Electronic Supplementary Material (ESI) for Integrative Biology

Supplementary Information

Breast tumour initiating cell fate are regulated by microenvironmental cues from extracellular matrix

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Table S1. Water contact angle measurements.

ECM substrates	Water contact angle (Deg)
Coll-I	35.7 ± 2
Fn	48.2 ± 2
PDL/Lam-I	54.4 ± 3
PDL	35.1 ± 2

Table S2. Primer sequences for real time PCR.

Gene Name	Forward Sequence 5′-3′	Reverse Sequence 5′-3′
Gapdh	TGCCGCCTGGAGAAACCTGC	CAGCCCCGGCATCGAAGGTG
Ck18	TCCGCGCCCAGTATGAAGCG	GAGGGTGCGTCTCAGCTCCG
Ck14	CGCCGTCTGCTGGAGGGAGA	GGAGACCACCTTGCCATCGTGC
Sma	GGCTCCATCCTGGCTTCGCT	GGAGGCGCTGATCCACAAAACG
Abcg2	TTCACTTGTATATTATACTTCATGTTAGGACTG	CGATTGTCATGAGAAGTGTTGCT
Tp63	ACAGACTGCAGCATTGTCAGTTTC	CTTCAGACTTGCCAAATCATCCA
Aldh1	GCTGGGGTGGTGTGGGTT	GACCATGTTCACCCAGTTCTCTTC
Gli1	TCGGACCCACTCCAATGAGAA	CTCGATGCCGCTTGGTCAC
CD133	TTATATGGTGTTCACAATCCTGTTATGAC	GCTTTCCATGTTGACTATCTTGTTGTTC

Foxc2	GTGTCCACTGGATAAGGTTTCGTCT	CATCTACTGTACAAAGCCATGCACTTC
Il6	AAATCGTGGAAATGAGAAAAGAGTTGT	TTTCCTGATTATATCCAGTTTGGTAGCA
Snail	GCCTTGTGTCTGCACGACCTG	TCTTCACATCCGAGTGGGTTTG
Twist	CCACGAGCGGCTCAGCTAC	CCTTCTCTGGAAACAATGACATCTAGG
Pai	AGGTAAACGAGAGCGGCACAGT	GCCCATGAAGAGGATTGTCTCTGTC
Cdh2	GCTGATCCTTGTTCTCATGTTTGTG	GCTGGCTCAAGTCATAGTCCTGGT
Zeb1	AGACACAAATATGAGCACACAGGTAAGA	GCTTGCCACACTTGTCACATTG
CD49f (Iα6)	GGCTCTATTAGTGTTTTTACTGTGGAAGTG	AATACTATGCATCGGAAGTAAGCCTCTC
CD61 (Iβ3)	GAGCCAAGTGGGACACAGCA	CGTCATCTGAAGATGGTCTCATTAAGT
Iα1	AACTGGATGGTCATCTTCTGATAATGT	CTGGATTGTGCCTCTTTTGAGAAC
Iα2	TAACATCACCTGCTGGCTGAAAG	CTGCACAGTCTGGAAAGTCGAAG
Iα5	CCCCAAAAGAAACTTCAGGTGG	TTTGAAGAAGCCGAGCTTGTAGAG
Iαv	GGATCTCTTCAACTCTACACTAGTCACC	CGTTTGAAAAAGCCCATCCTG
Iβ1	CAAGTGGGACACGGGTGAA	CTACTGTGACTAAGATGCTGCTGCTG
Iβ4	GAGCCCTTCCTCATGGATGGT	GAACTGTTGGTCCATATGAGTGCTG

