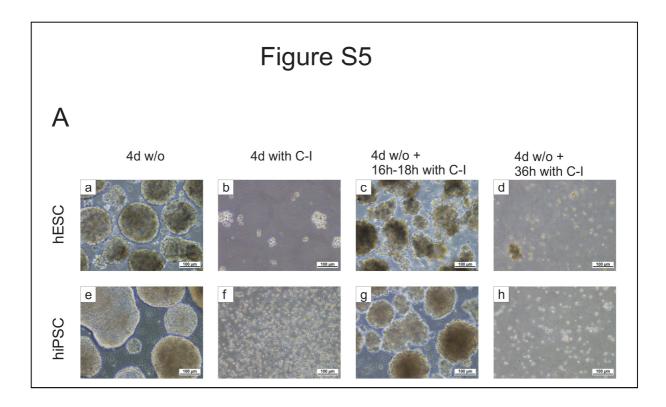


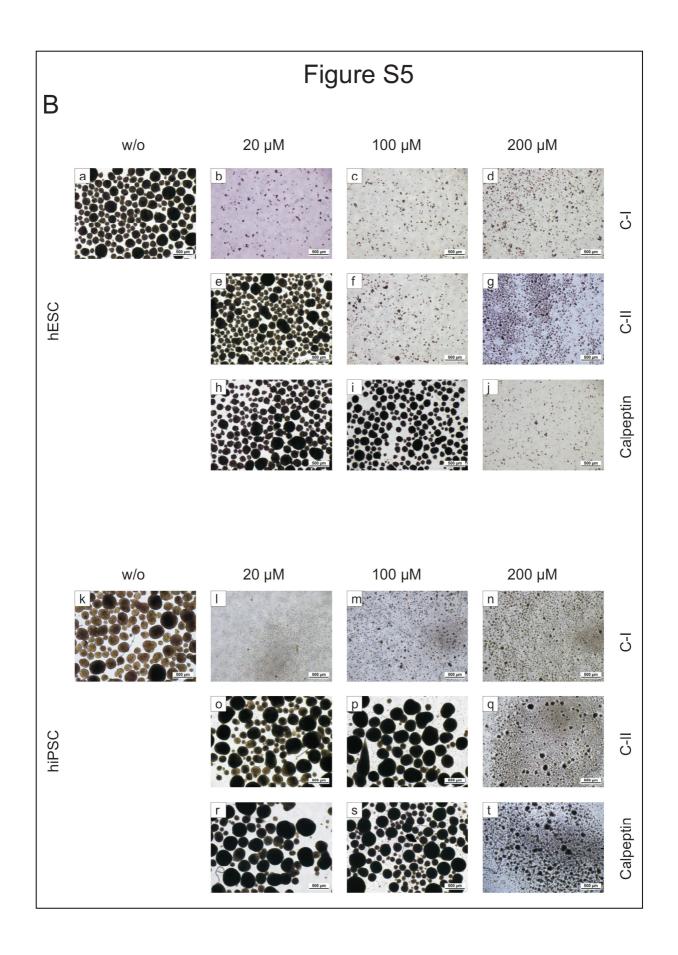
SUPPL. FIG S3 related to Fig. 2. Differentiation of SILAC-labeled cells into derivatives of all three germ layers. *A*, immunofluorescence microscopy for expression of the skeletal and cardiac muscle (mesoderm)-specific marker α -actinin after cardiomyogenic differentiation of SILAC-labeled hESCs and hiPSCs. The analysis was performed for each SILAC labeling condition (MEF-CM "light", "medium", and "heavy"). *B*, immunofluorescence microscopy for analysis of expression of the mesodermal/endodermal marker ISL-1 in hESCs and hiPSCs after initiation of endodermal differentiation. C, immunofluorescence microscopy for expression of the neuroectodermal markers neural cell adhesion molecule (NCAM) and polysialic acid (PSA). The scale bar represents 50 μ m (A-C). D, light microscopy of SILAC labeled hESCs upon cardiomyogenic differentiation at 10-fold magnification. Supplemental Movie S1 (movieS1.mov) is associated with this figure showing autonomous contraction typical for cardiomyocytes. Beating cells do also develop from "light" and "heavy" labeled hESCs as well as from SILAC labeled hiPSCs upon cardiomyogenic differentiation (data not shown).

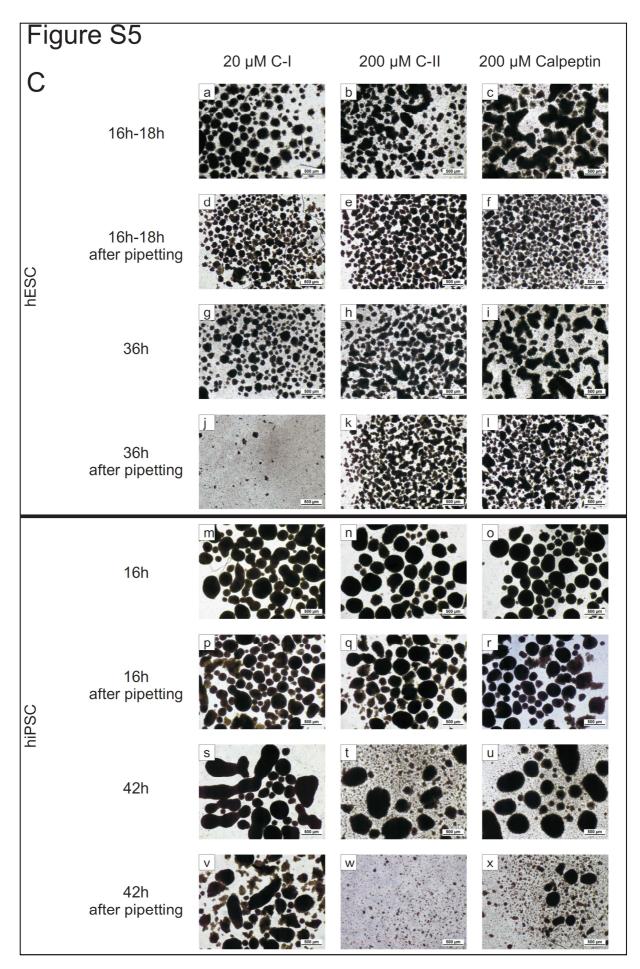


SUPPL. FIG S4 related to Fig. 3 and 4. Bioinformatic analysis of proteomics and transcriptomics for hESCs and hiPSCs being taken together as hPSCs. A, proteins significantly up- (green) or downregulated (red) comparing 3D vs. 2D culture with hESCs and hiPSCs being analyzed together. From the pooling of the differentially SILAC-labeled samples eight ratios of a 3D sample versus a 2D sample could be directly obtained from the MaxQuant analysis (four intra-cell-line comparisons [hESC 3D vs. hESC 2D or hiPSC 3D vs. hiPSC 2D] and four inter-cell-line comparisons [hESC 3D vs. hiPSC 2D or hiPSC 3D vs. hESC 2D] (Fig. 1)). The heatmap was generated with MeV V4.8.1 (http://www.tm4.org/mev/) from the eight 3D vs. 2D ratios for hESCs (e) and hiPSC (i) of 716 proteins that were significantly (p<0.05) regulated above the threshold of 1.5-fold and found in at least four comparisons. Significance and mean ratio were calculated from the eight different comparisons as indicated. Ratios were log2 transformed and hierarchical clustering was performed using "Pearson Uncentered" as "Distance Metric Selection". B, Volcano plot of 3521 different proteins identified by two or more peptides in at least four out of eight comparisons in a combined analysis of hESCs and hiPSCs. C, Volcano plot of 1298 different transcripts that were significantly (p<0.05) up- (504) or down- (794) regulated (>1.5-fold) in a combined analysis of hESCs and hiPSCs. D, comparison of ratios of the proteomic as well as the transcriptomic approach for proteins or transcripts that were identified significantly (p<0.05) above 1.5-fold by both approaches. E, representation of the KEGG pathway "focal adhesion" (blue boxes) including gene expression data (boxes directly below the blue boxes) and protein expression data (boxes shifted to the right below the blue boxes). Upregulation in 3D culture is indicated by greenish boxes, downregulation by reddish with the intensity of the green or red colour depending on the height of regulation. InCroMAP 1.4 software (http://www.ra.cs.uni-tuebingen.de/software/InCroMAP/) was used for importation of the KEGG pathway map as well as for importation and visualization of expression data. Final graphical layout was done manually with yEd Graph Editor Software Version 3.10.1 (http://www.yworks.com/de/products_yed_about.html).

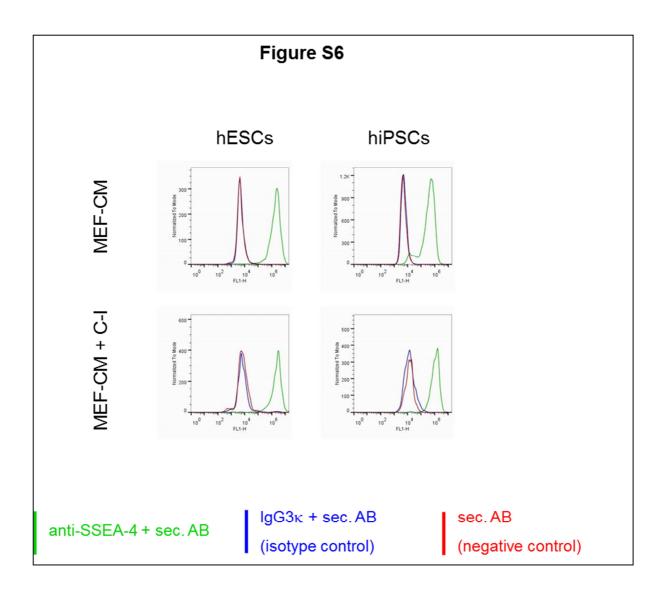
14







SUPPL. FIG S5, related to FIG. 7. Effects of calpain inhibitors on spheroid genesis and on pre-formed spheroids. A, this Figure is equivalent to Fig. 7, but pictures were taken at 10-fold magnification. B, addition of calpain inhibitors to the inoculum of suspension cultures inhibited spheroid formation. The suspension culture inoculum of hESCs or hiPSCs was supplemented with 20 µM, 100 µM or 200 µM of calpain inhibitor I (C-I), calpain inhibitor II (C-II) or calpeptin. For hESCs 20 µM C-I (b), 100 µM C-II (f) and 200 µM calpeptin (j) were sufficient to completely block spheroid formation. In case of hiPSCs spheroid formation was blocked with 20 µM C-I (I), 200 µM C-II (q) and 200 µM calpeptin (t). 100 µM C-II were sufficient to block spheroid formation of hESCs (f) but not of hiPSCs (p). Under standard culture conditions (w/o) spheroids developed as expected and spheroids of hiPSCs were slightly larger than those of hESCs. Solvents had no effect on spheroid formation (data not shown). Images were taken at 4-fold magnification. C, pre-formed spheroids could be disassembled by addition of calpain inhibitors to the culture media. 20 µM calpain inhibitor I (C-I), 200 µM calpain inhibitor II (C-II) or 200 µM calpeptin were added to 4d old pre-formed spheroids of hESCs and hiPSCs. Spheroid integrity was assessed after 16h to 18h and after 36h (hESCs) or 42h (hiPSCs). Images were taken at 4-fold magnification. To show loose cell-cell interactions of spheroids incubated with calpain inhibitors, they were pipetted up and down after the indicated times. Pipetting was not sufficient to disrupt spheroids grown in the absence of calpain inhibitors.



