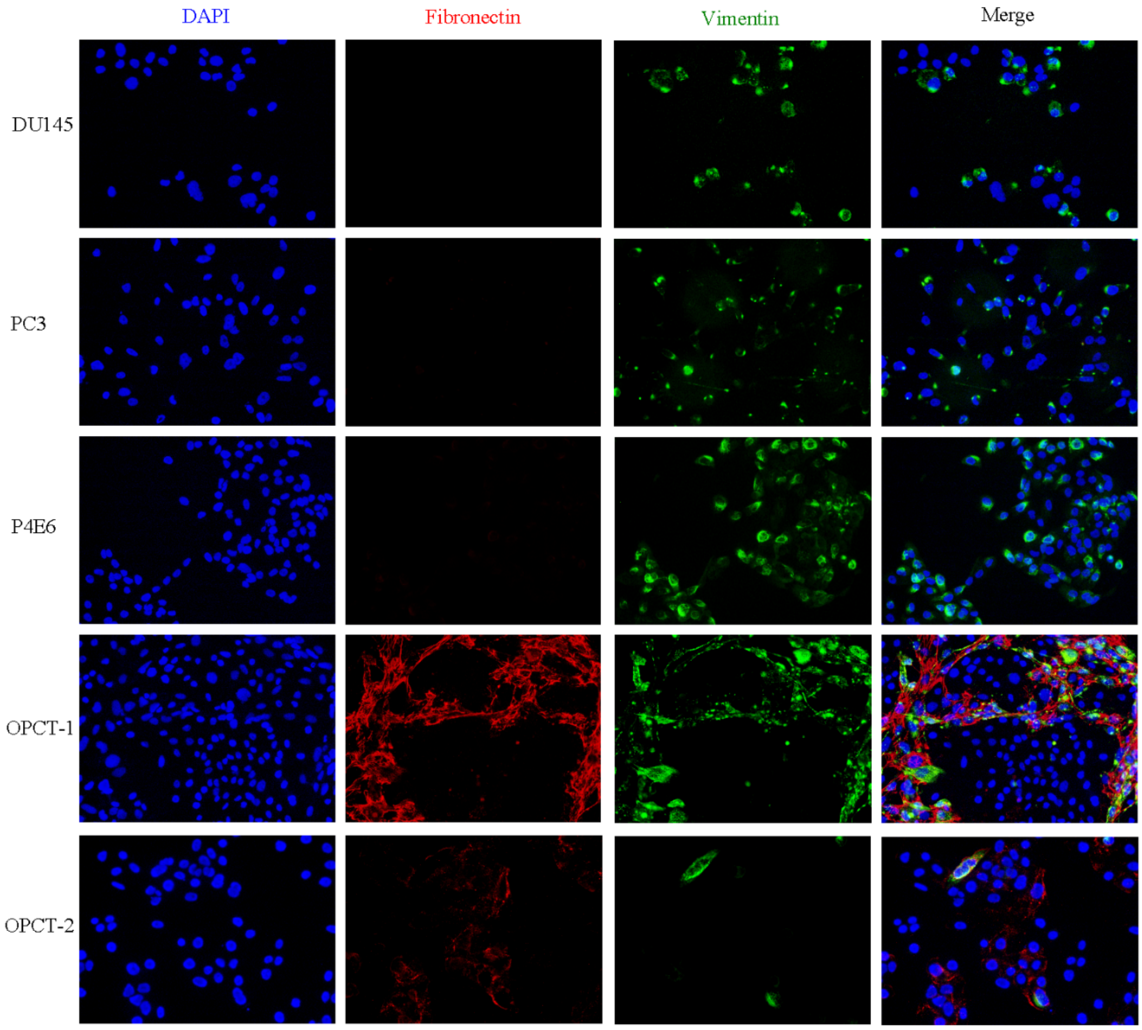
Supplementary Information:

A novel spontaneous model of epithelial-mesenchymal transition (EMT) using a primary prostate cancer derived cell line demonstrating distinct stem-like characteristics

