Supplementary Methods

Western blot (WB) analysis

The hES cells and embryoid bodies (EBs) were lysed in the RIPA buffer containing 10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.1% SDS, 0.3% Triton X-100, 0.3% sodium deoxycholate, 2 mM EDTA, and the Complete protease inhibitor cocktail (Roche Diagnostics) and stored at -20 ˚C. The concentration of the proteins in the samples was measured with the BCA Protein Assay Kit (Thermo Fisher Scientific) and equal amounts of protein were electrophoresed in an 8% SDS polyacrylamide gel and transblotted with the Mini Trans-blot Cell system (Bio-Rad, Hercules, CA) onto a polyvinylidene difluoride membrane (Millipore, Billerica, MA). The membranes were blocked with a 5% (w/v) non-fat dry milk powder (AppliChem) solution in PBS containing 0.1% Tween-20 (blocking solution). The incubation with primary antibodies was performed in the same blocking solution overnight at 4 ˚C, which was followed by an incubation with a secondary antibody in a blocking solution for 1h at RT. The membranes were probed with rabbit anti-integrin α6 antibodies, mouse anti-integrin β1 antibodies (Santa Cruz Biotechnology), and mouse anti-E-cadherin antibodies (Abcam, Cambridge, MA, USA), which was followed by the inclusion of the horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse secondary antibodies (Cell Signaling). The mouse anti-β-actin antibody (Abcam) was used for detecting the loading control. The binding of the antibodies was detected with the Immobilon Western Chemiluminescent HRP Substrate solution (Millipore Corporation). The membranes were exposed to the x-ray films (Agfa, Belgium) used for the chemiluminescent signal detection. Plots were analysed using the ImageJ gel plot analysing tool.