SUPPL. FIG S6 related to Fig. 7. Analysis of pluripotency of hESCs and hiPSCs treated with calpain inhibitor I. *A*, calpain inhibitor I was added to the inoculum of suspension cultures (MEF-CM + C-I). After 24 hours, control cells (MEF-CM) started formation of suspension culture spheroids whereas C-I treated cells remained as single cells. Flow cytometric analysis of hESCs and hiPSCs revealed that expression of SSEA-4 was unchanged in C-I treated cells compared to control. The green line represents the respective hPSC sample signal. The isotype control and the negative control are represented by the blue line and the red line, respectively.

SUPPL. TABLE S1, related to FIG. 1. SILAC-based proteomic results.

This table contains separate Excel sheets for proteins upregulated in 3D (i-iii) for hESCs (i), hiPSCs (ii) and hPSCs (iii, comprising a joint analysis of hESCs and hiPSCs) as well as for proteins downregulated in 3D (iv-vi) for hESCs (iv), hiPSCs (v) and hPSCs (vi). "Protein Names", "Gene Names", "Uniprot", "Peptides", "Sequence Coverage [%]", "Mol. Weight [kDa]", and "PEP" are direct output data of MaxQuant. "Gene Names" were searched at HGNC (HUGO Gene Nomenclature Committee, http://www.genenames.org/) in order to have a nomenclature comparable to transcriptomics data and are shown in row "HUGO Gene Names". "Fold Change 2D/3D" represents the ratiometric mean of the respective protein amount under 2D conditions divided by the value under 3D culture conditions. This value was calculated from the three different biological repeats for hESCs and hiPSCs, each and for hPSCs, which is a joint analysis of hESCs and hiPSCs. Accordingly, the "Standard Deviation" and a "Student's t-Test" were calculated.

SUPPL. TABLE S2, related to FIG. 1. Deep-sequencing-based transcriptomic results.

This table contains separate Excel sheets for transcripts upregulated in 3D (i-iii) for hESCs (i), hiPSCs (ii) and hPSCs (iii, comprising a joint analysis of hESCs and hiPSCs) as well as for transcripts downregulated in 3D (iv-vi) for hESCs (iv), hiPSCs (v) and hPSCs (vi). "id" depicts the nomenclature according to HGNC (HUGO Gene Nomenclature Committee, http://www.genenames.org/). "Base_Mean_2D" and "Base_Mean_3D" is a measure for the transcript amount in the sample. "Fold Change 3D/2D" is calculated as quotient of Base-Mean_3D vs. Base_Mean_2D and "Fold Change 2D/3D as quotient of Base-Mean_2D vs. Base_Mean_3D. Genes for which no transcript was detected either under 3D or 2D conditions are written in italics and the respective "Fold Change" was set to "Inf". The three biological replicates for each, hESCs and hiPSCs, were pooled, and the pools of hESCs and hiPSCs, were analysed individually. Results are shown for hESCs, hiPSCs and for hPSCs,

which is a joint analysis of hESCs and hiPSCs. The "p-Value" rather reflects the technical significance of the analysis than the biological.

