Supplementary information: A materials-based approach for interrogating human prostate cancer cell adhesion and migratory potential using a fluoroalkylsilica (FS) culture surface.

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1.0 – RT-PCR primer information

1.A RNA isolation and reverse transcription conditions

RNA was isolated into RNase free water (ThermoFisher AM9932) using the RNeasy™ Mini kit (Qiagen 74104) following the manufacturer's protocol. RNA concentration for each sample was quantified using a NanoDrop™ 8000. Reverse transcription (RT) of samples was performed in a volume of 10 µL, consisting of 2 µg RNA, 1 µL Oligo(dt)15 primer (Promega, C110A) and the remaining volume RNase free water. Samples were heated at 70°C for 5 min and placed on ice for 5 min. To each sample was added 15 µL of RT solution comprising 5 µL RT buffer (Promega, M531A), 1 µL RT enzyme (Promega, M170A), 0.7 µL RNasin™ (Promega, N2111), 1 µL dNTP (Sigma, D4788) and 7.3 µL of RNase free water. Tubes were incubated at 38°C for 90 min after which the reaction was stopped by heating to 95°C for 5 min. The cDNA was stored at -20°C until use.

1.B RT-PCR reaction conditions and data analysis

RT-PCR was conducted using the SYBR™ Green (Bio-Rad cat#172-5124), conducting 40 cycles per run with a Qiagen rotor gene Q thermocycler. Reactions were conducted in 13 µL, consisting of 1 µL of cDNA template (~40 ng/reaction) or H_2O for no template controls, 0.2 µM of forward and reverse primer and 6.75 µL SYBR™ Green Master mix. Cycling followed a hold period of 5 min at 95°C and was as follows: 95°C for 20 sec, 62°C for 20 sec and 72°C for 20 sec. Melt curve analysis was conducted from 62°C – 95°C. Data were analysed using the PFAFFL equation. [Pfaffl, 2001] The relative expression ratio was calculated from RT-PCR efficiencies and the crossing point of an unknown sample (target gene) *vs.* the control (reference gene). A 5-fold serial dilution of cDNA was conducted to determine the amplification efficiency of each primer pair, with each dilution being used as a template for PCR. The slope of the standard curve was used to calculate efficiency values through Rotor gene Q software (Table A.1.). Pooled cDNA derived from OPCT-1, P4B6B and P5B3 was used to determine efficiency and specificity. For each primer pair, the following acceptance criteria were defined: efficiency ≥80%, single product detected on melt curve, single band detected through agarose gel electrophoresis (Fig. A1) and a Ct value ≤35. In the case of gene induction (i.e. a sample produced a Ct value >35 under condition 1 but ≤35 under condition 2), the data from both samples was incorporated if the all other acceptance criteria were met for both samples under comparison.

Table S1. EMT panel and reference RT-PCR primers.

Primer pair	Forward/reverse sequence	Tm (°C)	GC content (%)	Efficiency (%)
E-cadherin	5'-TGCCCAGAAAATGAAAAAGG-3' 5'-GTGTATGTGGCAATGCGTTC-3'	53.2 57.3	40.0 50.0	99
Vimentin	5'-GAGAACTTTGCCGTTGAAGC-3' 5'-GCTTCCTGTAGGTGGCAATC-3'	57.3 59.4	50.0 55.0	102
Fibronectin	5'-CAGTGGGAGACCTCGAGAAG-3' 5'-TCCCTCGGAACATCAGAAAC-3'	61.4 57.3	60.0 50.0	83
N-cadherin	5'-ACAGTGGCCACCTACAAAGG-3' 5'-CCGAGATGGGGTTGATAATG-3'	59.4 57.3	55.0 50.0	95
ZEB1	5'-GGCATACACCTACTCAACTACGG-3' 5'-TGGGCGGTGTAGAATCAGAGTC-3'	62.4 62.1	52.2 54.5	99
Slug	5'-GGGGAGAAGCCTTTTTCTTG-3' 5'-TCCTCATGTTTGTGCAGGAG-3'	57.3 57.3	50.0 50.0	92
HPRT	5'-TGACACTGGCAAAACAATGCA-3' 5'-GGTCCTTTTCACCAGCAAGCT-3'	55.9 59.8	42.9 52.4	93
YWHAZ	5'-ACCGTTACTTGGCTGAGGTTGC-3' 5′-CCCAGTCTGATAGGATGTGTTGG-3′	63.2 61.0	54.6 52.2	98

