Supplemental Information

Characterization of Integrin Engagement during Defined Human Embryonic Stem Cell Culture

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Table S1. Oligonucleotide sequences of primers used for RT – PCR.

Gene	Forward Primer	Reverse Primer	Length (base pairs)
Integrin α_1	5'-TCCAGTGAGATTTCAGAGACC -3'	5'-GTGATTTCCTGTGTTTTCGTCG-3'	117
Integrin α_2	5'-AACTCTTTGGATTTGCGTGTG - 3'	5'-TGGCAGTCTCAGAATAGGCTT-3'	82
Integrin α_{IIb}	5'-AGCTGCGAGGGAACTCCTT- 3'	5'-GGTGTGCTCCACTTTGGGT-3'	104
Integrin α_4	5'-ACTGTGAAGGCACGAGTGTG - 3'	5'-TGCTGGTTCGGAGGAATAG-3'	103
Integrin α_5	5'-AGAGAGACAATCAGTGGTTGG - 3'	5'-TCAGTTCTGTTCGTAAATCAGG-3'	168
Integrin α_6	5'-TGCCTCCCTCACCATCTTC - 3'	5'-TGCTTCTGCCAGTCCAGC-3'	171
Integrin α_8	5'-TGATCGAAATTCCTACCCTGATG - 3'	5'-TAATCACAGGCCGGGATCTG-3'	81
Integrin α_V	5'-GGGCAGCTACTTCGGCTAC - 3'	5'-GGCTCCCCCTTCCACGATA-3'	110
Integrin β_1	5'-CAAAGGAACAGCAGAGAAGC - 3'	5'-ATTGAGTAAGACAGGTCCATAAGG-3'	168
Integrin β_3	5'-GTGGTAGAAGAGCCAGAGTGTCC -3'	5'-CGTGGATGGTGATGAGGAGTTTC-3'	126
Integrin β_4	5'-GTGGTAGAACCAGAGTGTCC-3'	5'-CGTGGATGGTGATGAGGAGTTTC-3'	521
Integrin β_5	5'-AAGTCCACCCATTGAAGA-3'	5'-CCACAGCCATTTTTGACAAGG-3'	135
Integrin β_6	5'-TAACCAGCAACTTTAGACTGGGC-3'	5'-TCAGCATTTGTAATGCA-3'	181
Integrin β_8	5'-TTTGTCTGCCTGCAAAACGAC-3'	5'-GCCCAGTCCAAGAACAAGTGA-3'	84
Oct3/4	5'-CTGGGTTGATCCTCGGACCT-3'	5'-CACAGAACTCATACGGCGGG-3'	128
Nanog	5'-AAAGAATCTTCACCTATGCC-3'	5'-GAAGGAAGAGGAGAGACAGT-3	110
Pax6	5'-GTCCATCTTTGCTTGGGAAA-3'	5'-TAGCCAGGTTGCGAAGAACT-3'	814
MAP2	5'-CAGGTGGCGGACGTGTGAAAATTGAGAGTG-3'	5'-CACGCTGGATCTGCCTGGGGACTGTG-3'	221
Msx1	5'-CGAGAGGACCCCGTGGATGCAGAG-3'	5'-GGCGGCCATCTTCAGCTTCTCCAG-3'	307
GAPDH	5'-GTGGACCTGACCTGCCGTCT-3'	5'-GGAGGAGTGGGTGTCGCTGT-3'	153

We gratefully acknowledge the primer sequences for pluripotency and germ layers provided by Shinya Yamanaka, Kyoto University.

Table S2 Integrin antibodies used: specific clones, host species, and manufacturer.

Antibody Target	Clone	Host / Isotype	Source
Integrin α_1	FB12	mcl mouse IgG1	Chem: MAB1973z-20
Integrin α_2	P1E6	mcl mouse IgG1	Chem: MAB1950z-20
Integrin α_3 (CD49c)	ASC-1	mcl mouse IgG1	Chem: MAB2056-20
	P1B5	mcl mouse IgG1	Chem: MAB1952Z
Integrin α4	P4C2	mcl mouse IgG3	Chem: MAB1955
Integrin α5	P1D6	mcl mouse IgG3	Chem: MAB1956Z
Integrin α_6 (CD49f)	NKI-GoH3	mcl rat IgG2a	Chem: MAB1378-20
Integrin α_v		pcl rabbit	Chem: MAB1930
Integrin β_1	P4G11	mouse IgG1	Chem: MAB1951z-20
Integrin β_3		pcl rabbit	Chem: AB1932
Integrin β_4	ASC-3	mouse IgG1	Chem: MAB2058-20
Integrin α2β1	BHA2.1		Chem: MAB1998Z
Integrin $\alpha_v\beta_3$	LM609	mouse IgG1	Chem: MAB1976z
Isotype		Purified mouse IgG1	Caltag: MG100
Isotype		Normal rabbit IgG	Upstate: 12-370
Isotype		purified rat IgG2a	Caltag: R2A00
α-mouse IgG Alexa 488		goat	Mole Prob: A11029
α-rabbit IgG Alexa 488		goat	Mole Prob: A11034
α-rat IgG Alexa 488		donkey	Mole Prob: A21208

mcl = monoclonal; pcl = polyclonal; Chem = Chemicon; Mole Prob = Molecular Probes; Caltag = Caltag Lab; Upstate = Upstate (Millipore).