Housekeeping genes ARPC1A / actin related protein 2/3 subunit 1A ARPC1A fw GTGGGCACCACACTCATTTCT ARPC1A / actin related protein 2/3 subunit 1A ARPC1A fw GTGGGCACCACACTCATTTCT ARPC1A / actin related protein 2/3 subunit 1A ARPC1A fw GTGGGCACCACACTCATTTCT ARPC1A / actin related protein 2/3 subunit 1A ARPC1A fw GTGGGCACCACACTGCACGAAA SDHB / succinate dehydrogenase complex, SDHB rev CATGTGTGGAAGAGCUGGAAAA SOX2 OCT3/4 / POUSF1 / POU class 5 homeobox 1 OCT4 / rev CTTCCCAAATTGGACCCGTACCC SOX2 / SRY (sex determining region Y)-box 2 SOX2 rev CTGCGTGAGTGGAATCGT TCC SOX2 / SRY (sex determining region Y)-box 2 SOX2 rev CTGCGTGAGTGGAACGCGTACCC LIN28 homolog A LIN28 rw TTGGAGAGAGGGGAAGGG NANOG rev TGCAGTTGGACGAGAGGG TTGGAGGAGAGGGGAAGGG PODXL / podcalyxin-like PODXL rev ACGGGTCGACGGATGG PODXL / podcalyxin-like PODXL rev ACGGGTCGAGAGGGTGG DNMT3B / DNA (cytosine-5-)-methyltransferase 3 DNMT3B rev ATGTGTGCAGAAGGGGTAGTCC CMD1 / ry AAGGCGTCCAGGAGTGGTCACCCA DVMT3B rev ATGGTGCAGAGGGTAGTCCCCCC CMD1 / cyclin D1	Target gene name / full name Primer name Primer sequence (5' to 3')						
ARPC1A / actin related protein 2/3 subunit 1A ARPC1A, tw GTGGAGCACGACTCATTTCT ARPC1A, rev TGATCCTGCTGCCAGCAAAA SDHB, rev TCGATCATCCAAGAAAA SDHB, is uccinate dehydrogenase complex, sDHB, tw CTTCCGAAGACTACTCCAGCAGAAA SDHB, tw CTTCCGAAGACTACTCCAGCACAAAA CT3/4 / POU5F1 / POU class 5 homeobox 1 OCT4 fev CTTCCCAAATAGAACCCCCA OCT4 rev CTTCCCAAATAGAACCCCCA SOX2 / SRY (sex determining region Y)-box 2 SOX2 rev CTCGCGTGATTAGACCCCCA CCCGCGTGATTAGACCCCCA SOX2 / SRY (sex determining region Y)-box 2 SOX2 rev CTCGCGTGATTAGACCCCCA CCCGCGTGATAGCTCCACCC LIN28 / In-28 homolog A LIN28 rev TTTGCCGAGACAGCAGCGCCGC CACACCGGCGCACACAGCGCCACACGC NANOG / Nanog homeobox NANOG rev TGCGATTGCACAGAGCACTGG CDXL, rev CTTGCTCAACTCGTGTCTGCG DNMT3B / DNA (cytosine-5-)-methyltransferase 3 DNMT3B rev ATGGAGAGAGCCCCCA DNMT3B rev ATGGAGAGAGCCCCCA MWt target genes CCND1 / rev AAGCGTCCACGAAAATAGT ETV4 / FAA3 / GGAGCACGGCACACAGAAAATAGT ETV4 / FAA3 / GGAGACAGGCCACGACGAAAATAGT SLUG / Suail homolog 2 SLUG / rev GAAGAGGGCACACGGCACACGAAAATAGT ETV4 / rev CAGCAGGGCACACG							
ARPC1A_rev TGATCCTGCTGCCAGCAAAA Subunit B, iron sulfur (lp) SDHB / w TCTCCGAAGCACATGCAGAG subunit B, iron sulfur (lp) SDHB rev CATGTGTGGAAGAGGGTAGA Putripotency markers OCT3/4 / POUSF1 / POU class 5 homeobox 1 OCT4 / fw AGAAGGAGAAGCUGGAGCAA OCT3/4 / POUSF1 / POU class 5 homeobox 1 OCT4 / rev CTTCCCAAATGGCTGCAACCCCA SOX2 / SRY (sex determining region Y)-box 2 SOX2 / rev CTGCGTGATTAGACCCCCA SOX2 / fw GGACACTGGCTGAATCCCC LIN28 homolog A LIN28 fw ATGGAGAAACCCGGTGACGC NANOG / Nanog homeobox NANOG / w TTGAGGAGCAGACAGGCAGAGTGG NANOG / wanog homeobox NANOG / w TTGAGGAGACAGGCAGAGTGG DNMT3B / DNA (cytosine-5-)-methyltransferase 3 DNMT3B / w AAGTTGTGGCAGAGAGGGTGGT DMMT3B / DNA (cytosine-5-)-methyltransferase 3 DNMT3B / w AAGTTGTGGCAGAGAGGGTGTGTG DIMIT3B / DNA (cytosine-5-)-methyltransferase 3 DNMT3B / w AAGTGTGGCAGAGAGGGGCAGGTGGTGGTGTGGT CCND1 / Cvclin D1 CCND1 / w CTTGTCTGCAGAGGGGCAGGTGGTGTGGAGTGTGTGCT CCND1 / Cvclin D1	Housekee	eping genes					
SDHB_/succinate dehydrogenase complex, SDHB_rev CATGTGTGGAAGAGCATGATGCAGG Subunit B, iron sulfur (lp) Pluripotency markers OCT3/4 / POU5F1 / POU class 5 homeobox 1 OCT4_rev CTTCCCAAATAGAACCCCCA OX2 / SRY (sex determining region Y)-box 2 SOX2 / w GGAACATGGCTGAATCCT TCC SOX2 rev CTTGCGAAAAACCCGGTGAATCCT TCC SOX2 rev CTGGGTGATAGGACCGCGTGAATCCT TCC SOX2 rev CTGGGTGGTGGGAGGG LIN28 / lin-28 homolog A LIN28 rev TTTGCGGGAGCAGCAGCAGCAGGCAGCAGCAGCAGCAGCAGCA	ARPC1A / actin related protein 2/3 subunit 1A						
Subunit B, iron sulfur (lp) SDHB_rev CATGTGTGGAAGAGGGTAGA Pluripotency markers OCT3/4 / POU5F1 / POU class 5 homeobox 1 OCT4_trev AGAAGGAAGCLGGAGCAA OCT4 rev CTTCCCAAATAGAACCCCCA S0X2 / SRY (sex determining region Y)-box 2 S0X2_rev CTGCGTGATTAGGCTCCAACC LIN28 / Im-28 homolog A LIN28_rev TTTGCGTGACGTGGGTGGGTAGGC NANOG / Nanog homeobox NANOG rev TGCAGGACAGGCAGGCAGGCAGGGAGGGG NANOG / Nanog homeobox NANOG rev TGCATTGGACAGGCAGGCAGGCAGGCAGGGAGGGGG PODXL / podocalyxin-like PODXL rev CTTCCACTCTGTGTCTCTGGTGGT DNMT3B / DNA (cytosine-5-)-methyltransferase 3 DNMT3B rev AAGCGGTCCAGGTAGGTCAT CCND1 / Cyclin D1 CCND1_frev AATGTGTGCAGGAGGAGCAGCAGCAGCA CCND1 / cyclin D1 CCND1_rev AAGCGGTCCAGGTAGTCAT c-MYC / v-myc myelocytomatosis viral oncogene c-MYC_rev GTGCTAAGGCAACCAGCACCAGAACT ETV4 / FEA3 / ets variant 4 ETV4_rev CAGCAAGGCACACCAGAACT ETV4 / FEA3 / ets variant 4 ETV4_rev CAGCAAGGCACCCAGAACT CD41 / w AGGGGTCAAGGTCAGGTCAT CD44 rev ATGGGCAGGCACACAACT							
Pluripotency markers OCT3/4 / POU5F1 / POU class 5 homeobox 1 OCT4_frev CTTCCCAAATAGAACCCCGA SOX2 / SRY (sex determining region Y)-box 2 SOX2_rev CTCGCTGATTAGGCTCCAACC LIN28 / Iin-28 homolog A LIN28_rev TTTGAGGAGAAGCCGGTGAATCCT TCC SOX2 / LW GGACACTGGCTGAATCCCAACC LIN28_rev TTTGAGGAGCAGGCGAGGTGG NANOG / Nanog homeobox NANOG rev TTGAGGAGCAGGCAGAGGTGG PODXL / podocalyxin-like PODXL rev TTCGCATTGGTGTCTGTGTGTGTGTG PODXL / podocalyxin-like PODXL rev ACGGGTCCAGGTGGGT DNMT3B / DNA (cytosine-5-)-methyltransferase 3 DNMT3B_rev ATCGAGTTCGACTGGTGGT Wht target genes CCND1 / LV CCND1 / LV AGCGGTCAGGTAGGCAGAGGGCC C/MYC / v-myc myelocytomatosis viral oncogene -CMYC / W CTTCTGTAGGAGGAGAGGCCCACCAGAATT ETV4 / PEA3 / ets variant 4 ETV4_rev CTCGCGAGACGGAGAGGCCCACCAGAATT SLUG / Snail homolog 2 SLUG rev GAAGAGGAGAGAGGCACCAT SLUG / Snail homolog 2 SLUG rev CACGGGCAGACGCACCACATT CD44/ rCD44 antigen, Epican CD44 rev ATTGGGCGAGACGCACCACT CD44/ rev ATTGGGCG							
OCT3/4 / POU5F1 / POU class 5 homeobox 1 OCT4_rev CTTCCCAAATAGAACCUCGAACCCCA SOX2 / SRY (sex determining region Y)-box 2 SOX2 rev CTCCCAAATAGAACCCCCA SOX2 / IN-28 homolog A LIN28 rev TTTGCGTGAGGTGGAATCCT TCC LIN28 / lin-28 homolog A LIN28 rev TTTGCGTGAGGTGGGAATCGC NANOG / Nanog homeobox NANOG rev TTTGCGTGGACAGCAGGCAGCAGGGAGGAGG PODXL / podocalyxin-like PODXL fw ACGCAGCTCGACCGCAGCAGGAGGGG PODXL / podocalyxin-like PODXL rev CTTCTCACTCTGTGTCTCACCGG DNMT3B // NACGytosine-5-)-methyltransferase 3 DNMT3B rev ACTGCAGTCGAGGAGAGGAGGGGC CCND1 / Cyclin D1 CCND1_fw AATGTGTGCAGAAGGAGGACCA CCND1_rev CCND1 / Cyclin D1 CCND1_fw AATGTGTGCAGGAGGAGCACAGAAGTGGACCAT ETV4 / PEA3 / ets variant 4 ETV4 rev CAGGAAGCAGGAGAGGAGGACCCTCCT EVV4 / PEA3 / ets variant 4 ETV4 rev CAGGAGAGGAGAGAGGAGCCACCAGAAATT SLUG / Snail homolog 2 SLUG rev AATGAGAGAGAGAGAGAGGACCACACA SLUG / Snail homolog 2 SLUG rev AATGAGAGAGAGAGAGAGGACCACACA FOSL1 / FOS-like antigen 1 FOSL1 rev CAGGAGCAGGCAGGTGTGGACAACAC <tr< td=""><td>subunit B, iron sulfur (Ip)</td><td>SDHB_rev</td><td>CATGTGTGGAAGAGGGTAGA</td></tr<>	subunit B, iron sulfur (Ip)	SDHB_rev	CATGTGTGGAAGAGGGTAGA				
OCT4_rev CTTCCCAAATTGAACCCCCA SOX2 / SRY (sex determining region Y)-box 2 SOX2_fw GGACACTGGCTGAATCCT TCC SOX2 / Im-28 homolog A LIN28, fw ATGGAGAAAACCCGGTACGC LIN28, lim-28 homolog A LIN28, rev TTGGCTGAGTGTGAGTGGGGGG NANOG / Nanog homeobox NANOG_rev TTGACGAGCAGGCAGGCGGG PODXL / podocalyxin-like PODXL fw ACAGCAGCATCAACTACCCA PODXL / podocalyxin-like PODXL rev CTTGTCGTGTCTGTGTGTGTG DNMT3B / DNA (cytosine-5-)-methyltransferase 3 DNMT3B_rev ATCGAGTCGACAGGGGGC CCND1 / Cyclin D1 CCND1_rev AATGGTGCAGAGGGGCCCCCGGTCAGGTCAGTTCAT c-MYC / v-myc myelocytomatosis viral oncogene c-MYC fw CTTGTCTGAAAGGCCACCAGAAGT ETV4 / PEA3 / ets variant 4 ETV4, fw ATGAGAAACCCTGTGCACAAATT SLUG / rev CAGCAGGGCACCACGGAACT SLUG rev CAGCAGAGGGCACCA EV4 / CD44 antigen, Epican CD44 fw ATTGGCAGAGGGGACCT CD44 fw CD44 / CD44 antigen, Epican CD44 fw ATGGCAGGGCACCCGGAACGACT FOSL1 / FOS-like antigen 1 FOSL1 fw AACTGACGGGCACCCGGAACACT FOSL1 / FW TAGGGTCACAGGGGACCCTGTCGC	Pluripotency markers						
SOX2 / SRY (sex determining region Y)-box 2 SOX2 rev GGACACTGGCTGAATCCT TCC LIN28 / lin-28 homolog A LIN28 rev CTCGCTGATTAGGCTCCAACC LIN28 / lin-28 homolog A LIN28 rev TTTGCGTGAGTGGATGG NANOG / Nanog homeobox NANOG / w TTGACGACAGCAGCAGCAGCAGCAGCAGCATGG PODXL / podocalyxin-like PODXL rev TCTGCACTGTGTCGTCGTGGTGGTGG PODXL / podocalyxin-like PODXL rev CTCTGCACTCTGTGTGTGGTGGTGGT DNMT3B / DNA (cytosine-5-)-methyltransferase 3 DNMT3B rev ACGTCGACGAGAGGGGTC CCND1 / Cyclin D1 CCND1_fw AAGTGTGCAGAAGGAGGGTC c-MYC / v-myc myelocytomatosis viral oncogene c-MYC rev GTCGTAGTCGAGGGTAGTGCAT bomolog ETV4 / PEA3 / ets variant 4 ETV4 rev CTCACGGGAGCACCAGGAAATT SLUG / Snail homolog 2 SLUG rev GACAAGGAGAGAGCCCCATT CD44 / CD44 antigen, Epican CD44 rev ATGGGAGGAGGAGGACGCATT CD41 / FOS-like antigen 1 FOSL1 rev CACTGGTGGTGGAGCA FOSL1 / FOS-like antigen 1 FOSL1 rev CACAGGAGAGAGGCCCCATT CD44 rev ATGGGGGCCCTGGGTGTAGTC L1CAM rev 1 TAGGAGGAGAGAGCCCCCGCATT	OCT3/4 / POU5F1 / POU class 5 homeobox 1						
SOX2 rev CTCGCTGATTAGGCTCCAACC LIN28 lin-28 homolog A LIN28 rw ATGGAGAAAACCCGGGTACGC NANOG / Nanog homeobox NANOG_rw TTTGCGCGAGTGGACGGCCAAGTGG PODXL / podocalyxin-like PODXL_rev TGCATTGGACAAGCAGCTGCCAACTGG PODXL / podocalyxin-like PODXL_rev CTTGTCACTCTGTGTGTGTGTG DNMT3B / DNA (cytosine-5-)-methyltransferase 3 DNMT3B_rev ATCGAGTCCAACTACCCA Beta DNMT3B_rev ATCGAGTCCAAGGGAGGTC CCND1 / Cyclin D1 CCND1_rw AATGTGTGCAGAAGGGAGGTC CCND1 / Cyclin D1 CCND1_rw AATGGTGCAGAAGGGAGGTC CAHYC / v-myc myelocytomatosis viral oncogene c-MYC fw CTTGTGTGAGGGGCACAGA ETV4 / PEA3 / ets variant 4 ETV4, rev CAGCAAGCGCAACCACA SLUG / Snail homolog 2 SLUG / w ACTGCAGGAGAGAGAGACCACA SLUG / Snail homolog 2 SLUG / w ATGGAGAGGAGAGAGAGAGCCATT CD44 / TPCAJ PEAUR ATGGAGAGAGAGAGAGAGAGCACACA FOSL1 / FOS-like antigen 1 FOSL1 rev CATGCAGGGTCTGTGGACT CD44 rev ATGGGGCACGAGCTGTGTGGACT FOSL1 rev CATGGAGGGTCTGTGGAGCT FOSL1 / FOS-like an							