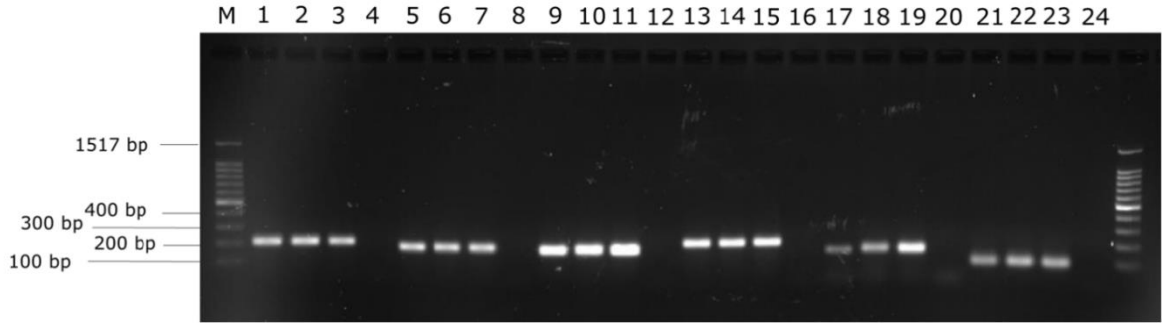

Figure S2. The ECAD primer products produced when using OPCT-1, P5B3 and P4B6B derived cDNA are shown in lanes 1, 2 and 3 respectively, with the no template control sample in lane 4. Similarly, results are shown for FN (lanes 5-8), VIM (lanes 9-12), NCAD (lanes 13-16), ZEB1 (lanes 17-20) and HPRT in lanes 21-24. The molecular weight ladder is shown in lane M.

2.0 – Immunofluorescence

OPCT-1, P5B3 and P4B6B cells ($1x10^4$) were seeded upon fluorescence compatible 96-well plates (BD Biosciences cat #353219) in 200 µL of medium. Plates were incubated for 24 h under SC, after which wells were washed x3 with 300 µL of PBS. Cells were fixed for 15 min with 200 µL of 4% w/v formaldehyde (Sigma cat #F8775) in PBS. Cells were washed x3 with 250 µL of PBS supplemented with 0.1% v/v Tween 20 and then blocked in wash buffer supplemented 10% v/v FCS for 1 h at RT. Primary staining was conducted with 200 µL/well in blocking buffer, overnight at 4°C. Cells were stained for the expression of fibronectin (FN) using a polyclonal rabbit anti-human fibronectin primary antibody (Sigma cat #F3648, 1:400 dilution) and for E-cadherin, vimentin and β-catenin using

monoclonal rabbit antibodies (Abs) from the Cell Signalling Technology EMT Ab sampler kit (cat #9782), all at the recommended dilution.

Wells were washed x3 with wash buffer and detection binding of primary antibody detected using an FITC-conjugated polyclonal swine anti-rabbit immunoglobulin secondary antibody (Dako cat #F005401-2, now Agilent, 1:40 dilution (in blocking buffer) for 2 h at RT). Plates were washed once prior to the addition of DAPI (VECTASHIELD™, VECTOR Laboratories cat #H-1200) and imaged using a Leica TCS SP5 LSCM. Z-stacks were prepared for each sample (2 μM steps) with the same laser power/gain between samples. Background signal was assessed by excluding the primary antibody from the staining protocol for each condition (Fig. B.1).