Relevant information from the manufacturers' factsheets.

The FB12 antibody clone, which reacts with the I domain (Val_{151}-Ala_{364}) of the human α_1 integrin (CD49a), was used for the α_1 integrin antibody. It has been found to inhibit the binding of activated human lymphocyte to laminin, collagen IV, and fibronectin.

The P1E6 clone was used for the α_2 integrin antibody. This antibody has been found to inhibit adhesion of fibroblasts, epithelial cells, endothelial cells, and non-activated platelets on collagens I, III, IV, VI, and laminin.

The ASC-1 clone was used for the α_3 (CD49c) integrin antibody, and this antibody has been found to inhibit cell attachment to laminin, but not fibronectin or collagen IV.

The NKI-GoH3 antibody was used for the α_6 integrin antibody, against the CD49f antigen. This clone has been found to react with a variety of cell types, including platelets, T lymphocytes, epithelial cells, peripheral nerves, and endothelial cells.

The MAB1930 polyclonal antibody was used for the α_v integrin subunit, and it recognizes the intracellular cytoplasmic domain of the protein. The antibody was found to not cross react to α_1, α_2, α_3, α_4, or α_6 integrins.

The P4G11 antibody clone was used for the β_1 integrin antibody, and its epitope recognition is Ca^{2+} dependent and has been found to be activating for ECM binding.

The MAB1932 polyclonal antibody was used for the β_3 integrin subunit, and importantly does not cross react with β_1 or β_2 integrins.

The ASC-3 clone was used for the β4 integrin antibody. This antibody was found to inhibit adhesion of some cell types to laminin, but not to collagen IV or fibronectin.The LM609 monoclonal antibody was used for the $\alpha_v\beta_3$ integrin antibody, and it is found to be reactive for the vitronectin receptor complex and to inhibit adhesion to vitronectin coated surfaces.

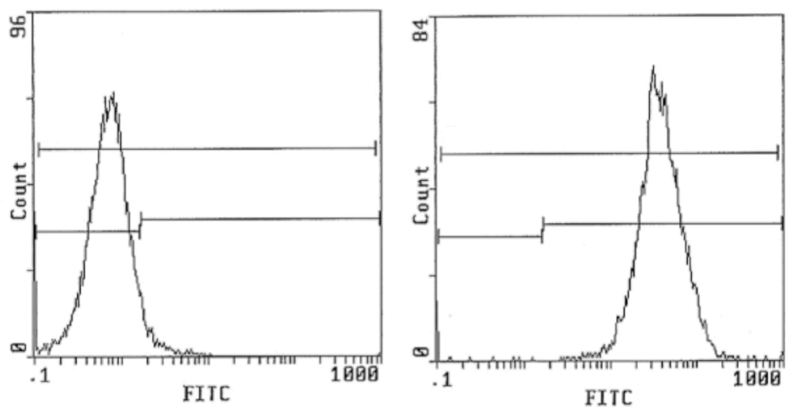

Figure S1 Histograms of the mouse IgG1 isotype control (left) and β1 integrin (right), indicating integrin expression above the background by >90% of the cells.