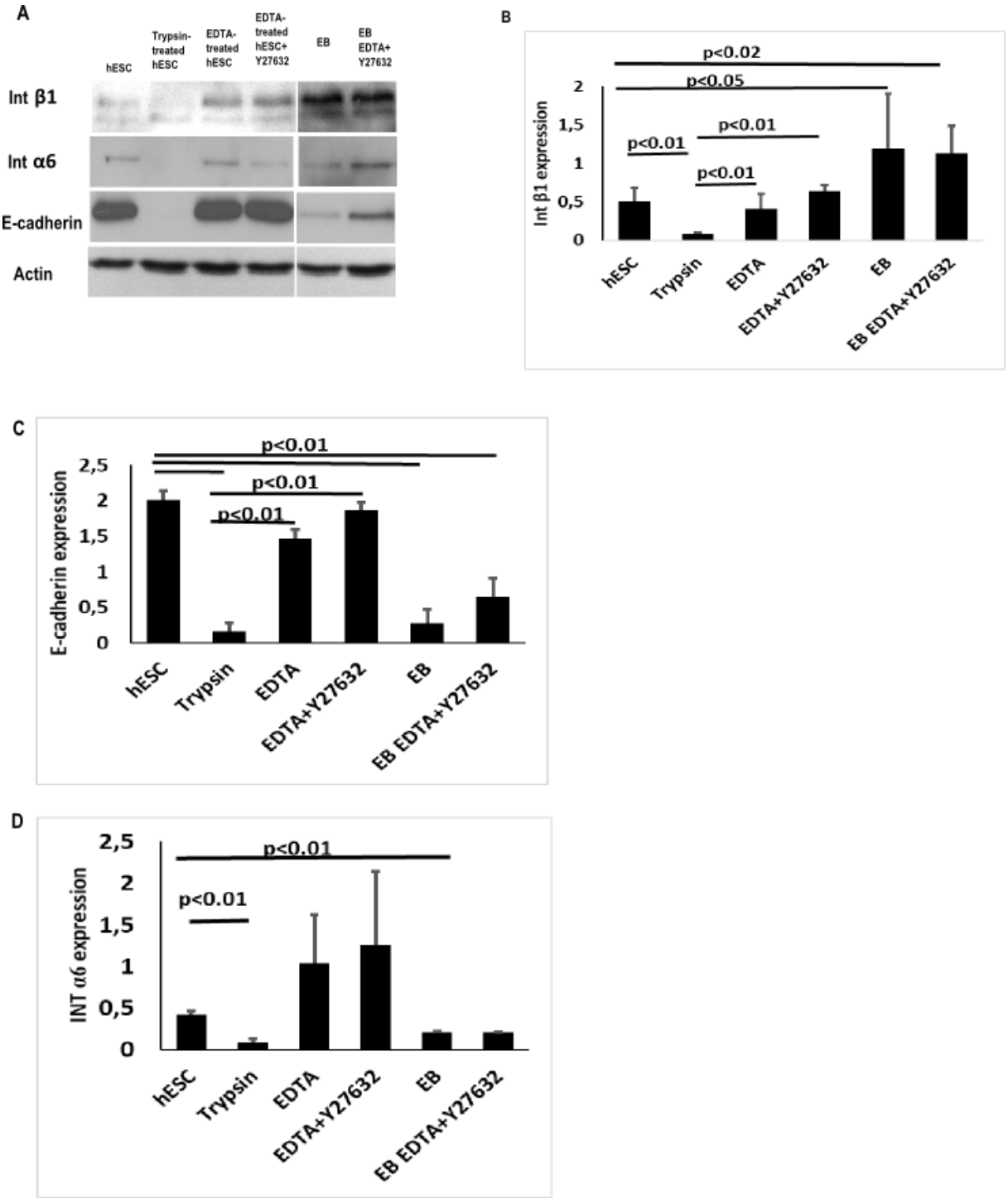

Fig. S1. The levels of integrins β1 and α6 as well as E-cadherin of the hES cells detached with different methods measured by Western blot/WB analysis.

(A) The WB analysis of the hES cells detached with different methods and the embryoid bodies (EB) formed from the hES cells detached manually or with EDTA and in the presence of the Rock inhibitor.

(B) Relative quantification of integrin β1 levels to β-actin levels using the ImageJ gel plot analysing tool. The data have been collected from three independent experiments and are shown as mean ± SD. P <0.01 is considered to be statistically significant.

(C) Relative quantification of integrin α6 level to β-actin level using Image J gel plot analysing tool. The data have been collected from three independent experiments and are shown as mean ± SD. P <0.01 is considered to be statistically significant.

(D) Relative quantification of E-cadherin level to β-actin level using Image J gel plot analysing tool. The data have been collected from three independent experiments and shown as mean ± SD. P <0.01 is considered to be statistically significant.