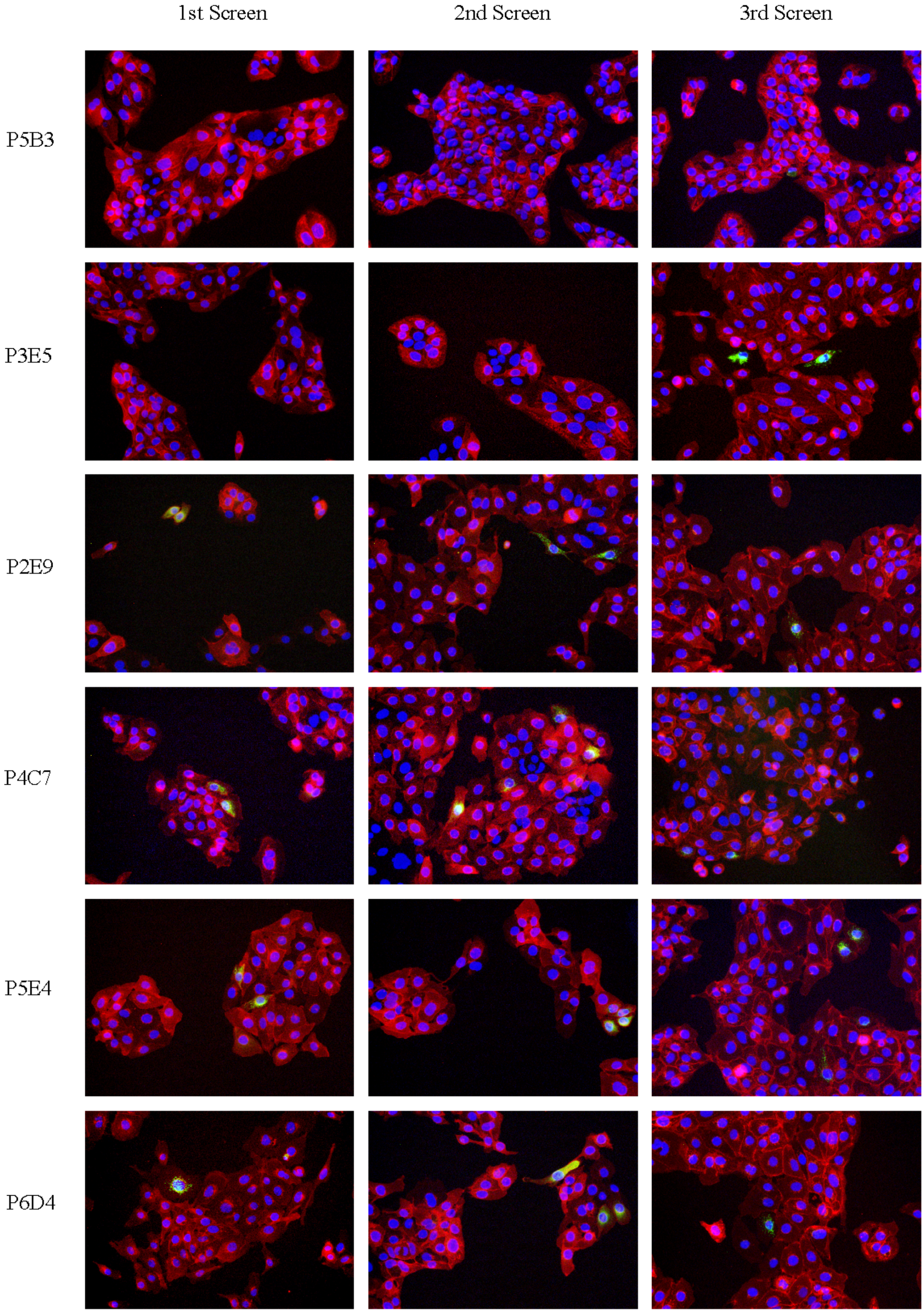
Naomi Dunning-Foreman, Jayakumar Vadakekolathu, Stéphanie A. Laversin, Morgan G. Mathieu, Stephen Reeder, A. Graham Pockley, Robert C. Rees, David J. Boocock