LIN28 / lin-28 homolog A LIN28 / lw ATGGAGAAAACCCGGTACGC NANOG / Nanog homeobox NANOG / lw TTGAGGAGCAGGCAGGAGGG NANOG / podcalyxin-like PODXL / fw TGAGGAGCAGGCAGCAGCAGGCAGGG PODXL / podocalyxin-like PODXL / fw ACAGCAGCAGCAACAACCCCA PODXL / podocalyxin-like PODXL / fw ACAGCAGGCAGCAACAACCACCAACTACCACCA PODXL / fw ACAGCAGGCATCAACTACCCA PODXL / fw ACAGCAGGCATCAACTACCCA DINMT3B / DNA (cytosine-5-)-methyltransferase 3 DINMT3B / lw AAGGTTCGACTGGGTCACAGG DINMT3B / rev ATGGAGCAGGCAGCAGCAGGGGT CCND1 / fw AATGTGGAGGGTCAACGGTCACAGG CCND1 / Cyclin D1 CCND1 / fw AATGTGGAGGGTCAGGGTAGTCAT CCND1 / fw AATGGGCCAGGAGGGGCATAGTCAT CMYC / v-myc myelocytomatosis viral oncogene -MYC / fw CTGCTGAAGGGCCATAGTCAT CTGCTGGAGGCAAAGGCCCACAGG ETV4 / PEA3 / ets variant 4 ETV4 / fw ATGGGAGAGGCCACAGCAGAAGTCGGCAAA ETV4 / fw ATGGGAGAGAGGCCCATAGT SLUG / rev CAGCAAGGCCACGCACAAGC ETV4 / fw ATGGGAGGAGAGGCCATT CD44 / fw TGGGGCAGGCAGGCCATT CD44 / CD44 antigen, Epican CD44 / fw TGGGCGCAGCTGGGCTGGCT	SOX2 / SRY (sex determining region Y)-box 2						
LIN28 rev TITTECGTGAGGTGGATGG NANOG / Nanog homeobox NANOG r. TTGAGGAGCAGGCAGGCAGGG PODXL / podocalyxin-like PODXL rev ACAGCAGCATCAACTACCCA PODXL rev CTTCTCACTCTGTGTCGTG DNMT3B / NAGTTCAACTAACCAAGG DNMT3B / DNA (cytosine-5-)-methyltransferase 3 DNMT3B rev ATCGAGTTCGACTAGGGAGGTC CCND1 / Cyclin D1 CCND1 rev AAGTGTCGAGGTAGGAGGAGGTC c-MYC / v-myc myelocytomatosis viral oncogene c-MYC rev GTGGTGAGCAGGAGGAGCCA ETV4 / PEA3 / ets variant 4 ETV4 rev CTTCTCTGAAAGGCACTCTCCT CD44 / CD44 antigen, Epican CD44 rev ATGGGCAGCAGCAGCAGCACACGACACTG CD44 rev ATGGGCAGCTGTGGTGGTGC L1CAM fw 1 TAGGGCAGCACTGCTGGACT L1/CAM / L1 cell adhesion molecule L1CAM fw 1 TAGGCAGGTCTGGTGC L1CAM fw 1 L1/CAM / L1 cell adhesion molecule L1CAM fw 1 TAGGCAGGACCAGCAGGAGGCCATT CD44 rev TTGGGCAGGTGTGAGAGCCACAC PLAUR / plasminogen activator, urokinase PLAUR rv 1 GCAGGTGCGGGGTGCAGGCTGGT L1CAM fw 1 TAGGCAGGTGGTGAGGCACAC DKK1 / dickkopf 1 homolog DKK1 rev GCAGGTGCAGGGCCATTGAGCCCGGAGACGGGACCAGACGGAGGAGGACCGA DKK4							
NANOG / Nanog homeobox NANOG_fw TTGAGGAGCAGGCAGGCAGTGG PODXL / podocalyxin-like PODXL_rev TGCATTTGGACAGAGCATGG PODXL_rev CTTCTCACTCTGGTGCTCAGG DNMT3B / DNA (cytosine-5-)-methyltransferasa 3 DNMT3B_rev ATCGAGTTCGACTGGTGCTCAGG DNMT3B / DNA (cytosine-5-)-methyltransferasa 3 DNMT3B_rev ATCGAGTTCGACTGGTGGT Wint target genes CCND1 / Cyclin D1 CCND1_rev AAGCGGTCCAGGAGGAGGTC CMYC / v-myc myelocytomatosis viral oncogene c-MYC fw CTTCTGAAAGGCTCCTCCT homolog CTW4 / PEA3 / ets variant 4 ETV4, rev AGCGGACCACAGAGGCCACCA SLUG / Snail homolog 2 SLUG rev GACGAAGGGAGAGAGGAGCACT SLUG / Snail homolog 2 SLUG rev GACTGCAGGAGGTCTGGACCAC GD44 rev ATTGGGCAGCTGTGGCACCA FOSL1 / FOS-like antigen 1 FOSL1 rev AACTGACCGACTTGGCGACCA FOSL1 / FOS-like antigen 1 FOSL1 rev AACTGGCGGTGTGGAGACCT L1CAM fw 1 TATGGGCAGGTCTGGACCA PLAUR // 1 plasminogen activator, urokinase PLAUR fw 1 TAGGAGTACATTGGGAGACCACC PLAUR fw 1 TAGGAGTCAGAGAGAGAGAGAGTG DKK1 / dickkopf homo	LIN28/ IIn-28 homolog A						
NANGG_rev TGCATTTGGAACGAGCATGG PODXL / podocalyxin-like PODXL rev CACGCAGCATCAACTACCCA PODXL rev CTTCTCACTCTGTGTGTGTGTGTGTGTGTGTGTGTGTGTG							
PODXL / podocalyxin-like PODXL_fw ACAGCAGCATCAACTACCAC DNMT3B / DNA (cytosine-5-)-methyltransferase 3 DNMT3B_rw AAGTTCTGCTGCTCACAGG beta DNMT3B_rev ATGGAGTTCGACTTGGTGGT Wnt target genes CCND1 / Cyclin D1 CCND1_fw AATGTGTGCAGAAGGAGGTCCTCAT c-MYC / v-myc myelocytomatosis viral oncogene c-MYC_fw CTTCTCTGAAAGGCTCATCAT c-MYC / v-myc myelocytomatosis viral oncogene c-MYC_rev GTCGTAGTGCAGGCACAA ETV4 / PEA3 / ets variant 4 ETV4 rev CAGCAAGGCCACCAGAAATT SLUG_fw ATGAGGAGCAACACA SLUG_fw ACTACACGCGACACAACA SLUG_fw ACTACACGCGACACACA SLUG_fw ACTACACGCGACTGGACCACA CD44 fw TGGGTTCATAGAAGGCCACTG CD44 fw TGGGTTCATAGAAGGCCACAT CD44 fw TGGGTCATGGAGTCTGCACCA FOSL1 rev ACTGCAGCTGGTGTGC LICAM fw 1 TATGGGCTGGTGTGACT CD44 fw TGGGTTCATAGAAGCCACAC FOSL1 / FOS-like antigen 1 <td< td=""><td>NANOG / Nanog nomeobox</td><td></td><td></td></td<>	NANOG / Nanog nomeobox						
PODXL_rev CTTCTCACTCTGTGTCTGTG DNMT3B / DNA (cytosine-5-)-methyltransferase 3 DNMT3B / w AAGTTTCTGCTGCTCACAGG beta DNMT3B / w AAGTTTCGTGCTCACAGG Wht target genes CCND1 / Cyclin D1 CCND1 / fw AAGCGGTCCAGGTAGTTCAT c-MYC / v-myc myelocytomatosis viral oncogene c-MYC_rev CTTCTCGAAGGCTCATCAT c-MYC / v-myc myelocytomatosis viral oncogene c-MYC_rev CTGCTGTGCAGGGTCATAGT ETV4 / PEA3 / ets variant 4 ETV4 / fw ATGAGAAACCTCTGCGACCA ETV4 / Fw ATGAGAAACCTCTGCGACCA SLUG / rev GAAAGAGGAAGAGGCCACCAGAAATT SLUG / rev GAAAGAGGGAAGAGGCCACTAG CD44 fw ATTGAGCAAGGGCAGGACACCA SLUG / rev GAAAGAGGAAGAGGCCATT CD44 fw ATTGGCCAGGACAGCACCACCA SLUG / rev GAAGAGGGAAGAGGCCACTACA FOSL1 fw AACTGCCGCAGCAGCACCACCA FOSL1 fw AACTGGCCGAGGTCTGTGCACAC FOSL1 fw AACTGGCCGAGGTCTGCTGCACAC FOSL1 fw TATGGCCTTGGT	RODYL / podoooluvin like						
DNMT3B / DNA (cytosine-5-)-methyltransferase 3 beta DNMT3B / rev AAGTTTCTGCTGCTCACAGG Wnt target genes ATCGAGTTCGACTTGGTGTC CCND1 / Cyclin D1 CCND1 / fw AATGGTGTGCAGAAGGAGGTC CCND1 / Cyclin D1 CCND1 / rev AAGCGGTCCAGGTAGTTCAT c-MYC / v-myc myelocytomatosis viral oncogene homolog C-MYC / rev GTCGTAGTCGAGGCCACAGAAGTAGT ETV4 / PEA3 / ets variant 4 ETV4 / rev CAGCAGGCACCACAAAATT SLUG / Snail homolog 2 SLUG / rev GAAGAAGAGGAGAGAGGCCACT CD44 / CD44 antigen, Epican CD44 fw TGGGTCATAGAAGGCCAGCT CD41 / CD44 antigen 1 FOSL1 rev CACGGGACCAGGGCCAGTG FOSL1 / FOS-like antigen 1 FOSL1 rev CACGGGCCCGGGGTCT L1CAM / L1 cell adhesion molecule L1CAM fw 1 TATGGGCTGTGTGGGGGTACAGCTT L1CAM / L1 cell adhesion molecule L1CAM fw 1 GCAGTGCAGGGGGGACCAAC PLAUR / plasminogen activator, urokinase PLAUR fw 1 GCAGTGCAGAGCAGAGGAGGAGCTT DKK4 / dickkopf 1 homolog DKK1 rev TACGAGCAGACAGAGAGAGGAGCTT DKK4 / dickkopf 1 homolog 4 DKK4 rev CACGAGGAGCAGCACATTGGAGACCT DKK4 / dickkopf homolog 4 DKK4 rev	FODAL / podocalyxIII-like						
beta DNMT3B_rev ATCGAGTTCGACTTGGTGGT Wnt target genes CCND1 / Cyclin D1 CCND1_rev AAGCGGTCCAGGAGGTGCTCCCC C-MYC / v-myc myelocytomatosis viral oncogene c-MYC_rev GTCGTAGTCGAGGGTCATAGT bottomolog c-MYC_rev GTCGTAGTCGAGGGTCATAGT ETV4 / PEA3 / ets variant 4 ETV4 fw ATGGGAAAGCCTCTCCGGACCA EUUG / Snail homolog 2 SLUG_fw ACTACAGGCGAACTGGACACA SLUG / Snail homolog 2 SLUG_fw ACTACAGGCGAACTGGACCACA SLUG / Snail homolog 2 SLUG_fw ACTACAGGCGAACTGGACACA SLUG / Snail homolog 2 SLUG fw ACTACAGGCGAACTGGACCACA SLUG / CD44 antigen, Epican CD44 fw TGGGTTCATAGAAGGGCATT CD44 rev ATTGGGCAGGTCTGTGACT FOSL1 fw AACTGACGAGCATGGACACA FOSL1 / FOS-like antigen 1 FOSL1 fw AACTGACGAGCATGGACACAC PLAUR rev 1 TATGGCATTGGGAACCAAC PLAUR fw 1 GCACGAGGTGTGAGACT PLAUR rev 1 TATGGCAGTAGAGCAACACT PLAUR rev 1 TCTCTGAGGAGACAGGTT Wnt antagonists CER1_fw GAAGTACATTGG	DNIMT3B / DNIA (cytosine-5-)-methyltransferase 3	—					
Writ target genes CCND1 / Cyclin D1 CCND1_fw AATGTGTGCAGAAGGAGGTC CCND1_rev AAGCGGTCCAGGTAGTTCAT c-MYC / v-myc myelocytomatosis viral oncogene c-MYC fw CTTCTTGGAAGGCTCCCT c-MYC / v-myc myelocytomatosis viral oncogene c-MYC / w CTTCTTGGAAGGCTCATAGT ETV4 / PEA3 / ets variant 4 ETV4 fw ATGGGCAAGCCCACAGGTCATAGT ETV4 / FeV CAGCAAGGCCACCAGGACACA SLUG fw ACTACAGGCCACCAGGACACA SLUG rev GAAAGAGGAGAGAGGCCATG CD44 / W TGGGTGCACCACA CD44 fw TGGGCAGGCCATG CD44 rev ATTGGGCAGGTCTGCTGCA CD44 rev ACTGGCAGGCTGGCACCAG FOSL1 rev CACTGGCAGGCTGTGCCCGCAGACGACTG CD44 rev ACTGGCAGGCTGTGCTCTCTGCGAC LICAM rev 1 TAGGAGCAGGAGGCACGACCACC PLAUR fv 1 CCCGCAGCAGGCCTGCTGCCTGCCGCACACACC </td <td></td> <td></td> <td></td>							
CCND1 / Cyclin D1 CND1_fw AATGTGTGCAGAAGGAGGTC c-MYC / v-myc myelocytomatosis viral oncogene c-MYC_fw CTTCTCTGAAAGGCTCTCCT homolog c-MYC_fw CTCGTAGTGAAGGCTCTCCT ETV4 / PEA3 / ets variant 4 ETV4_fw ATGAGAAACCTCTGCGAACA ETV4 / PEA3 / ets variant 4 ETV4_fw ATGAGAAACCTCTGCGAACA ETV4 / PEA3 / ets variant 4 ETV4_rev CAGCAAGGCCAACCAGAGAAATG SLUG / Snail homolog 2 SLUG_rev GAAAGAGGAGAGGAGCACTG CD44 / CD44 antigen, Epican CD44 fw TGGGTTCATAGAGAGGCACTG CD44 rev ATTGGGCAGGTCTGTGGACT FOSL1 rev CACTGGTACTGCCGCA FOSL1 / FOS-like antigen 1 FOSL1 rev CACTGGTACTGCCTGTGTC L1CAM / L1 cell adhesion molecule L1CAM rev 1 TATGGCCTGGTGTAAGACCAAC VLAUR / plasminogen activator, urokinase PLAUR rev 1 TCTCTGAGAGGAGAGAGCAGCATT VMt antagonists CER1 rev CACAGGAGACCAGGACCAAC CK41 / dickkopf 1 homolog DKK1 rev TCCTGAGCGAGACCAGGACCACAC DKK4 / dickkopf homolog 4 DKK4 fw AACATCAGGAGACCATTGAGAACCAGAC DKK4 / dickkopf homolog 4 DKK4 fw AACATCAGGA		_	21002011002011001001				
CCND1_rev AAGCGGTCCAGGTAGTTCAT c-MYC / v-myc myelocytomatosis viral oncogene c-MYC_fw CTTCTGAAAAGCCTCTCCT homolog c-MYC_fw CTTCTGAAAAGCCTCTCCT ETV4 / PEA3 / ets variant 4 ETV4_rev ATGAGAAACCTCTGCGAACAA SLUG / Snail homolog 2 SLUG_fw ACTACAGCGAACTGGACACAA SLUG_rev GAAAGAGGAGAGAGGCCATT CD44 fw TGGGTTCATAGAAGGGCATG CD44 / CD44 antigen, Epican CD44 fw TGGGTTCATAGAAGGGCATG FOSL1 / FOS-like antigen 1 FOSL1 fw AACTGACCGACTTGTGACT FOSL1 / FOS-like antigen 1 FOSL1 fw AACTGACCGACTTGTGTGCAC FOSL1 / FOS-like antigen 1 FOSL1 fw AACTGACGACTGTGTGTGTCT L1CAM / L1 cell adhesion molecule L1CAM fw 1 TATGGCCTTGTTGTGAGCT PLAUR / plasminogen activator, urokinase PLAUR fw 1 GCATGCAGGAGACACAC receptor PLAUR fw 1 GCATGGAGCAGGAGGAGGAGGTAT DKK1 / dickkopf 1 homolog DKK1_fw GTACCAGAGCACAGAGACTTG DKK4 / dickkopf 1 homolog 4 DKK4_fw AACACCAGGAGACAGAACATTGGCAGAGAGAGAGAGAGAG		get genes					
c-MYC / v-myc myelocytomatosis viral oncogene homolog c-MYC_fw CTTCTCTGAAAGGCTCTCCT ETV4 / PEA3 / ets variant 4 ETV4_rev GTCGTAGTCGAGGTCATAGT ETV4 / PEA3 / ets variant 4 ETV4_rev GTCGTAGTCGAGGCACACA SLUG / Snail homolog 2 SLUG fw ACTACAGCGAACTGGACACA SLUG / Snail homolog 2 SLUG fw ACTACAGCGAACTGGACACA SLUG / CD44 antigen, Epican CD44 fw TGGGTTCATAGAAGGGCACTG CD41 / CD44 antigen, Epican CD44 fw ACTGGACGCACTGGTGTGACT FOSL1 / FOS-like antigen 1 FOSL1 fw AACTGGCCGCACTGGTGTGTGACT FOSL1 / FOS-like antigen 1 FOSL1 rev CACTGGTACTGGCAGGTCT L1CAM / L1 cell adhesion molecule L1CAM fw 1 TAGGAGCTGGGGATCT L1CAM / L1 cell adhesion molecule L1CAM fw 1 GCATGCAGTGTAAGACCAAC PLAUR / plasminogen activator, urokinase PLAUR fw 1 GCATGCAGGAGAGAGAGCCTG fmomolog CER1 fw GAAGTACATTGGGAGACCTG DKK1 / dickkopf 1 homolog DKK1_rev TGCAGGCAGAGAGAGAGAGTACATTG DKK4 / dickkopf homolog 4 DKK4_fw AACTCAGGAGACAGATTGGACAGATT DKK4 / dickkopf homolog 4 DKK4_rev CAC	CCND1 / Cyclin D1						