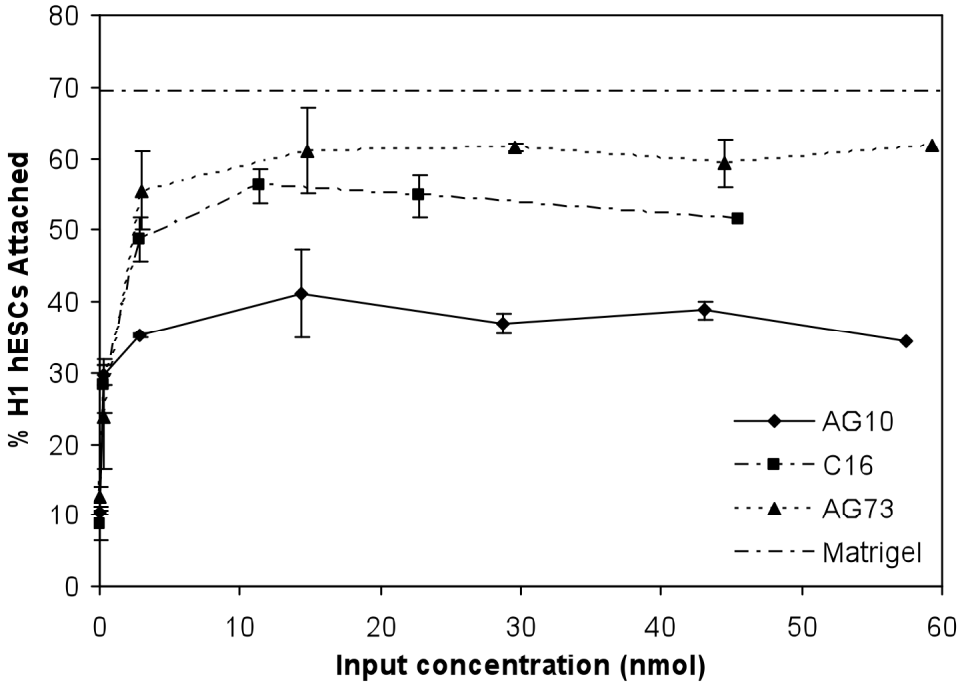

Figure S2 Percent attachment of H1 cells to peptide-coated substrata as a function of the amount of peptide added. Three peptides, AG10, C16, and AG73 known to engage $\alpha_6\beta_1$ and $\alpha_V\beta_3$ integrins and Syndecan-1, respectively, were adsorbed at various concentrations and shown to support hESC adhesion. As a comparison, the dashed line represents the percent attachment observed on Matrigel™-coated substrata. Cell adhesion increased with input concentration of peptide, until a threshold value of ~10 nmol/well was reached, above which no further increase of cell attachment was observed. Values of 0 nmol/well input concentration represent substrates blocked with BSA.

Table S3 Human ES colony properties on individual peptides (AG10, C16 & AG73) and binary and ternary peptide combinations. A nonparameteric method was used to record the percentage of the culture well covered by the colonies and number of colonies. The number of Oct4(+) cells within areas of the culture surface containing colonies was counted as well. These data and similar analyses were used to select the AG10:C16:AG73 (60:16:24) surface for more thorough analyses.

Peptide Coating	Percentage Culture Area Covered by Colonies	Number of Colonies	Number of Oct4(+) Colonies in 5 Randomly Selected Colony Areas
AG10	50-75	25-50	9
C16	< 25	< 25	16
AG73	50-75	< 25	14
AG10:C16:AG73 (60:16:24)	50-75	25-50	19
AG10:C16:AG73 (20:32:48)	< 25	< 25	21
C16:AG73 (40:60)	< 25	< 25	20

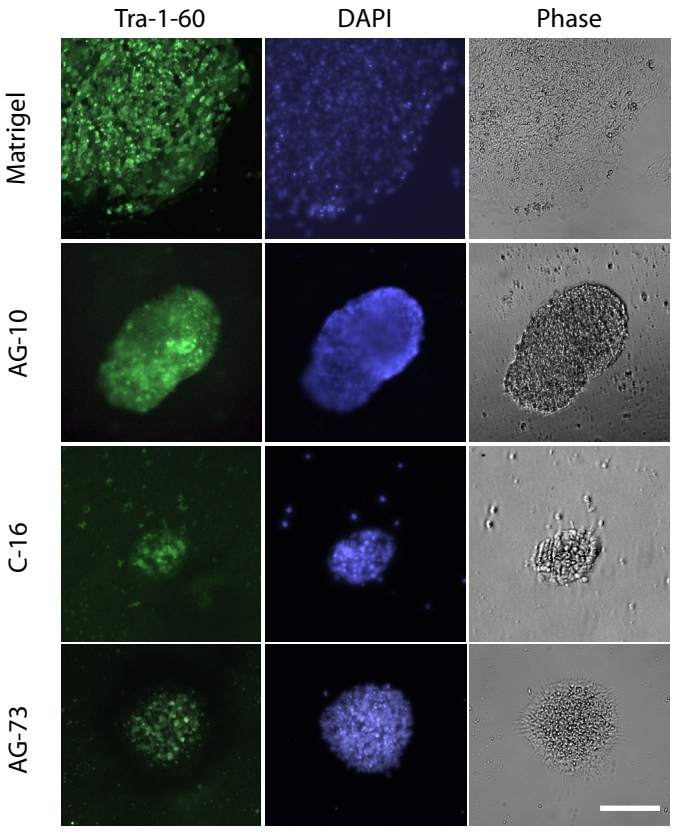

Figure S3 H1 hES cells were cultured on peptide-coated substrata for 7 days and Tra-1-60 marker expression was assessed by immunofluorescence. Scale bar: 500 μm.