Supplementary Figure 1

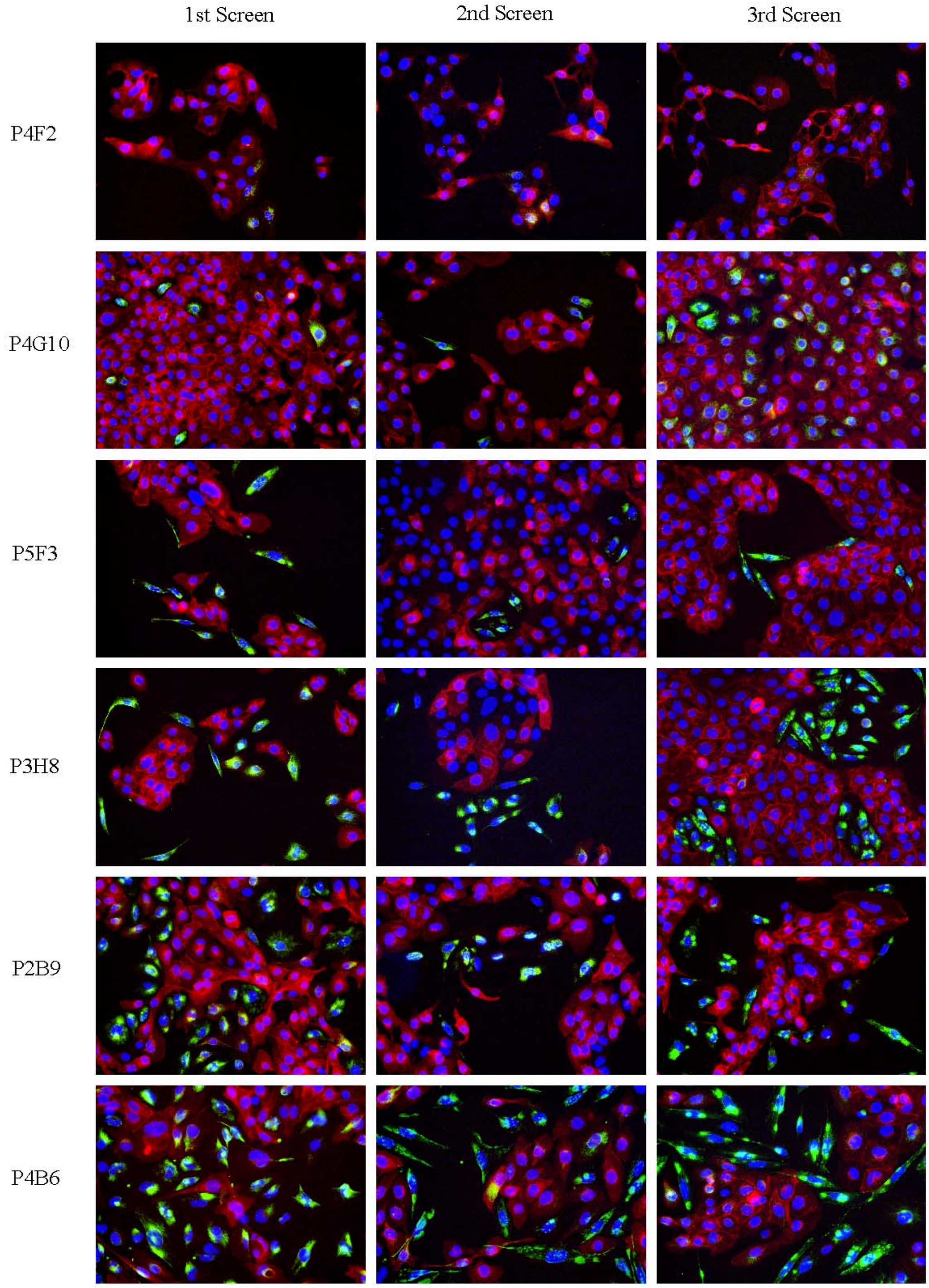


Dual immunofluorescent staining of DU145, PC3, P4E6, OPCT-1 and OPCT- 2 using a murine monoclonal antibody against cellular fibronectin and a rabbit polyclonal antibody against the mesenchymal marker, vimentin. E-cadherin was labelled with an anti-mouse Alexa Fluor®568-conjugated secondary antibody and vimentin was labelled with an anti-rabbit Alexa Fluor®488-conjugated secondary antibody. Nuclear staining was achieved using mounting media with DAPI. Representative images. Image magnification at x20.

Supplementary Figure 2:

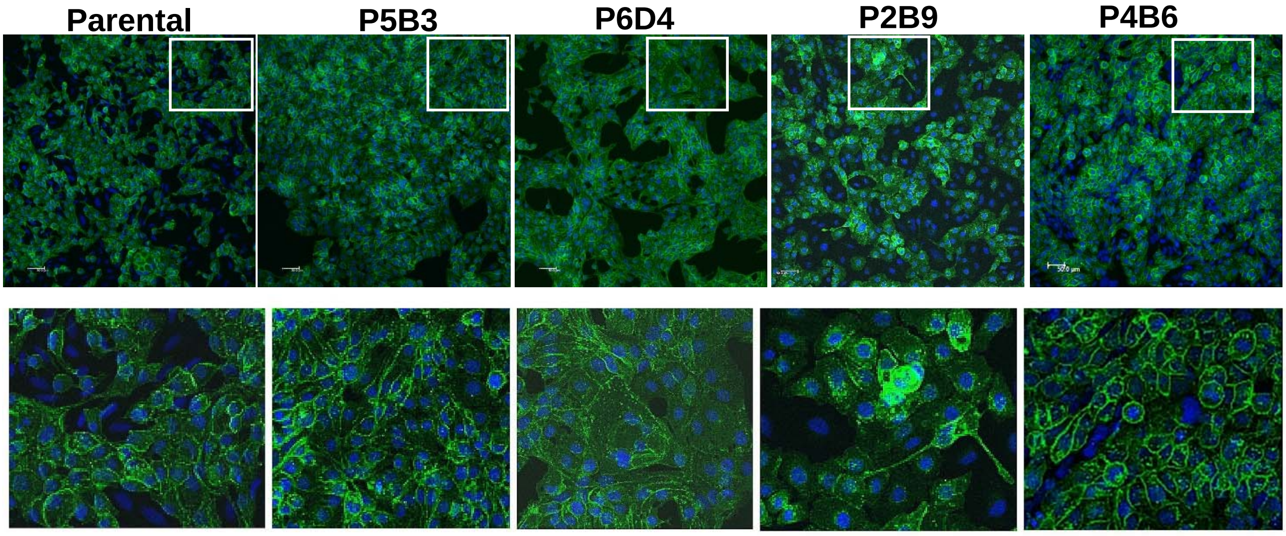


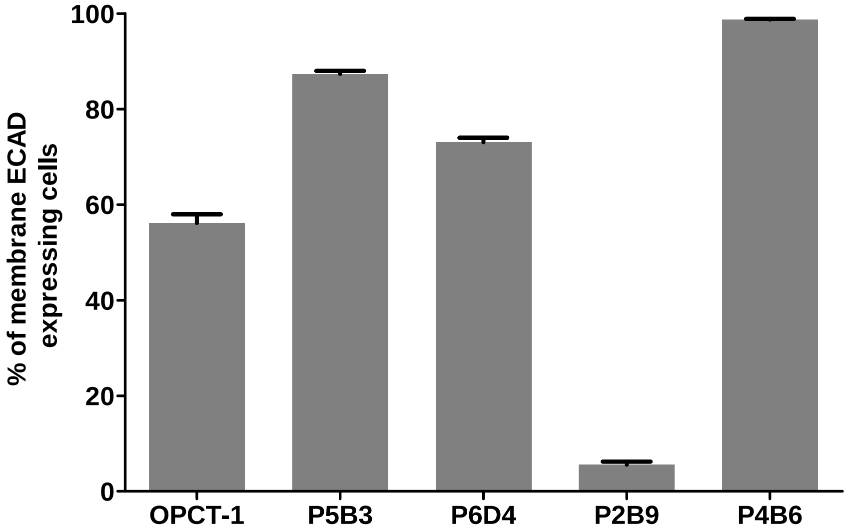
Supplementary Figure 2 (Continued)



Supplementary Figure 2: *Dual immunofluorescent staining of 1-12 OPCT-1 clones of interest; P5B3, P3E5, P2E9, P4C7, P5E4, P6D4, P4F2, P4G10, P5F3, P3H8, P2B9 and P4B6, using a murine monoclonal antibody against the epithelial marker, E-cadherin and a rabbit polyclonal antibody against the mesenchymal marker, vimentin. E-cadherin was labelled with an anti-mouse Alexa Fluor®568-conjugated secondary antibody and vimentin was labelled with an anti-rabbit Alexa Fluor®488-conjugated secondary antibody. Nuclear staining was achieved using mounting media with DAPI. 1st screen represents the initial screening of the 51 clones, from which these clones were selected (P2), 2nd screen represents the second screening conducted after defrosting and passaging the cells (P3) and 3rd screen represents the third screening conducted after defrosting and passaging the clones for a second time (P4). (n=3). Representative images. Image magnification at x20.*