homolog c-MYC_rev GTCGTAGTCGAGGTCATAGT ETV4 / PEA3 / ets variant 4 ETV4_fw ATGGAAACCTCTGCGACCA ETV4 / PEA3 / ets variant 4 ETV4_fw ATGGAAACCTCTGCGACCA ETV4 / PEA3 / ets variant 4 ETV4_fw ATGGAAACCTCTGCGACCA SLUG / Snail homolog 2 SLUG_fw ACTACAGCGAACTGGACACA CD44 / CD44 antigen, Epican CD44 fw TGGGCAGGTCTGTGACT CD41 / CD44 antigen 1 FOSL1 / w ACTGACAGGGACTGGCCACTTCCTGCA FOSL1 / FOS-like antigen 1 FOSL1 rev CACTGGTACTGCCTGTGACT L1CAM / L1 cell adhesion molecule L1CAM fw 1 TATGGCCTGTTGGGGATCA PLAUR / plasminogen activator, urokinase PLAUR fw 1 GCAGCAGTGAAGACCAAC PLAUR rev 1 TCTCTGAGTGGAGACCTG DKK1 fw GTACCAGAGCAGAGACTT 0 Wnt antagonists CER1_rev ACAGTGAGACAGGAGAGATT 0 DKK1 / dickkopf homolog 4 DKK1_fw GTACCAGACCAGACACACT 0 DKK4 / dickkopf homolog 4 DKK4_rev CACGGCAGACAGAGACT 0 DKK4 / dickkopf homolog 4 FRZB /w GAAGTAGCAGAGAAGACT 0 ACATCAGGAGAACACTCAGGAGAAC <td></td> <td>—</td> <td></td>		—					
ETV4_PEA3 / ets variant 4 ETV4_fw ATGAGAAACCTCTGCGACCA SLUG / Snail homolog 2 SLUG_fw ACTACAGCGAACGGCAACAGAAATT SLUG_rev GAAAGAGGAGAGAGGACGACACA CD44 / CD44 antigen, Epican CD44 fw TGGGTTCATAGAAGGGCAGTG CD44 rev ATTGGGCAGGTCTGTGACT FOSL1 / FOS-like antigen 1 FOSL1 fw AACTGACCGACACTTCCTGCGA FOSL1 / FOS-like antigen 1 FOSL1 rev CACTGGTACTGCCTGTGTGC L1CAM / L1 cell adhesion molecule L1CAM fw 1 TATGGCCTTGTTGTGGGATCT L1CAM / L1 cell adhesion molecule L1CAM fw 1 TATGGCAGTGTAAGACCAAC PLAUR / plasminogen activator, urokinase PLAUR fw 1 GCATGCAGTGTAAGACCAAC receptor PLAUR fw 1 GCATGCAGTGGAGACCTG Momolog CER1_rev ACATGAGAGAGACACATTGGGAGACCTG homolog DKK1_rev TGCAGGCGAGACAGATTTG DKK1 / dickkopf 1 homolog DKK1_rev TGCAGGCGAGACGAGAGATTG DKK4 / dickkopf homolog 4 DKK4_fw AACATCAGGGAACCACACTGAGAG FRZB / Frizzled-related protein B FRZB_fw GAAGATGAGGAACGATCCAG FRZB / Frizzled-related protein B FRZB_fw GAAGTGAGGAACGATGAGAGAGAT WIF1/WNT inhibitory factor 1							
ETV4_rev CAGCAAGGCCACCAGAATT SLUG / Snail homolog 2 SLUG_rw ACTACAGGCAACGGACACAA SLUG_rev GAAAGAGGAGAAGAGGCCATT CD44 / CD44 antigen, Epican CD44 fw TGGGTTCATAGAAGGGCATG CD44 rev ATTGGGCAGGTCTGTGACT FOSL1 / FOS-like antigen 1 FOSL1 fw AACTGACCGACTTCCTGCA L1CAM / L1 cell adhesion molecule L1CAM fev 1 TATGGCTTGTGTGGGGTCT L1CAM / L1 cell adhesion molecule L1CAM fev 1 TATGGCCTTGGTGAGCT PLAUR / plasminogen activator, urokinase PLAUR fw 1 GCATGCAGTGGGGTACAGCTT Wht antagonists CER1 / cerberus 1, cysteine knot superfamily, homolog CER1_rev ACAGTGAGAGCAGGAGCATG DKK1 / dickkopf 1 homolog DKK1_fw GAAGTACAGGAACAGTTGACACTT DKK4 fw AACATCAGGAACCATTGACAACT DKK4 / dickkopf homolog 4 DKK4_fw AACATCAGGAACAGTTCCAG FRZB / Frizzled-related protein B FRZB_rev GAAGTTCGACCAGGAACAGTTCAGA FRZB / Frizzled-related protein B FRZB_rev GAAGTTCGACCAGGAACAGTTCAG FRZB_rev AACATCAGCAGAACCATTGAGAAC FWF1 / WNT inhibitory factor 1 WIF1_rev TGTATGAGGCTGGCTTCAG SFRP1_rev AAAGGGCACAGATT	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~						
SLUG / Snail homolog 2 SLUG_fw ACTACAGCGAACTGGACACA CD44 / CD44 antigen, Epican CD44 fw TGGGTTCATAGAAGGGCATG CD44 / CD44 antigen, Epican CD44 fw TGGGTTCATAGAAGGGCATG FOSL1 / FOS-like antigen 1 FOSL1 fw AACTGACCGACTTCCTGCACT FOSL1 / FOS-like antigen 1 FOSL1 fw AACTGGCCAGGTCTGTGTGC L1CAM / L1 cell adhesion molecule L1CAM fw 1 TATGGCCTTGTTGTGGGATCT L1CAM / L1 cell adhesion molecule L1CAM fw 1 TATGGCCTTGTTGTAGCT PLAUR / plasminogen activator, urokinase PLAUR rev 1 TCTCTGAGTGGGTACAGCTT Veceptor Wnt antagonists CER1 / cerberus 1, cysteine knot superfamily, homolog CER1_fw GAAGTAGAGAGAGAGCACTG DKK1 / dickkopf 1 homolog DKK1_rev TGCAGGCGAGACAGAACT DKK1_rev TGCAGGCGAGACAGAACT DKK4 / dickkopf homolog 4 DKK4_rev CACGACATTGGACAGAGAC FRZB / Frizzled-related protein B FRZB_rev GAAGTAGAGGCACGAGACCTG FRZB / Frizzled-related protein B FRZB_rev GAAGTGAGGACCCTGGACAGAGAT GAAGTACATGGGCACAGAGACCTGAGAGACCCAGAGAC WIF1/WNT inhibitory factor 1 WIF1_rev GAAGTAGAGGCCGAGACAGATTAA SFRP1_rev AA	ETV4 / PEA3 / ets variant 4						
SLUG_rev GAAAGAGGAGAGGAGGCCATT CD44 / CD44 antigen, Epican CD44 fw TGGGTTCATAGAAGGGCATG FOSL1 / FOS-like antigen 1 FOSL1 fw AACTGACCGACTTCCTGCA FOSL1 / FOS-like antigen 1 FOSL1 fw AACTGACCGACTTCCTGCA FOSL1 rev CACTGGTACTGCCTGTGTC L1CAM / L1 cell adhesion molecule L1CAM fw 1 TATGGCCTTGTCTGGGGACT L1CAM / L1 cell adhesion molecule L1CAM fw 1 TATGGCCTGGTTGTAGCT PLAUR rev 1 TAGGAGCTCTGGTTGAGCT PLAUR / plasminogen activator, urokinase PLAUR fw 1 GCATGCAGTGGAGACCAAC PLAUR rev 1 TCTCTGAGTGGGAGACAACCAAC receptor Whatatagonists CER1_rev ACAGTGAGAGCAGGAGAGCATG ACAGTGAGAGCAGGAGGAGAT homolog DKK1 / cerberus 1, cysteine knot superfamily, homolog CER1_rev ACAGTGAGAGCAGGAGCAT <i>DKK1</i> / dickkopf 1 homolog 4 DKK1_rev TGCAGGCGAGACAGATTG DKK4_rev CACGGCAGACAGATTG <i>DKK4</i> / dickkopf homolog 4 DKK4_rev CACGGCATGAGCAACGT GAAGATGAGGAACGTTCCAG <i>FRZB</i> / Frizzled-related protein B FRZB_rev GAAGATGAGGAACGTTCCAG FRZB_rev GAAGATGAGGAACGTTCCAG <i>WIF1</i> / WNT inhibitory factor 1 </td <td></td> <td></td> <td></td>							
CD44 / CD44 antigen, Epican CD44 fw TGGGTTCATAGAAGGGCATG FOSL1 / FOS-like antigen 1 FOSL1 / w AACTGACCGACTTCCTGCACT FOSL1 / FOS-like antigen 1 FOSL1 rev CACTGGTACTGCCTGTGACT L1CAM / L1 cell adhesion molecule L1CAM fw1 TATGGCCTGGTGTGGGACTT L1CAM / L1 cell adhesion molecule L1CAM fw1 TATGGCCTGGTTGTAGCT PLAUR / plasminogen activator, urokinase PLAUR fw1 GCATGCAGTGAGGGTACAGCTT VMt antagonists Vent artagonists CER1 / cerberus 1, cysteine knot superfamily, homolog CER1_rev ACAGTGAGAGCAGGAGCAGGAGGTAT DKK1 / dickkopf 1 homolog DKK1_fw GTACCAGGACCAGGACAGACTTGACAACT DKK1_rev TGCAGGCGAGACAGATTGACAACT DKK4 / dickkopf homolog 4 DKK4_fw AACCTCAGGAGCACAGAACC DKK4_rev CACGACATGTAGCACAGAAC FRZB / Frizzled-related protein B FRZB_rev GACATCAGGAGACAGTTCAGG GAAGATGAGGAACCTT WIF1 / WNT inhibitory factor 1 WIF1_fw AAACGCCAGCAGGAGACCT WIF1_rev TGTATGAGGCTGGCTTCAG CDH1 / Cadherin-1 / E-cadherin CDH1_rev CTGTGTACGTGCTGTTTATAGG CDH1_rev CTGTGTACGTGCTGTTCTC CDH1 / Gatherin-1 / E-cadherin CDH	SLUG / Snail homolog 2						
CD44 rev ATTGGGCAGGTCTGTGACT FOSL1 / FOS-like antigen 1 FOSL1 fw AACTGACCGACTTCCTGCA FOSL1 rev CACTGGTACTGCCTGTGTC L1CAM / L1 cell adhesion molecule L1CAM fw 1 TATGGCCTTGTCGGGATCT L1CAM / L1 cell adhesion molecule L1CAM rev 1 TAGGAGCCTTGGTGTGAGCT PLAUR / plasminogen activator, urokinase PLAUR fw 1 GCATGCAGTGTAAGACCAAC Peceptor Wht antagonists CER1 / cerberus 1, cysteine knot superfamily, homolog CER1_rev ACAGTGAGAGCAGGAGGTAT DKK1 / dickkopf 1 homolog DKK1_fw GAAGTACATTGAGAGCAGCAGCAGTTG DKK1_rev TGCAGGCGAGACCAGATTG DKK4 / dickkopf homolog 4 DKK4_fw AACATCAGGAGCACGTGCTGCTGA DKK4_rev CACGACATGTAGCACAGAAC FRZB / Frizzled-related protein B FRZB_fw GAAGATGAGGAACGTTCCAG FRZB_rev GAAGTAGGCACAGAGGAGCT WIF1 / WNT inhibitory factor 1 WIF1_rev TACTGGCCCGAGACAGATTAA SFRP1_rev AAACTCGCTGGCACAGAGAGT WIF1 / WNT inhibitory factor 1 WIF1_rev TACTGGCCGAGACACATTTATGG GDH1_rev TGTATGAGGCTGGCTTCTGTA CDH1 / Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGGG CDH1_rev<	OD44/OD44 antigent Enigen	—					
FOSL1 / FOS-like antigen 1 FOSL1 fw AACTGACCGACTTCCTGCA L1CAM / L1 cell adhesion molecule L1CAM fw 1 TATGGCCTTGTCTGGGATCT L1CAM / L1 cell adhesion molecule L1CAM fw 1 TATGGCCTTGTCTGGGATCT L1CAM / L1 cell adhesion molecule L1CAM fw 1 TATGGCCTTGTCGGGATCT L1CAM rev 1 TAGGAGCCTTGTGAGCT PLAUR rev 1 TAGGAGCTGTGAGACCAAC Peceptor PLAUR rev 1 TCTCTGAGTGGGAGACCAGC Wtt antagonists CER1 / cerberus 1, cysteine knot superfamily, homolog CER1_rev ACAGTGAGAGCAGAGAGAGATT DKK1 / dickkopf 1 homolog DKK1_fw GAAGTACATTGGGAGACCAGCAGAGATT DKK4 / dickkopf homolog 4 DKK4_fw AACTCAGGAGCACGAGACATTGGAACCAGAAC FRZB / Frizzled-related protein B FRZB_fw GAAGATGAGGAACGTTCCAG FRZB / Frizzled-related protein B FRZB_fw GAAGATGAGGAACGTTCCAG FRZB / Frizzled-related protein 1 SFRP1_rev AAACTCGCTGGCACAGAGAT WIF1 / WNT inhibitory factor 1 WIF1_rev TACTGGCCGAGACACATTTAGG WIF1 / WNT inhibitory factor 1 WIF1_rev TGTATGAGGCTGGCTTCGTA CDH1 / Cadherin-1 / E-cadherin CDH1_rev CTGTGTACGTGCGTGCTGTTCTC CDH1 / Cadherin-1 / E-cadherin CDH1_rev </td <td>CD44 / CD44 antigen, Epican</td> <td>-</td> <td></td>	CD44 / CD44 antigen, Epican	-					
FOSL1 rev CACTGGTACTGCCTGTGTC L1CAM / L1 cell adhesion molecule L1CAM fw 1 TATGGCCTTGTCTGGGATCT L1CAM / L1 cell adhesion molecule L1CAM fw 1 TATGGCCTTGTCTGGGATCT PLAUR / plasminogen activator, urokinase receptor PLAUR fw 1 GCATGCAGTGTAAGACCAAC PLAUR rev 1 TCTCTGAGTGGGACCAGCTT Wnt antagonists CER1 / cerberus 1, cysteine knot superfamily, homolog CER1_rev ACAGTGAGAGCAGAGAGCAGA DKK1 / dickkopf 1 homolog DKK1_fw GTACCAGACCATTGACAACT DKK4 / dickkopf homolog 4 DKK4_fw AACATCAGGAGCACGAGACAGATTGG DKK4 / rev TGCAGGCGAGAACAGATTGG DKK4_rev CACGGACATGTAGCACAGAAC FRZB / Frizzled-related protein B FRZB_fw GAAGATGAGGAACGTTCCAG GACTTCTGACTGGGGA SFRP1 / Secreted frizzled-related protein 1 SFRP1_fw TACTGGCCCGAGAGAGCT GACTGCTGGCACAGAGAT WIF1/WNT inhibitory factor 1 WIF1_fw AAAGTCGCTGGCTTCGTA SFRP1_rev AAACTCGCTGGCACAGAGACCT WIF1/Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CDH1_rev CTGTGTACGTGCTGTTCTC CTNNB1_fw CTGCTAAATGACGAGGAGCACA CTGTGTACGTGCTGTTCTC CT							
L1CAM / L1 cell adhesion molecule L1CAM fw 1 TATGGCCTTGTCTGGGATCT PLAUR / plasminogen activator, urokinase receptor PLAUR fw 1 GCATGCAGTGTAAGACCAAC PLAUR / plasminogen activator, urokinase receptor PLAUR fw 1 GCATGCAGTGTAAGACCAAC PLAUR rev 1 TCTCTGAGTGGGGTACAGCTT PLAUR rev 1 TCTCTGAGTGGGGACCAGCTT Wnt antagonists Wnt antagonists CER1 / cerberus 1, cysteine knot superfamily, homolog CER1_rev ACAGTGAGAGCAGGAGCAGGAGGTAT DKK1 / dickkopf 1 homolog DKK1_fw GTACCAGACCATTGACAACT DKK1_rev TGCAGGCGAGACAGATTG DKK4 / dickkopf homolog 4 DKK4_fw AACATCAGGAGCTCTGCTGA DKK4_rev CACGACATGTAGCACAGAAC FRZB / Frizzled-related protein B FRZB_rev GAAGATGAGGAACGTTCAG FRZB_rev GAACTCGGCCGAGAACGTTCAG SFRP1 / Secreted frizzled-related protein 1 SFRP1_rev AAACTCGCCGAGAGACCT WIF1_rev TGTATGAGGCTGGCTTCATA WIF1/WNT inhibitory factor 1 WIF1_fw AAAGGTTACCAGGGAGACCAT WIF1_rev TGTATGAGGCTGGCTTCGTA CDH1 / Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CDH1_rev CTGTGTACGTGCTGTTCTTC CTNNB1 / β-catenin	FOSL1 / FOS-like antigen 1						