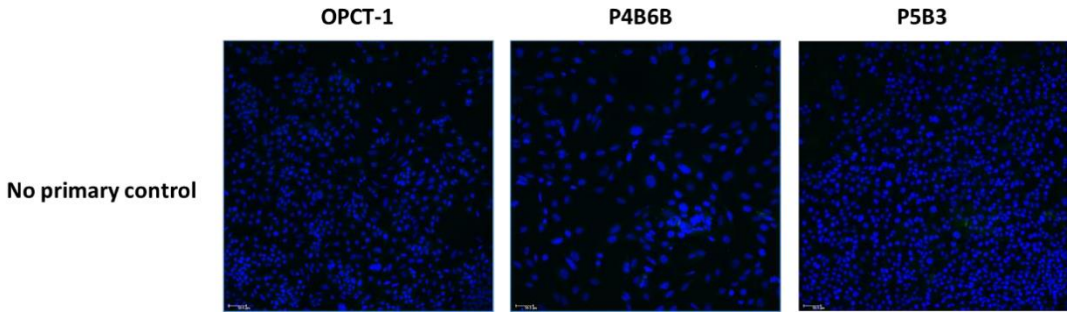

Figure S3. No primary control micrographs for OPCT-1, P4B6B and P5B3. Cells were stained with an FITC-conjugated polyclonal swine anti-rabbit immunoglobulin secondary antibody (Dako cat #F005401-2, now Agilent, 1:40 dilution (in blocking buffer) and DAPI only. Fluorescence micrographs were taken at the maximum 488 nm laser gain used for FITC detection.

3.0 – Flow cytometry gating

The presence of $CD44^{+}CD24^{-/low}$ cells was determined by washing OPCT-1, P5B3 and P4B6B cells x2 with PBS, after which they were disassociated using Accutase® (Sigma cat #A6964). Cells ($1x10^{6}$) were washed twice with 1 mL of PBS, pelleted and suspended in 100 μL of PBS. The presence of viable cells was detected by incubating cells for 15 min with 1 mL of Invitrogen LIVE/DEAD® Fixable Violet Dead Cell Stain (ThermoFisher Scientific cat#L34955), after which cells were washed x2 with 1 mL of PBS, suspended in 100 μL of PBS and incubated for 1 h at RT with 5 μL of an FITC-conjugated rat anti-human CD44 monoclonal antibody (mAb) (clone IM7, ThermoFisher cat# eBioscience, 11-0441-82)

and an APC-conjugated mouse anti-human CD24 mAb (clone eBioSN3 (SN3 A5-2H10), ThermoFisher cat# 17-0247-41). Cells were washed with 2 mL of PBS and suspended in 500 µL of Beckman Coulter ISOTON™ II diluent prior to analysis using a Beckman Coulter Gallios™ flow cytometer. Individuals cells were identified using forward and side light scatter (FSc, SSc) and gated upon based on unstained samples, LIVE/DEAD™ only, LIVE/DEAD™ + CD44, LIVE/DEAD™ + CD24 and LIVE/DEAD™ + CD44 + CD24 (Fig. C.1, C.2, C.3). Data were acquired, analysed and presented using Beckman Coulter Kaluza™ acquisition and analysis software.