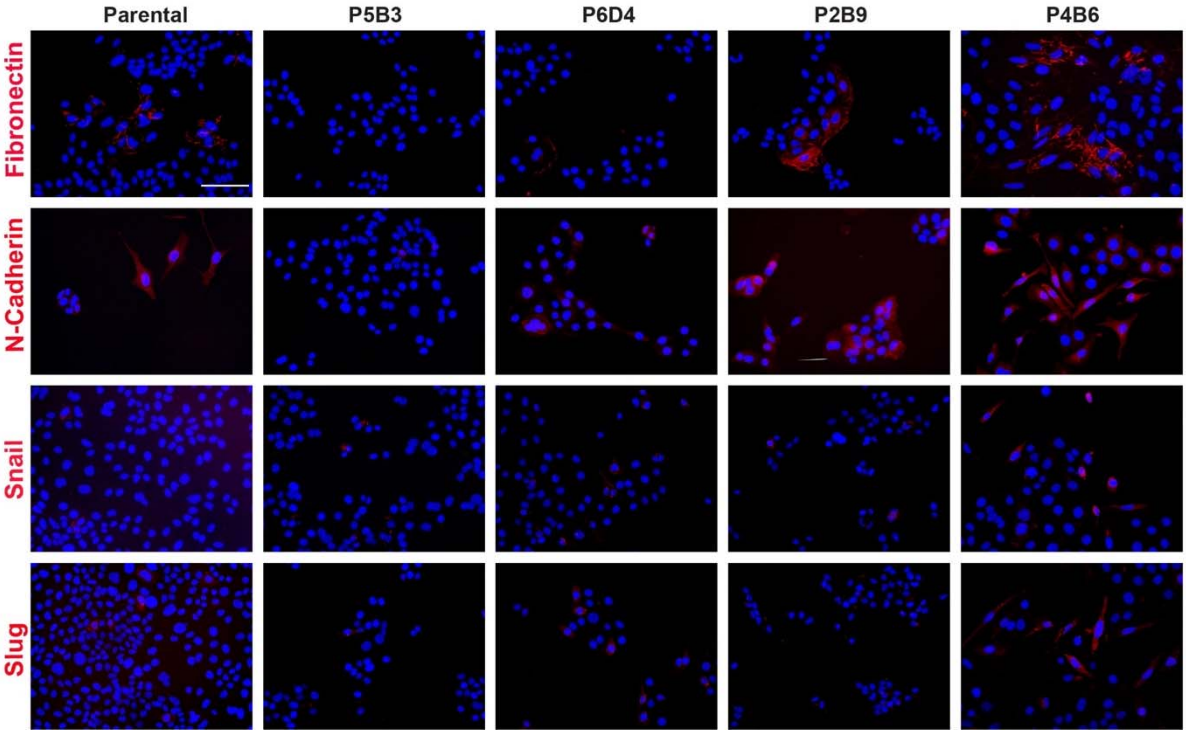
Supplementary Figure 3





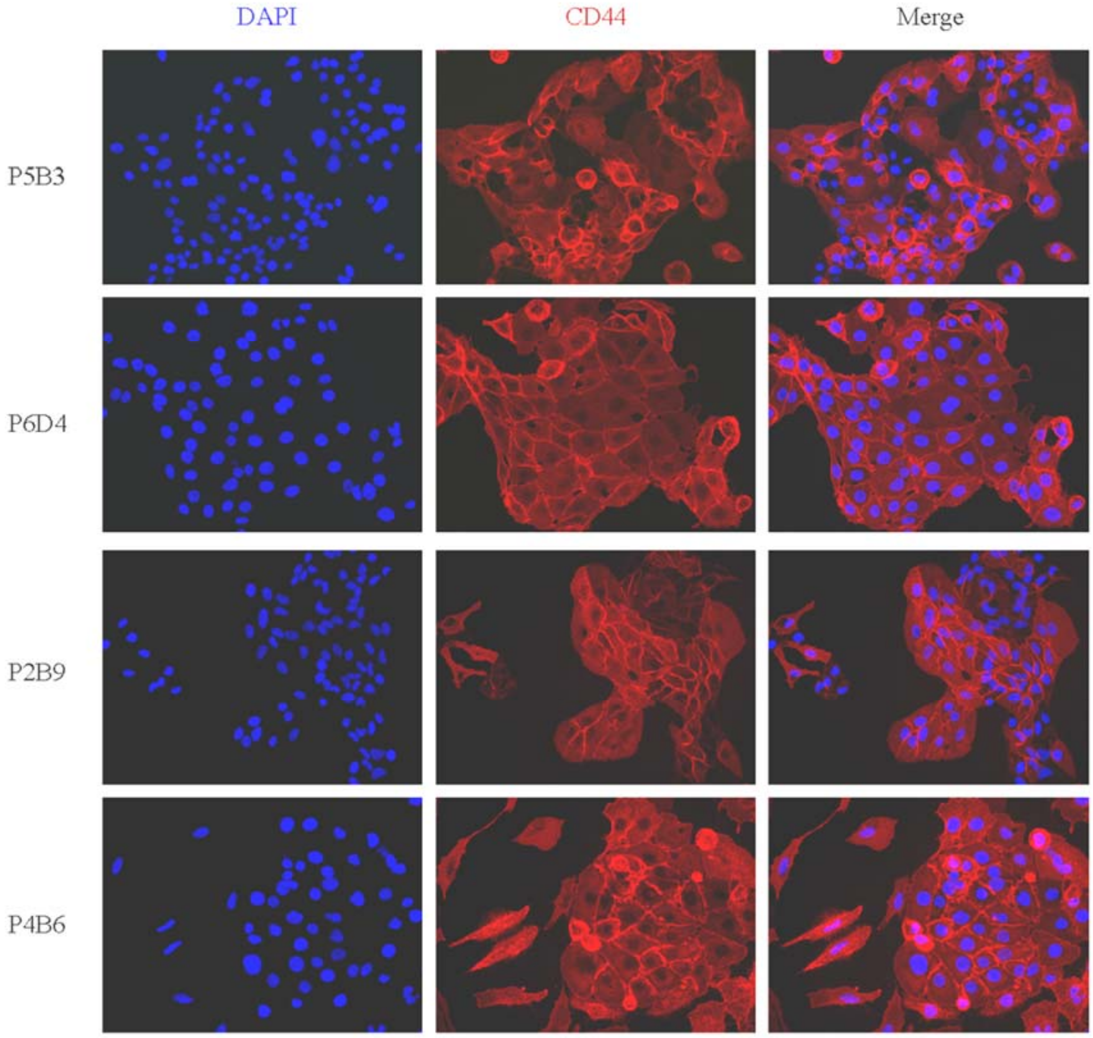
Supplementary Figure 3: Confocal images showing the localisation of E-cadherin in parental and clonal progenies of OPCT-1 and bar graph showing percentage of membrane localised E-cadherin for each clone and the parental line.(magnified images of selected regions also provided for better visualisation of E-cadherin localisation. Scale bar=50µM.