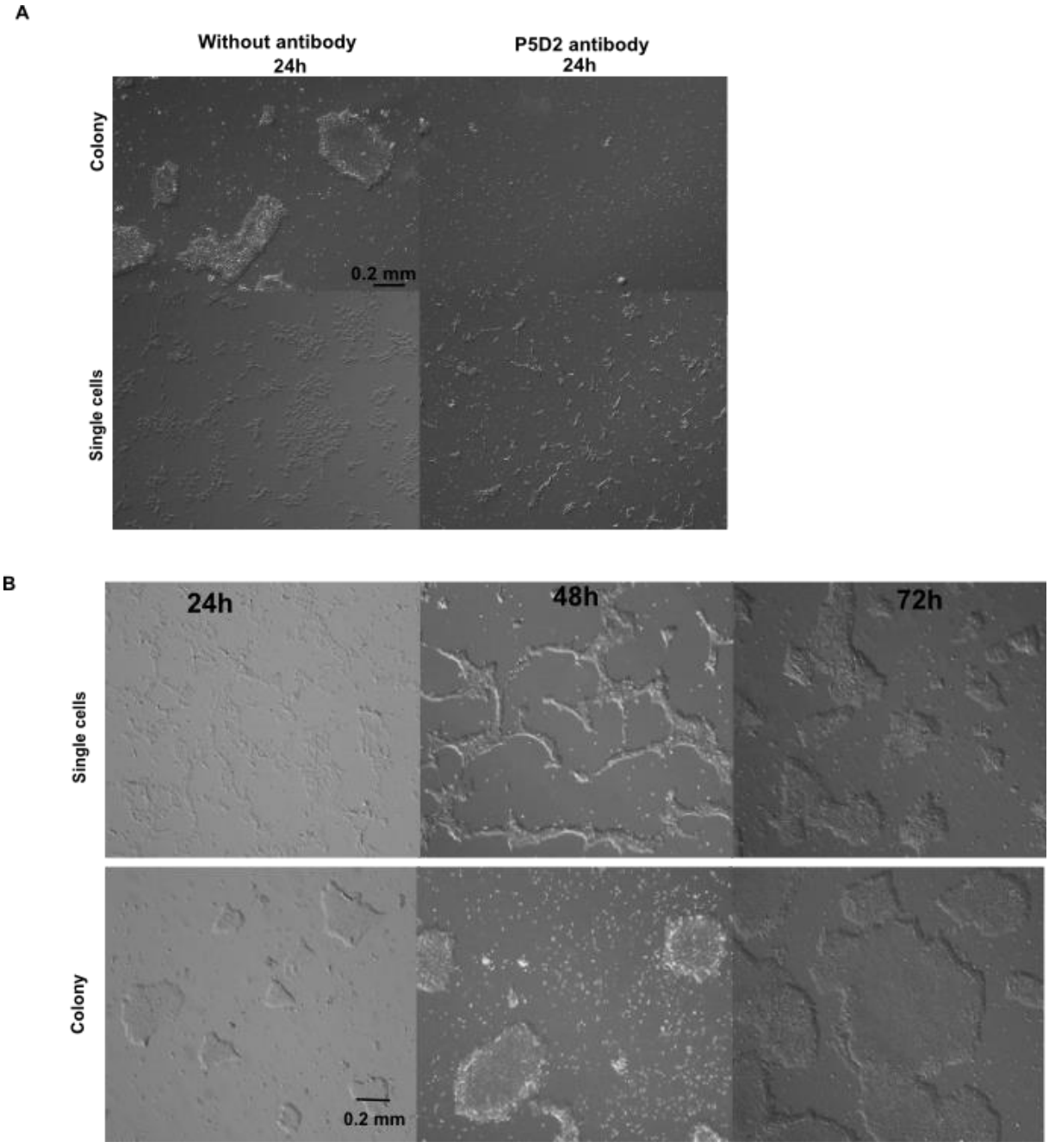

Fig. S2. The formation of the colonies of hES cells and growth in a single-cell culture.

(A) The effect of the integrin β1-blocking antibody P5D2 on the attachment of hES cells after having been detached manually or with EDTA in the presence of Y-27632 and re-seeded to the Matrigel®-coated 6-well plate.

(B) The effect of different detachment methods on the growth of hES cells during 72h.