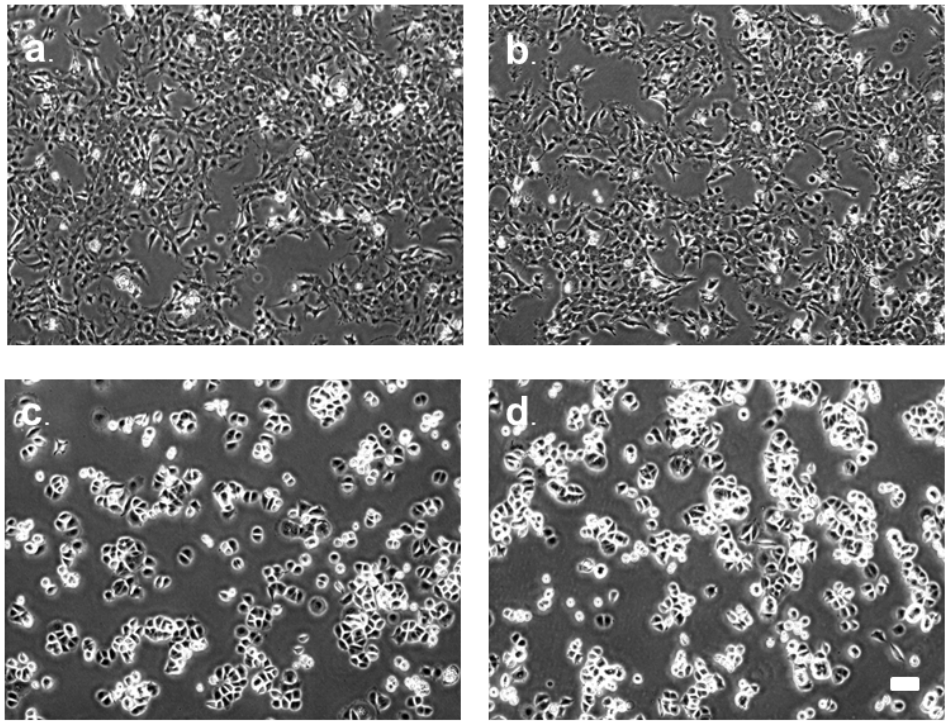

Fig. S1. Phase contrast micrographs of H6O5 cells on (a) Poly-lysine coated TCP, (b) on TCP and MCF-7cells on (c) Poly-lysine coated TCP, (d) on TCP. No difference in morphology was observed between cells on Poly-lysine coating and TCP. Scale bar = 50 μm.

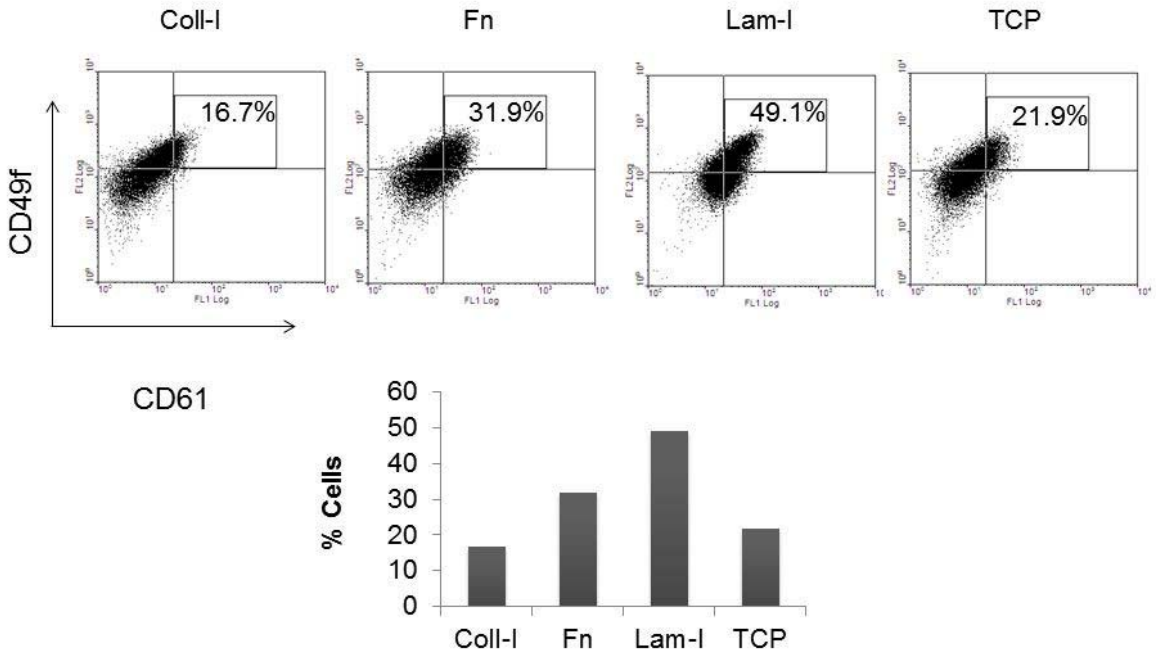

Fig. S2. FACS analysis showed that Lam-I and Fn maintained the majority of the TIC population whereas Coll-I and TCP cell cultures exhibited significant loss of TICs after two weeks of culture on coated substrates. The FACS analysis data were also plotted as a bar graph (the bottom panel).