Supplementary Figure 4



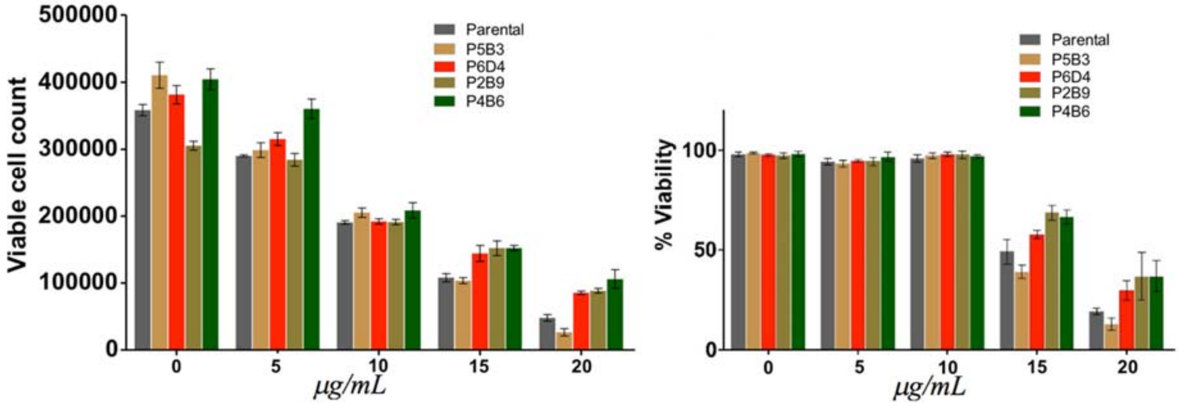
Supplementary Figure 4: Immunofluorescence of fibronectin, N-Cadherin, Slug and Snail in the parental OPCT-1 and the four clones. Magnification 20x; scale bar 50 μm.

Supplementary Figure 5



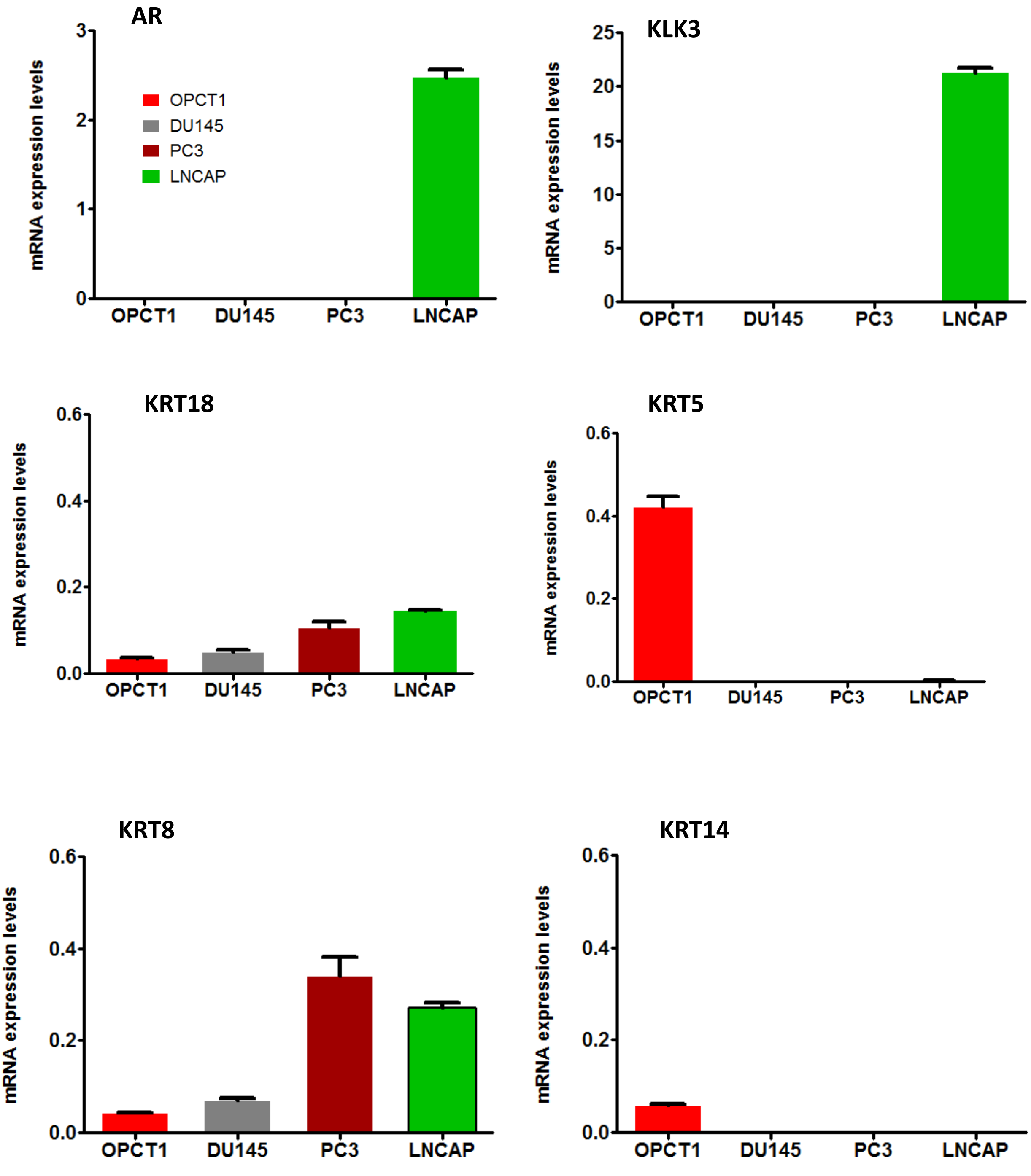
Supplementary Figure 5: *Immunofluorescent staining of clones P5B3, P6D4, P5F3, P2B9, and P4B6 using a mouse monoclonal antibody against CD44. Secondary antibody labelling was achieved using an anti-mouse AlexaFluor®568-conjugated secondary antibody and nuclear staining was performed using mounting media with DAPI (n=3). Representative images. Image magnification at x20.*