L1CAM rev 1 TAGGAGCTCTGGTTGTAGCT PLAUR / plasminogen activator, urokinase receptor PLAUR fw 1 GCATGCAGTGTAAGACCAAC PLAUR rev 1 TCTCTGAGTGGGTACAGCTT Wnt antagonists Vmt antagonists CER1 / cerberus 1, cysteine knot superfamily, homolog CER1_fw GAAGTACATTGGGAGACCTG DKK1 / dickkopf 1 homolog DKK1_fw GTACCAGACCATTGACAACT DKK4 / dickkopf homolog 4 DKK4_fw GTACCAGACCATTGACAACT DKK4 / dickkopf homolog 4 DKK4_rev CACGACATGAGAGCACAGAACG FRZB / Frizzled-related protein B FRZB_fw GAAGATGAGGAACGTTCCAG SFRP1 / Secreted frizzled-related protein 1 SFRP1_fw TACTGGCCCGAGACACAGATT WIF1/WNT inhibitory factor 1 WIF1_fw AAACTCGCTGGCACAGAGACCT WIF1/WNT inhibitory factor 1 WIF1_rev TGTATGAGGCTGGCTTCGTA CDH1 / Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CDH1 / Cadherin-1 / E-cadherin CDH1_fw CTGTGTACGTGCTGTTCTTC							
PLAUR plasminogen activator, urokinase PLAUR fw 1 GCATGCAGTGTAAGACCAAC PLAUR rev 1 TCTCTGAGTGGGTACAGCTT PLAUR rev 1 TCTCTGAGTGGGTACAGCTT Wnt antagonists CER1 / cerberus 1, cysteine knot superfamily, homolog CER1_fw GAAGTACATTGGGAGACCTG DKK1 / dickkopf 1 homolog DKK1_fw GTACCAGACCATTGACAACT DKK4 / dickkopf homolog 4 DKK4_fw AACATCAGGAGCACAGAACT DKK4 / dickkopf homolog 4 DKK4_fw AACATCAGGAGCACAGAAC FRZB / Frizzled-related protein B FRZB_fw GAAGATGAGGAACGTTCCAG FRZB / Frizzled-related protein B FRZB_fw GAAGATGAGGAACGTTCCAG SFRP1 / Secreted frizzled-related protein 1 SFRP1_fw TACTGGCCCGAGATGCTTAA SFRP1 / Secreted frizzled-related protein 1 SFRP1_rev AAACTCGCTGGCACAGAGAT WIF1 / WNT inhibitory factor 1 WIF1_fw AAAGGTTACCAGGGAGCCT WIF1_rev TGTATGAGGCTGGCTTCGTA CDH1 / Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CDH1 / p-catenin CDH1_fw CGAGGCCAGACACATTTATGG	L1CAM/ L1 cell adhesion molecule						
receptor PLAUR rev 1 TCTCTGAGTGGGTACAGCTT Wnt antagonists CER1 / cerberus 1, cysteine knot superfamily, homolog CER1_fw GAAGTACATTGGGAGACCTG DKK1 / dickkopf 1 homolog CER1_rev ACAGTGAGAGCAGGAGGAGTAT DKK1_fw GTACCAGACCATTGACAACT DKK4 / dickkopf homolog 4 DKK4_fw GAAGTACAGGAGCAGGAGCAGAACTTG DKK4 / dickkopf homolog 4 DKK4_fw AACATCAGGAGCACAGAACA FRZB / Frizzled-related protein B FRZB_fw GAAGATGAGGAACGTTCCAG FRZB / Frizzled-related protein B FRZB_rev GACTTCTGACTCTGAGTGGA SFRP1 / Secreted frizzled-related protein 1 SFRP1_fw TACTGGCCCGAGATGCTTAA SFRP1 / WNT inhibitory factor 1 WIF1_fw AAACTCGCTGGCACAGGAGACT WIF1 / WNT inhibitory factor 1 WIF1_rev TGTATGAGGCTGGCTTCGTA CDH1 / Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CDH1 / pev CTGTGTACGTGCTGTTCTTC CTNNB1 / β-catenin							
Wnt antagonists CER1 / cerberus 1, cysteine knot superfamily, homolog CER1_fw GAAGTACATTGGGAGACCTG DKK1 / dickkopf 1 homolog CER1_rev ACAGTGAGAGCAGGAGGAGTAT DKK1 / dickkopf 1 homolog DKK1_fw GTACCAGACCATTGACAACT DKK4 / dickkopf homolog 4 DKK4_fw AACATCAGGAGCAGAGACAGATTTG DKK4 / dickkopf homolog 4 DKK4_rev CACGACATGTAGCACAGAAC FRZB / Frizzled-related protein B FRZB_fw GAAGATGAGGAACGTTCCAG FRZB / Frizzled-related protein 1 SFRP1_fw TACTGGCCCGAGATGCTTAA SFRP1 / Secreted frizzled-related protein 1 SFRP1_fw TACTGGCCCGAGATGCTTAA SFRP1 / WNT inhibitory factor 1 WIF1_fw AAAGGTTACCAGGGAGACCT WIF1 / WNT inhibitory factor 1 WIF1_rev TGTATGAGGCTGGCTTCGTA CDH1 / Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CDH1 / Secretenin CDH1_fw GGAGCCAGACACATTTATGG	· · · · · · · · · · · · · · · · · · ·						
CER1 / cerberus 1, cysteine knot superfamily, homolog CER1_fw GAAGTACATTGGGAGACCTG DKK1 / dickkopf 1 homolog DKK1_fw GTACCAGACCATTGACAACT DKK4 / dickkopf homolog 4 DKK4_fw GAAGTACATGAGAGCAGAGATTG DKK4 / dickkopf homolog 4 DKK4_fw AACATCAGGAGCACAGATTG DKK4 / bickkopf homolog 4 DKK4_fw AACATCAGGAGCTCTGCTGA DKK4_rev CACGACATGTAGCACAGAAC DKK4_rev CACGACATGTAGCACAGAAC FRZB / Frizzled-related protein B FRZB_fw GAAGTTCTGACTCTGAGTGGA SFRP1 / Secreted frizzled-related protein 1 SFRP1_fw TACTGGCCCGAGATGCTTAA SFRP1 / Secreted frizzled-related protein 1 SFRP1_fw TACTGGCCCGAGATGCTTAA SFRP1 / WNT inhibitory factor 1 WIF1_fw AAAGGTTACCAGGGAGACCT WIF1/WNT inhibitory factor 1 WIF1_fw AAAGGTTACCAGGGAGACCT CDH1 / Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CDH1 / S-catenin CDH1_fw CTGTGTACGTGCTGTTCTTC CTNNB1 / β-catenin CTNNB1_fw CTGCTAAATGACGAGGACCA	receptor	PLAUR rev 1	TCTCTGAGTGGGTACAGCTT				
homologCER1_revACAGTGAGAGCAGGAGGTATDKK1 / dickkopf 1 homologDKK1_fwGTACCAGACCATTGACAACTDKK4 / dickkopf homolog 4DKK4_fwAACATCAGGAGCTCTGCTGADKK4 / dickkopf homolog 4DKK4_fwAACATCAGGAGGCACAGAACAFRZB / Frizzled-related protein BFRZB_fwGAAGATGAGGAACGTTCCAGFRZB / Frizzled-related protein 1SFRP1_fwTACTGGCCCGAGATGCTTAASFRP1 / Secreted frizzled-related protein 1SFRP1_fwTACTGGCCCGAGATGCTTAASFRP1 / WNT inhibitory factor 1WIF1_fwAAAGGTTACCAGGGAGACCTWIF1_revTGTATGAGGCTGGCTTCGTACDH1 / Cadherin-1 / E-cadherinCDH1_fwGGAGCCAGACACATTTATGGCDH1_revCTGTGTACGTGCTGTTCTTCCTNNB1 / β-cateninCTNNB1_fwCTGCTAAATGACGAGGACCA	Wnt an	tagonists					
homologCER1_revACAGTGAGAGCAGGAGGTATDKK1 / dickkopf 1 homologDKK1_fwGTACCAGACCATTGACAACTDKK4 / dickkopf homolog 4DKK4_fwAACATCAGGAGCTCTGCTGADKK4 / dickkopf homolog 4DKK4_fwAACATCAGGAGGCACAGAACAFRZB / Frizzled-related protein BFRZB_fwGAAGATGAGGAACGTTCCAGFRZB / Frizzled-related protein 1SFRP1_fwTACTGGCCCGAGATGCTTAASFRP1 / Secreted frizzled-related protein 1SFRP1_fwTACTGGCCCGAGATGCTTAASFRP1 / WNT inhibitory factor 1WIF1_fwAAAGGTTACCAGGGAGACCTWIF1_revTGTATGAGGCTGGCTTCGTACDH1 / Cadherin-1 / E-cadherinCDH1_fwGGAGCCAGACACATTTATGGCDH1_revCTGTGTACGTGCTGTTCTTCCTNNB1 / β-cateninCTNNB1_fwCTGCTAAATGACGAGGACCA	CER1 / cerberus 1, cysteine knot superfamily,	CER1_fw					
DKK1_rev TGCAGGCGAGACAGATTTG DKK4 / dickkopf homolog 4 DKK4_fw AACATCAGGAGCTCTGCTGA DKK4 / dickkopf homolog 4 DKK4_fw AACATCAGGAGCTCTGCTGA DKK4_rev CACGACATGTAGCACAGAAC FRZB / Frizzled-related protein B FRZB_fw GAAGATGAGGAACGTTCCAG SFRP1 / Secreted frizzled-related protein 1 SFRP1_fw TACTGGCCCGAGATGCTTAA SFRP1 / Secreted frizzled-related protein 1 SFRP1_rev AAACTCGCTGGCACAGAGAT WIF1 / WNT inhibitory factor 1 WIF1_fw AAAGGTTACCAGGGAGACCT WIF1_rev TGTATGAGGCTGGCTTCGTA CDH1 / Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CDH1_rev CTGTGTACGTGCTGTTCTTC CTNNB1 / β-catenin CTNNB1_fw CTGCTAAATGACGAGGACCA			ACAGTGAGAGCAGGAGGTAT				
DKK4 / dickkopf homolog 4 DKK4_fw AACATCAGGAGCTCTGCTGA DKK4_rev CACGACATGTAGCACAGAAC FRZB / Frizzled-related protein B FRZB_fw GAAGATGAGGAACGTTCCAG FRZB / Frizzled-related protein 1 FRZB_rev GACTTCTGACTCTGAGTGGA SFRP1 / Secreted frizzled-related protein 1 SFRP1_fw TACTGGCCCGAGATGCTTAA SFRP1 / WNT inhibitory factor 1 WIF1_fw AAACTCGCTGGCACAGAGACCT WIF1 / WNT inhibitory factor 1 WIF1_rev TGTATGAGGCTGGCTTCGTA CDH1 / Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CDH1_rev CTGTGTACGTGCTGTTCTTC CTNNB1 / β-catenin CTNNB1_fw CTGCTAAATGACGAGGACCA	DKK1 / dickkopf 1 homolog						
DKK4_rev CACGACATGTAGCACAGAAC FRZB / Frizzled-related protein B FRZB_fw GAAGATGAGGAACGTTCCAG FRZB_rev GACTTCTGACTCTGAGTGGA SFRP1 / Secreted frizzled-related protein 1 SFRP1_fw TACTGGCCCGAGATGCTTAA SFRP1 / Secreted frizzled-related protein 1 SFRP1_fw TACTGGCCCGAGATGCTTAA WIF1 / WNT inhibitory factor 1 WIF1_rev AAACTCGCTGGCACAGAGACCT WIF1 / WNT inhibitory factor 1 WIF1_rev TGTATGAGGCTGGCTTCGTA CDH1 / Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CDH1_rev CTGTGTACGTGCTGTTCTTC CTNNB1 / β-catenin CTNNB1_fw CTGCTAAATGACGAGGACCA							
FRZB / Frizzled-related protein B FRZB_fw GAAGATGAGGAACGTTCCAG FRZB_rev GACTTCTGACTCTGAGTGGA SFRP1 / Secreted frizzled-related protein 1 SFRP1_fw TACTGGCCCGAGATGCTTAA SFRP1_rev AAACTCGCTGGCACAGAGAT WIF1 / WNT inhibitory factor 1 WIF1_fw AAAGGTTACCAGGGAGACCT WIF1 / WNT inhibitory factor 1 WIF1_rev TGTATGAGGCTGGCTTCGTA CDH1 / Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CDH1_rev CTGTGTACGTGCTGTTCTTC CTNNB1 / β-catenin CTNNB1_fw CTGCTAAATGACGAGGACCA	DKK4 / dickkopf homolog 4						
FRZB_rev GACTTCTGACTCTGAGTGGA SFRP1 / Secreted frizzled-related protein 1 SFRP1_fw TACTGGCCCGAGATGCTTAA SFRP1_rev AAACTCGCTGGCACAGAGAT WIF1 / WNT inhibitory factor 1 WIF1_fw AAAGGTTACCAGGGAGACCT WIF1_rev TGTATGAGGCTGGCTTCGTA Adherens junction CDH1_fw GGAGCCAGACACATTTATGG CDH1 / Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CTNNB1 / β-catenin CTNNB1_fw CTGCTAAATGACGAGGACCA							
SFRP1 / Secreted frizzled-related protein 1 SFRP1_fw TACTGGCCCGAGATGCTTAA SFRP1_rev AAACTCGCTGGCACAGAGAT WIF1 / WNT inhibitory factor 1 WIF1_fw AAAGGTTACCAGGGAGACCT WIF1_rev TGTATGAGGCTGGCTTCGTA Adherens junction CDH1 / Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CDH1_rev CTGTGTACGTGCTGTTCTTC CTNNB1 / β-catenin CTNNB1_fw CTGCTAAATGACGAGGACCA	FRZB / Frizzled-related protein B						
SFRP1_rev AAACTCGCTGGCACAGAGAT WIF1 / WNT inhibitory factor 1 WIF1_fw AAAGGTTACCAGGGAGACCT WIF1_rev TGTATGAGGCTGGCTTCGTA Adherens junction CDH1_fw GGAGCCAGACACATTTATGG CDH1 / Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CDH1_rev CTGTGTACGTGCTGTTCTTC CTNNB1 / β-catenin CTNNB1_fw CTGCTAAATGACGAGGACCA							
WIF1 / WNT inhibitory factor 1 WIF1_fw AAAGGTTACCAGGGAGACCT WIF1_rev TGTATGAGGCTGGCTTCGTA Adherens junction CDH1 / Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CDH1_rev CTGTGTGCTGCTGTTCTTC CTNNB1 / β-catenin CTNNB1_fw CTGCTAAATGACGAGGACCA	SFRP1 / Secreted frizzled-related protein 1						
WIF1_rev TGTATGAGGCTGGCTTCGTA Adherens junction CDH1_fw GGAGCCAGACACATTTATGG CDH1_Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CDH1_rev CTGTGTACGTGCTGTTCTTC CTNNB1 / β-catenin CTNNB1_fw CTGCTAAATGACGAGGACCA	14/154/100 NIT in hit is in item ($5 - 5 - 5$	_					
Adherens junction CDH1 / Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CDH1_rev CTGTGTACGTGCTGTTCTTC CTNNB1 / β-catenin CTNNB1_fw CTGCTAAATGACGAGGACCA	<i>wir</i> i / wini inniditory factor 1						
CDH1 / Cadherin-1 / E-cadherin CDH1_fw GGAGCCAGACACATTTATGG CDH1_rev CTGTGTACGTGCTGTTCTTC CTNNB1 / β-catenin CTNNB1_fw CTGCTAAATGACGAGGACCA		vviF1_rev	TGTATGAGGCTGGCTTCGTA				
CDH1_rev CTGTGTACGTGCTGTTCTTC CTNNB1 / β-catenin CTNNB1_fw CTGCTAAATGACGAGGACCA	Adherens junction						
CTNNB1 / β-catenin CTNNB1_fw CTGCTAAATGACGAGGACCA	CDH1 / Cadherin-1 / E-cadherin						
CTNNB1_rev GTATTCTGCATGGTACGTAC	CTNNB1 / β-catenin						
		CTNNB1_rev	GTATTCTGCATGGTACGTAC				