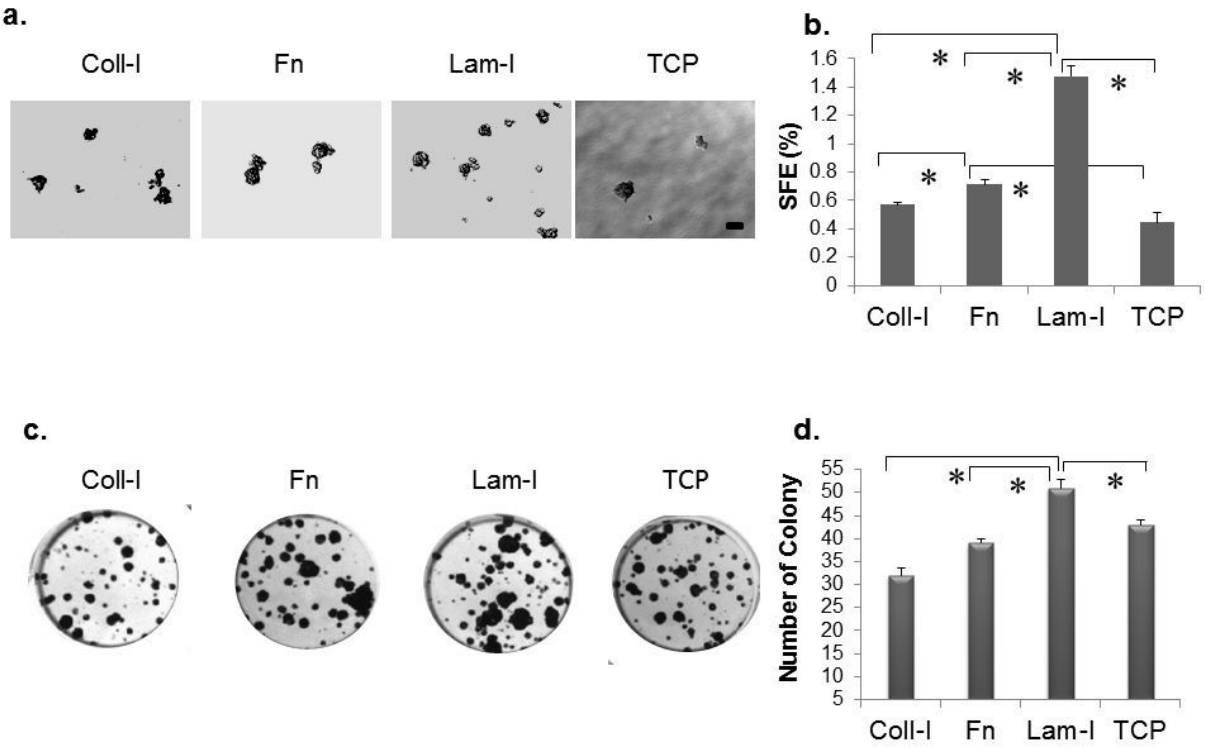

Fig. S3. TICs exhibit greatest self-renewal characteristics in Lam-I after two weeks of culture. (a) *In vitro* tumorsphere formation analysis of TICs retrieved from Coll-I, Fn, Lam-I and TCP. Scale bar = 50 μm. (b) Plot showing sphere formation efficiency (SFE) for each substrate. (c) *In vitro* clonogenic assay of TICs pre-cultured on Coll-I, Fn, Lam-I and TCP substrates. (d) Plot showing colony numbers for each substrate. All measurements were done in triplicates and data are indicated as mean ± standard deviation. (*) $p < 0.05$ indicates a statistically significant difference.