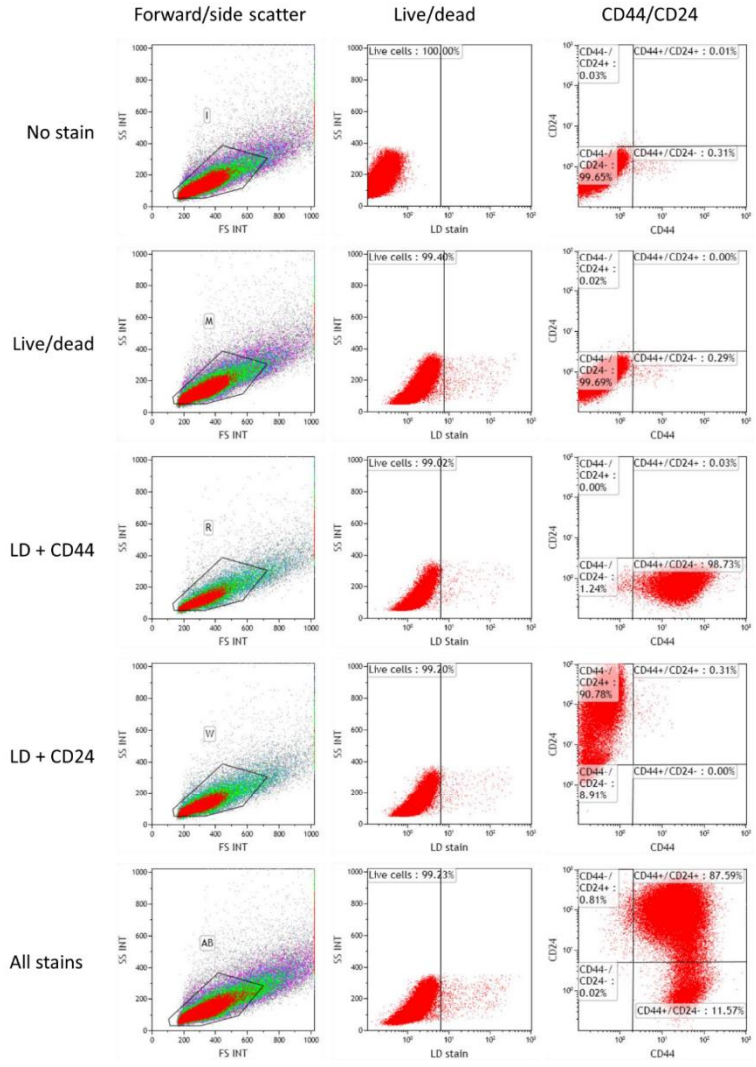

Figure S4. Representative gating strategy for determining CD44/CD24 expression by OPCT-1. Plots illustrate show forward/side scatter, LIVE/DEAD™ and CD44/CD24 staining for no stain, LIVE/DEAD™ stained, LIVE/DEAD™ + CD44, LIVE/DEAD™ + CD24 samples and samples with all stains.