SUPPL. TABLE S3, related to FIG. 4 and 5. Primers used for qPCR analyses.

SUPPL. TABLE S4, related to Fig. 4 and 5. Primary antibodies used for western blots,

flow cytometry and immunofluorescence.

Target	Host species (isotype)	Supplier	Supplier #	Clone	Immunogen / Epitope *	
		Pluripoten	cy markers			
OCT3/4 / Octamer- binding protein 3/4 / POU domain, class 5, transcription factor 1	mouse (IgG _{2b})	Santa Cruz Biotechnology	sc-5279	C-10	Raised against amino acids 1 - 134	
SSEA-4 Stage-specific embryonic antigen-4	mouse (IgG _{3κ})	BioLegend	330401	MC- 813-70	Detects a glycolipid: NeuAcα2- 3Galβ1-3GalNacβ1-R	
		E-Ca	dherin			
CDH1 / Cadherin-1 / E- Cadherin	rabbit	Cell Signaling Technologies	3195S	24E10	Raised against peptide around amino acid 780; the epitope is located C- terminal of amino acid 752, it contains at least amino acids 782 – 787 and ends C- terminal of amino acid 788 (personal communication with technical staff from Cell Signaling Technologies)	
	mouse (IgG _{2a})	BD Transduction Laboratories	C20820	C-36	Raised against C-terminal recombinant E-cadherin (cytosolic part); epitope: amino acids 773 – 791 (1)	
	goat	Santa Cruz Biotechnology	sc-1499	C-19	Raised against peptide within a C-terminal cytoplasmic domain; epitope: amino acids 863 – 882 (2)	
		β-Ca	tenin			
CTNNB1 / β-catenin	rabbit	Cell Signaling Technologies	8480P	D10A8	Recognizes endogenous levels of total β-catenin protein; raised against peptide around amino acid 714	
	rabbit	Millipore	ABE208		Epitope is corresponding to the consensus GSK3 phosphorylation site of β- catenin; detects total levels of β-catenin	
	mouse (IgG _{1κ})	Millipore	05-665	8E7	Epitope corresponds to amino acids $36 - 44$ and is specific for the active form of β - catenin, dephosphorylated on Ser37 or Thr41	
Other						
SFRP1 / Secreted frizzled-related protein 1	rabbit	Cell Signaling Technologies	3534S	D5A7	Detects endogenous levels of total SFRP1 protein	
SPTAN1 / Spectrin alpha chain	mouse (IgG ₁)	Millipore, Chemicon	MAB1622	AA6	Immunogen: Chicken red blood cell membranes purified by hypotonic lysis and mechanical enucleation	
PSA (polysialic acid)	mouse			735D4	Immunogen: polysialic acid	