Supplementary Figure 6



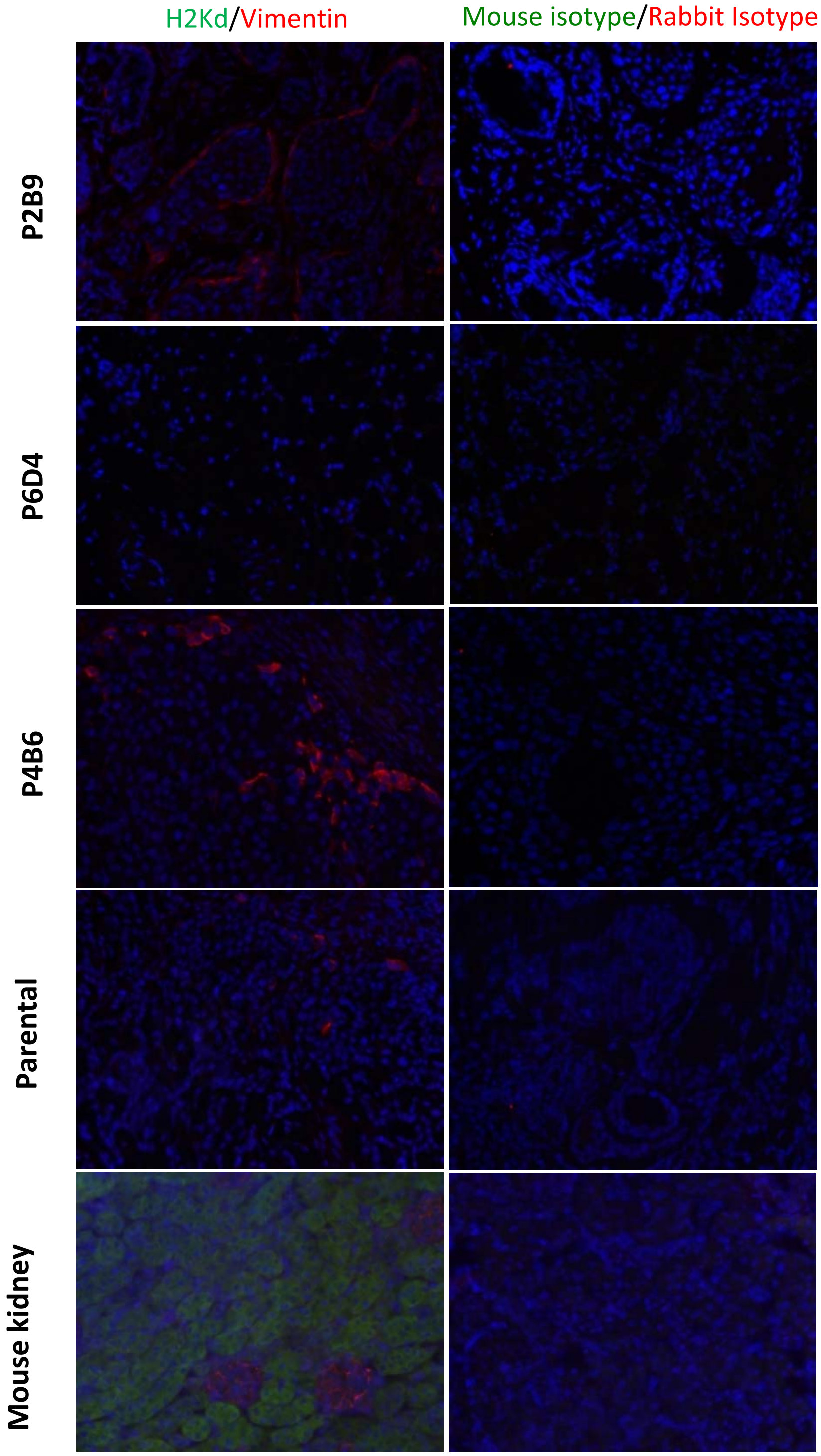
Supplementary Figure 6: *Optimal concentration of mitomycin for wound healing assay was determined using cultured cells (1.5x10^5 cells/well seeded overnight in a 24 well plate). the cells were treated with different concentrations of mitomycin for 2 h. after two hours the cells were washed three times with sterile PBS and the wells were replenished with normal tissue culture media. after 24 h in normal growth conditions the cells viability and number had been determined using an automated cell counter (NucleoCounter® NC-250™). 10 ug/mL of mitomycin for 2 h was found to be optimal for arresting cell proliferation without compromising viability.*