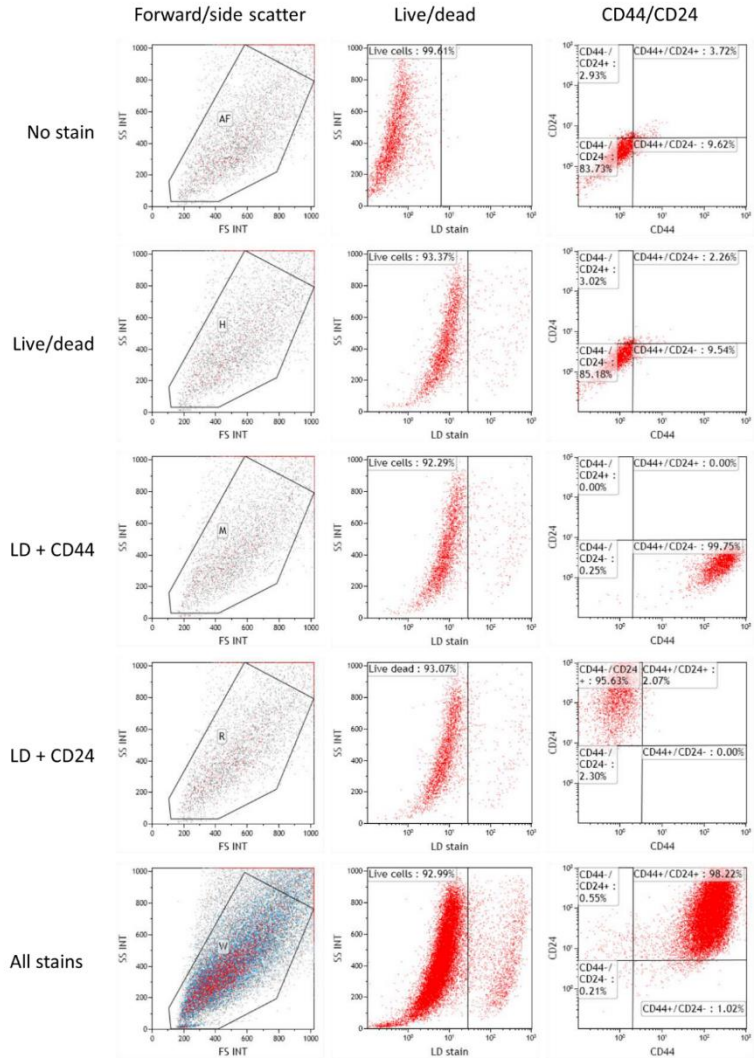

Figure S5. Representative gating strategy for determining CD44/CD24 expression by P4B6B. Plots illustrate show forward/side scatter, LIVE/DEAD™ and CD44/CD24 staining for no stain, LIVE/DEAD™ stained, LIVE/DEAD™ + CD44, LIVE/DEAD™ + CD24 samples and samples with all stains.

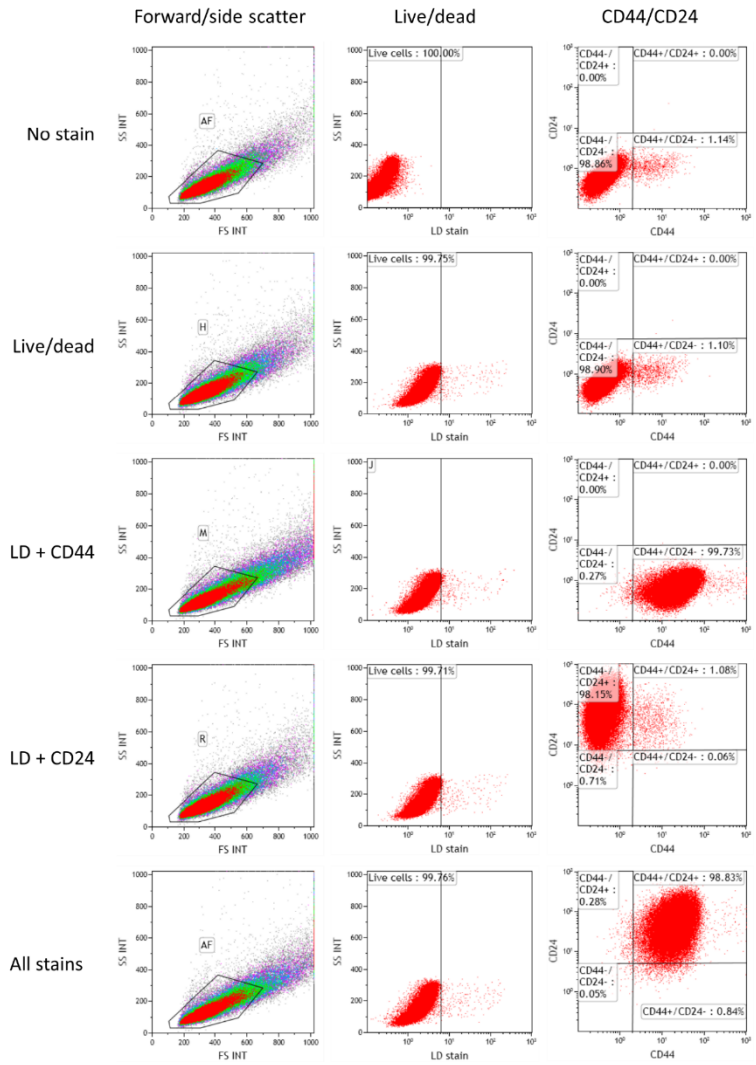

Figure S6. Representative gating strategy for determining CD44/CD24 expression by P5B3. Plots illustrate show forward/side scatter, LIVE/DEAD™ and CD44/CD24 staining for no stain, LIVE/DEAD™ stained, LIVE/DEAD™ + CD44, LIVE/DEAD™ + CD24 samples and samples with all stains.