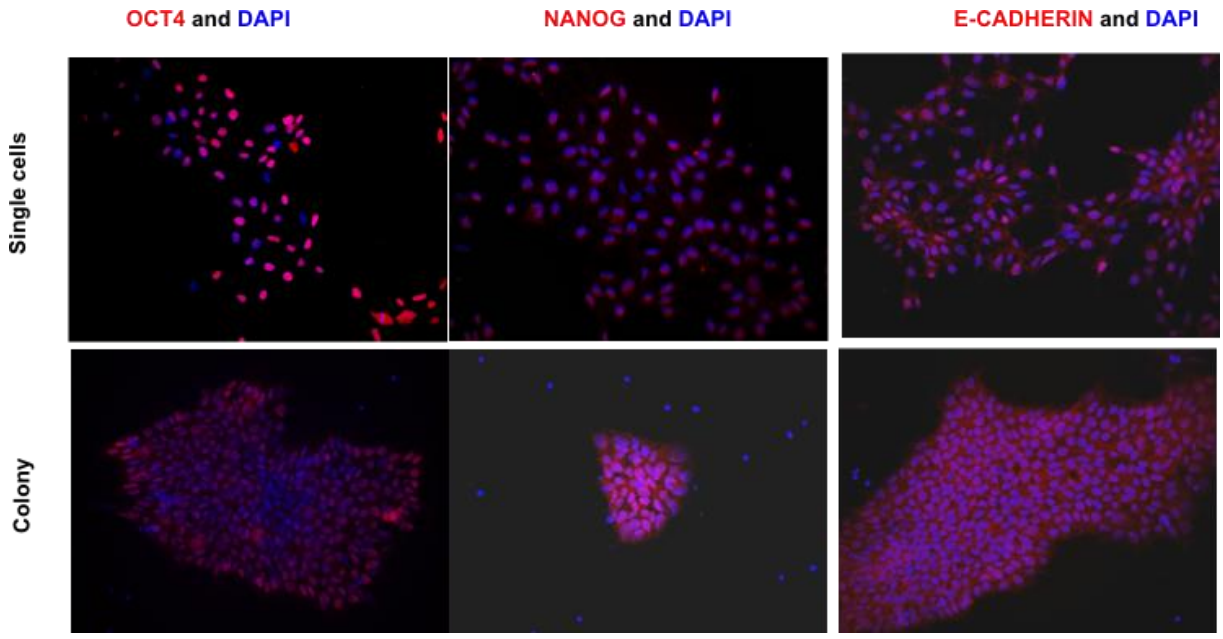

Fig. S3. Characterisation of pluripotency markers in hES cells in single-cell culture and in colony. Immunofluorescence staining of transcription factors NANOG, OCT4, and E-cadherin in hES cells 24h after re-seeding.

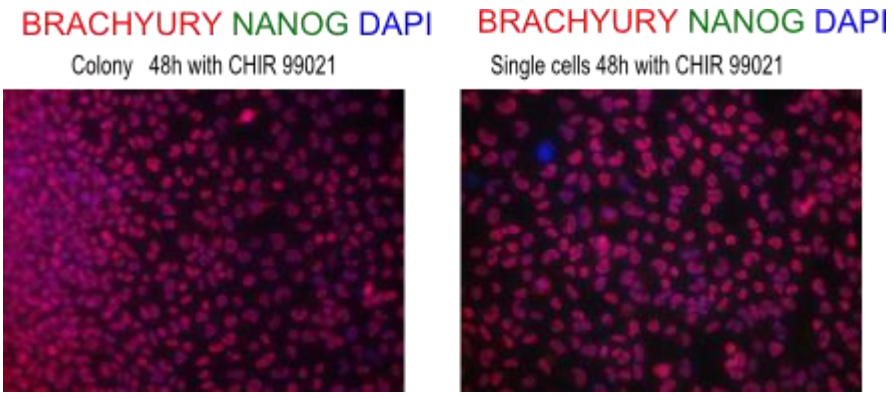

Fig. S4. Differentiation of hES cells into mesodermal lineage. The expression of the mesodermal marker BRACHYURY and the pluripotency marker NANOG in the cells differentiated with CHIR99201 in the Essential 6 medium for 48h. The hES cells were detached manually or grown as a single-cell culture for 24h before differentiation was initiated.

Table S1. Antibody clones used for different assays.

Target	Clone	Manufacturer	Assay
Integrin β1	P5D2	SC-13590	IF, neutralisation assay, internalisation assay
Integrin β1	12G10	SC- 59827	IF, internalisation assay
Integrin β1	B3B11	Ab77803	WB, FACS
Integrin α6		LSC 38650	IF, WB
Protein 4.1B	B-6	SC-398089	IF
RhoA	67B9	2117S Cell Signaling Technology	IF
TSC2		3612S Cell Signaling Technology	IF
Phosphorylated Myosin light chain 2 (T18/S19)		3674S Cell Signaling Technology	IF
Paxillin-TRITC (1-557)		610055 BD	IF
E-cadherin	3-10	SC-8426	IF, FACS
CD184		SC-53534	FACS
Nestin Alexa Fluor 647		560341 BD (lot 4346985)	FACS, IHC
CD184 PE		557145 BD	FACS, IHC
Nanog	N31-355	560483 BD	IF
OCT4	C-10	SC-5279	IF

Secondary antibodies

	Reference number	Manufacturer
Chicken anti-rabbit Alexa Fluor 488	A21441	Life Technologies
Goat anti-mouse Alexa Fluor 647	A21463	Invitrogen
Goat anti-mouse Alexa Fluor 555	A21424	Invitrogen
Donkey anti-rabbit Alexa Fluor 488	A21207	Invitrogen
Donkey anti-mouse Alexa Fluor 488	A210202	Invitrogen
Donkey anti-goat Alexa Fluor 594	11058	Invitrogen