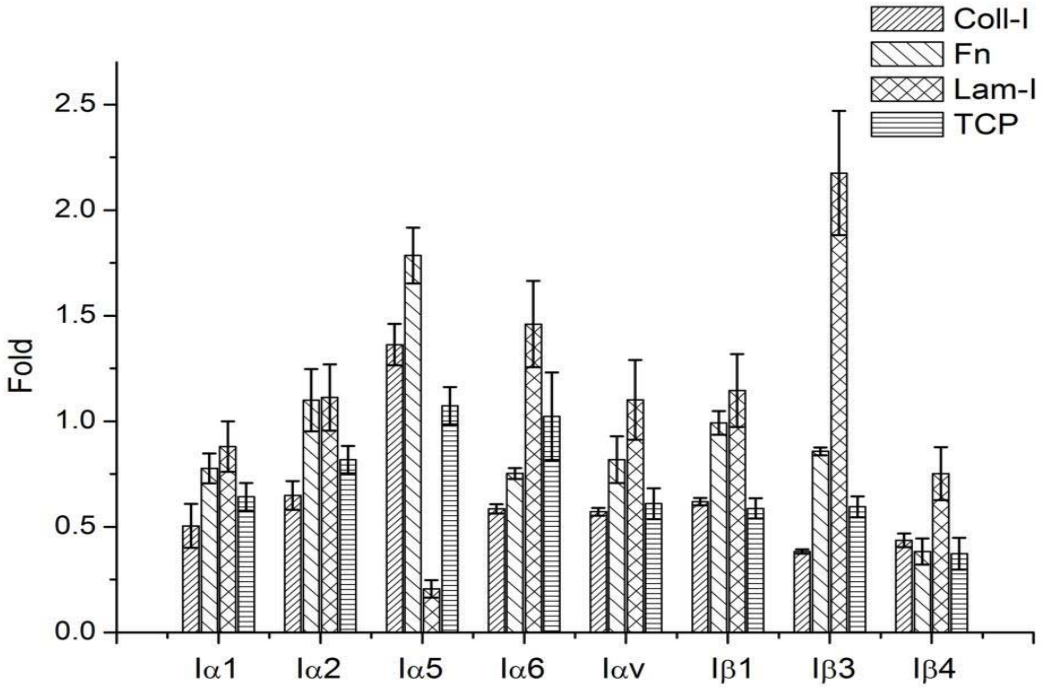

Fig. S4. Expression analysis of integrins in sorted cells cultured on Coll-I Fn, Lam-I and TCP substrates for one week using quantitative real-time reverse transcription PCR analysis. Each expression was normalized by using *Gapdh* as the internal control.

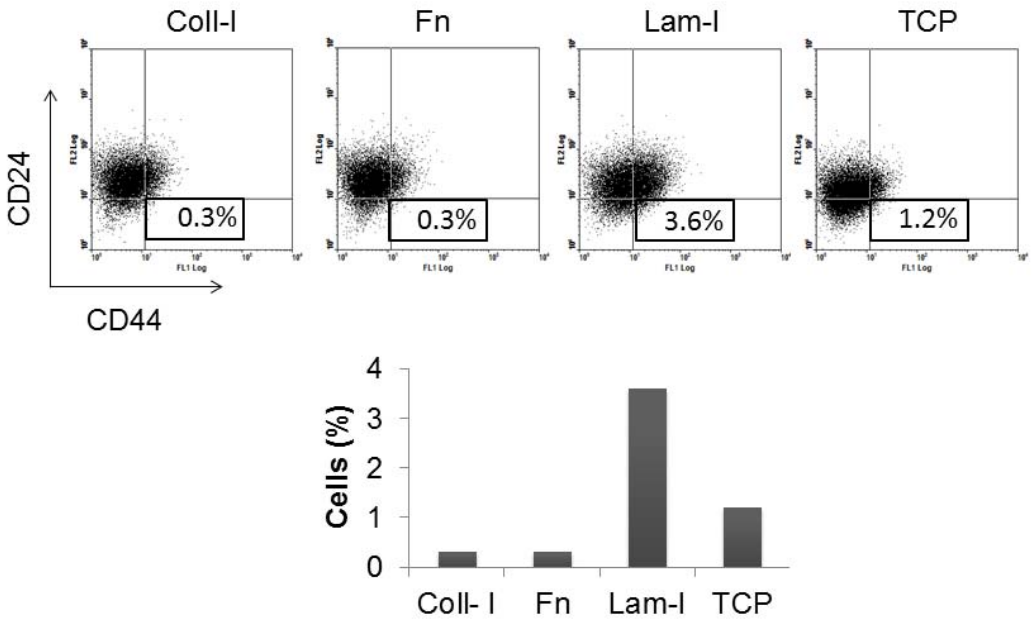

Fig. S5. FACS analysis for $CD44^{high}CD24^{low}$ population in MCF-7 cell line after one week of culture on coated substrates, showed increase in TICs population in Lam-I whereas Coll-I and Fn cultures exhibited significant loss of TICs. The FACS analysis data were also plotted as a bar graph (the bottom panel). MCF-7 is a human breast cancer cell line reported to have very low population of $CD44^{high}CD24^{low}$ that identified as a cancer stem cell like population.