For determining vimentin expression, OPCT-1, P5B3 and P4B6B cultures were washed twice with PBS and disassociated using Accutase®. Cells ($1x10^6$) were incubated with LIVE/DEAD® Fixable Violet Dead Cell Stain as described above. Cells were washed twice with 1 mL of PBS and fixed by re-suspending in 2% v/v formaldehyde solution (Sigma cat# F8775) for 15 min at RT. Cells were washed in 2 mL of PBS and permeabilised by suspending in 100 µL of 0.5% v/v Tween™ 20 in PBS (Sigma cat# P9416). Cells were washed in 2 mL of 0.1% v/v Tween™ 20, in 100 µL of which they were then resuspended. Cells were then incubated with 10 µL PE-conjugated murine anti-human vimentin mAb

(clone RV202, BD Biosciences cat# 562337) for 1 h at RT. Cells were washed in 2 mL of PBS and suspended in 500 µL ISOTON™ II diluent prior to analysis, as described. Gating was based on unstained samples, LIVE/DEAD™ only and LIVE/DEAD™ + vimentin (Fig. C.4, C.5 and C.6).

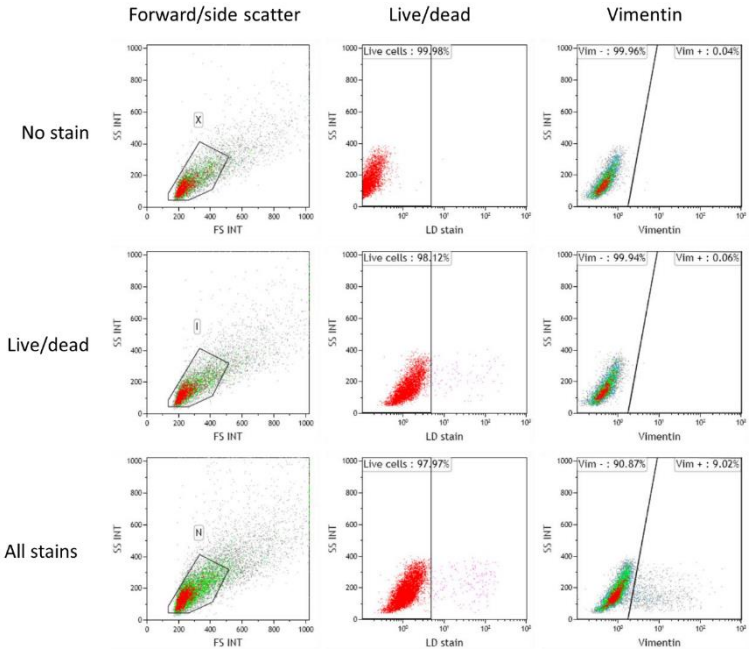

Figure S7. Representative gating strategy for determining Vimentin expression by OPCT-1. Plots illustrate show forward/side scatter, LIVE/DEAD™ and CD44/CD24 staining for no stain, LIVE/DEAD™ stained, LIVE/DEAD™ + CD44, LIVE/DEAD™ + CD24 samples and samples with all stains.

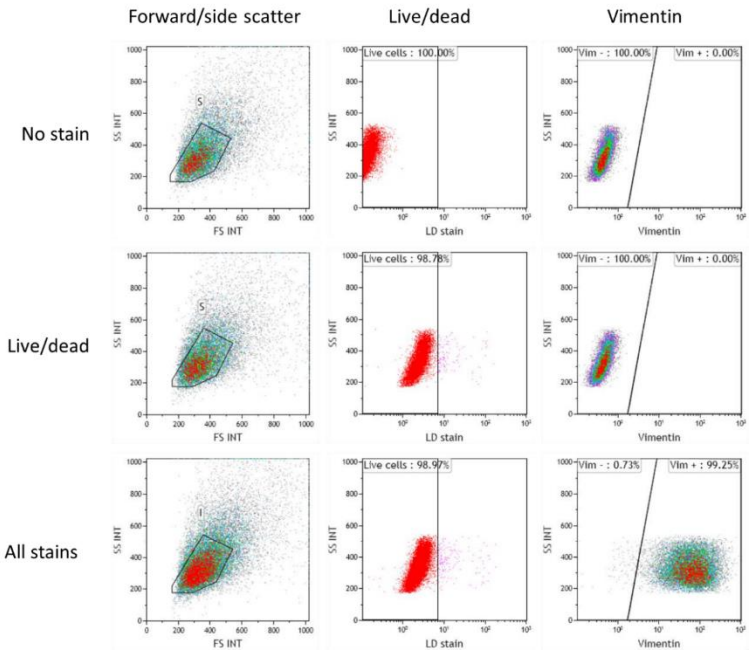

Figure S8. Representative gating strategy for determining Vimentin expression by P4B6B. Plots illustrate show forward/side scatter, LIVE/DEAD™ and CD44/CD24 staining for no stain, LIVE/DEAD™ stained, LIVE/DEAD™ + CD44, LIVE/DEAD™ + CD24 samples and samples with all stains.