	(IgG _{2a})				
NCAM	mouse (IgG ₁)			123C3	Immunogen: all isoforms of human NCAM
Islet-1	rabbit	Abcam	ab20670		Raised against peptide conjugated to KLH from within residues 300 to the C-terminus of human Islet-1
α-actinin (sarcomeric)	mouse (IgG ₁)	Sigma-Aldrich	A7811	EA-53	Raised against purified rabbit skeletal muscle α-actinin

* Numbers refer to amino acid positions of the respective human protein, if not stated

otherwise; information was obtained from supplier's data sheets, if not stated otherwise

SUPPL. TABLE S5, related to Fig. 4 and 5. Secondary antibodies used for western blots,

flow cytometry and immunofluorescence.

Target species (isotype)	Host species	Supplier	Supplier #	Fluorochrome	Application
goat IgG (H+L)	donkey	LiCor	926-32214	IRDye 800CW	Western blot (against CDH1 (C19))
anti-mouse IgG	goat	LiCor	926-32220	IRDye 680	Western blot
anti-mouse IgG	goat	LiCor	926-32210	IRDye 800CW	Western blot
anti-rabbit IgG	goat	LiCor	926-32221	IRDye 680	Western blot
anti-mouse IgG (H+L)	goat	Molecular Probes	A 11029	Alexa Fluor [®] 488	Flow cytometry (against SSEA-4)
anti-mouse IgG _{2a}	goat	Molecular Probes	A 21134	Alexa Fluor [®] 568	Immunofluorescence (against anti-PSA)
anti-mouse IgG _{2b}	goat	Molecular Probes	A 21141	Alexa Fluor [®] 488	Immunofluorescence (against anti-OCT3/4)
anti-mouse IgG₁	goat	Molecular Probes	A 21121	Alexa Fluor [®] 488	Immunofluorescence (against anti-NCAM)
anti-mouse IgG (H+L)	goat	Jackson Immuno Research Laboratories	115-165-003	Cy [™] 3	Immunofluorescence (against anti-SSEA-4)
anti-rabbit IgG (H+L)	donkey	Molecular Probes	A21206	Alexa Fluor [®] 488	Immunofluorescence (against anti-Islet-1)

SUPPLEMENTAL RESULTS

Genes with increased expression in suspension culture spheroids encode nuclear factors — STRING database-based enrichment analyses performed with proteins and transcripts upregulated (p<0.05, >1.5-fold) under 3D culture conditions in hPSCs (combined analysis of hESCs and hiPSCs) revealed that 29 transcripts matching to the KEGG pathway "systemic lupus erythematosus" were found to be enriched with high significance (p=6x10⁻¹³). All 29 matches were histone genes (Supplementary Table S2) and belong to the nucleosome. Gene ontology (GO)-enrichment analyses for "cellular component" and "biological process" confirmed the enrichment of transcripts upregulated in suspension culture to belong to the nucleosome or to be involved in chromatin assembly. With the exception of the core histone macro-H2A other histone proteins were not identified in the proteomic approach, because histones are generally smaller than 25 kDa, which was the lower size limit in the proteomic screen (Fig. 1B). Performing a search with the list of upregulated proteins for enrichment in "GO biological process" applying the STRING database, we identified categories like "telomere maintenance via semi-conservative replication", "telomere maintenance via recombination" or "chromosome organization" as the most significant hits. The nucleosome assembly protein 1-like 1 (NAP1L1) was found to be the most highly upregulated protein in the SILAC proteomic experiment. Other replication associated proteins including the endonuclease III-like protein 1 (NTH1), the core histone macro-H2A (H2AFY2), the DNA polymerase epsilon subunit A (POLE1) or the DNA polymerase delta subunit A were also among the highly upregulated proteins in 3D culture (Supplementary Table S1).

SUPPLEMENTAL DISCUSSION

Histone mRNAs are cell cycle-regulated and increase considerably when cells enter the S phase of the cell cycle where the chromosomal replication takes place. The histones are required in the S phase for packaging the newly synthesized DNA into chromatin. At the end of the S phase, before entering mitosis, histone expression drops to baseline levels (3). Pluripotent stem cells divide rapidly and have a considerably shortened G1 phase of the cell

cycle compared to somatic cells (4). Our finding of 3D induced upregulation of histone genes suggests that under 3D culture conditions more cells are in the S phase, which is indicative of higher proliferation rates and accelerated progression through the G1 phase. Calpains have been implicated in regulation of cell proliferation as calpain inhibitors have antimitogenic effects (5) and calpain activation shortens the G1 phase of transformed cells (6). Upregulation of the endogenous calpain inhibitor calpastatin as observed by us in 2D vs. 3D has been shown to decrease growth of CHO cells (7). We thus hypothesize that the observed activation of calpain might result in an increased proliferation of 3D cultured cells.

Reference List

- 1. Chitaev, N. A., and Troyanovsky, S. M. (1998) Adhesive but not lateral E-cadherin complexes require calcium and catenins for their formation. *J. Cell Biol.* 142, 837-846
- Rios-Doria, J., Day, K. C., Kuefer, R., Rashid, M. G., Chinnaiyan, A. M., Rubin, M. A., and Day, M. L. (2003) The role of calpain in the proteolytic cleavage of E-cadherin in prostate and mammary epithelial cells. J. Biol. Chem. 278, 1372-1379
- Marzluff, W. F., and Duronio, R. J. (2002) Histone mRNA expression: multiple levels of cell cycle regulation and important developmental consequences. *Curr. Opin. Cell Biol.* 14, 692-699
- Becker, K. A., Ghule, P. N., Therrien, J. A., Lian, J. B., Stein, J. L., van Wijnen, A. J., and Stein, G. S. (2006) Self-renewal of human embryonic stem cells is supported by a shortened G1 cell cycle phase. J. Cell Physiol 209, 883-893
- 5. Goll, D. E., Thompson, V. F., Li, H., Wei, W., and Cong, J. (2003) The calpain system. *Physiol Rev.* 83, 731-801
- 6. Frame, M. C., Fincham, V. J., Carragher, N. O., and Wyke, J. A. (2002) v-Src's hold over actin and cell adhesions. *Nat. Rev. Mol. Cell Biol.* 3, 233-245
- 7. Xu, Y., and Mellgren, R. L. (2002) Calpain inhibition decreases the growth rate of mammalian cell colonies. *J. Biol. Chem.* 277, 21474-21479