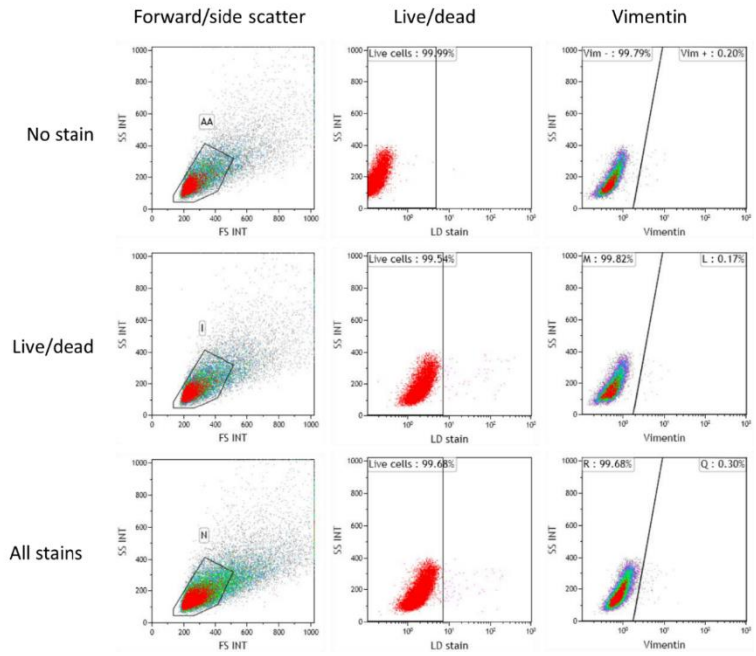

Figure S9. Representative gating strategy for determining Vimentin expression by P5B3. Plots illustrate show forward/side scatter, LIVE/DEAD™ and CD44/CD24 staining for no stain, LIVE/DEAD™ stained, LIVE/DEAD™ + CD44, LIVE/DEAD™ + CD24 samples and samples with all stains.

4.0 – Live cell imaging

OPCT-1, P5B3 and P4B6B cells ($1x10^6$) were seeded separately onto TCP and FS (6-well format) in 7 mL of medium. Cultures maintained under standard conditions and plates were imaged using a Leica TCS SP5 inverted laser scanning confocal microscope (LSCM). Bright-field micrographs were taken every 30 min for 96 h, videos were compiled from each point of interest using LAS-AF (Leica). For co-culture experiments interrogating the cross-regulation of responses, this process was performed for 72 h of using P5B3 cells ($1x10^6$), P4B6B cells ($1x10^6$) and a 1:1 mixture ($1x10^6$ total) of these lines.

Video S10: Composition of cell imaging of OPCT-1 on TCP with a data interval of 0.5 h.

Video S11: Composition of cell imaging of OPCT-1 on FS with a data interval of 0.5 h.

Video S12: Composition of cell imaging of P5B3 on TCP with a data interval of 0.5 h.

Video S13: Composition of cell imaging of P5B3 on FS with a data interval of 0.5 h.

Video S14: Composition of cell imaging of P4B6B on TCP with a data interval of 0.5 h.

Video S15: Composition of cell imaging of P4B6B on FS with a data interval of 0.5 h.

Video S16: Composition of cell imaging of a 1:1 mixture of P5B3 and P4B6B on TCP with a data interval of 0.5 h.

Video S17: Composition of cell imaging of a 1:1 mixture of P5B3 and P4B6B on FS with a data interval of 0.5 h.

5.0 References

Pfaffl, M.W.. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29 (2001) e45.