Supplementary Figure 7

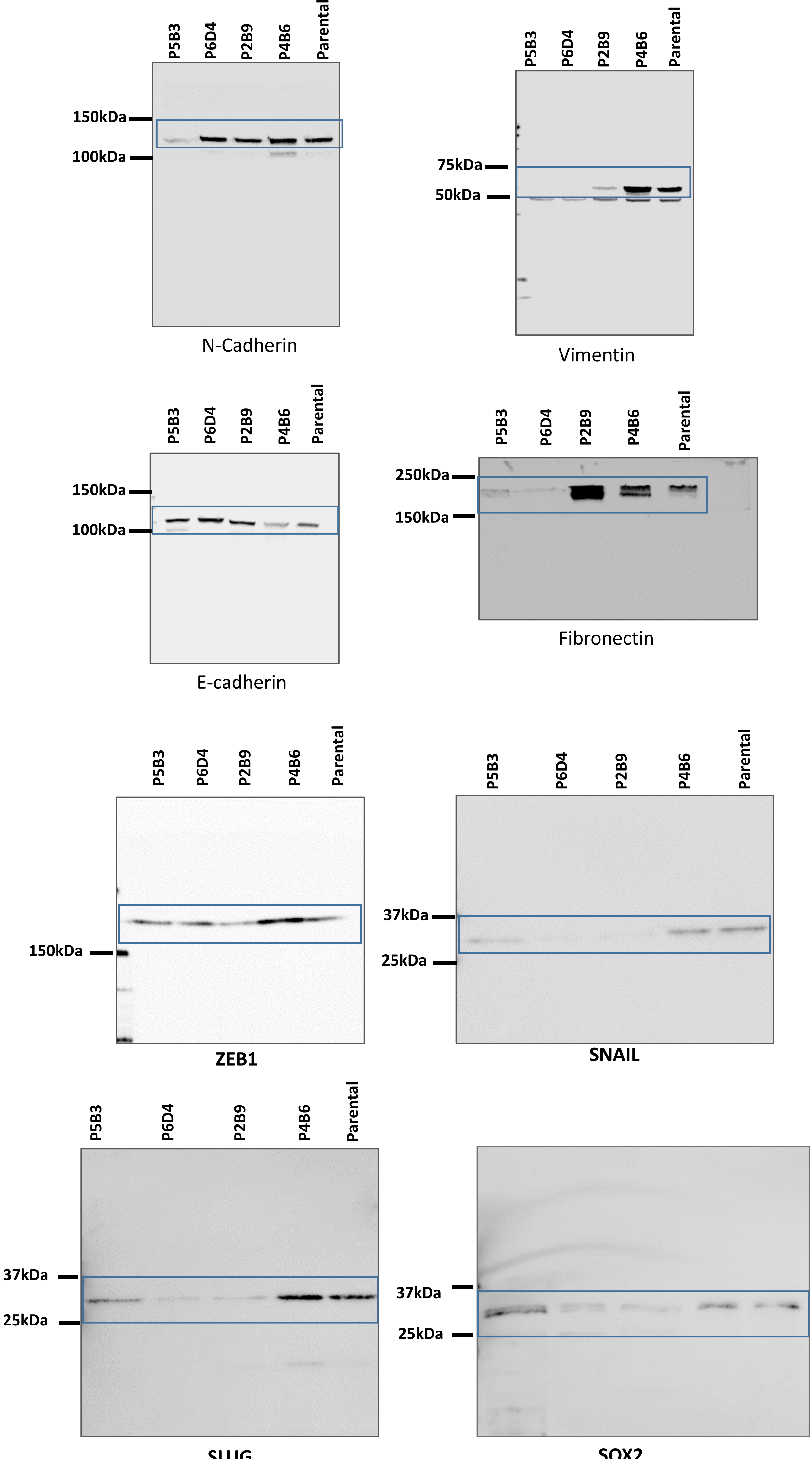


Supplementary Figure 7: *Expression of different markers used to differentiate basal, intermediate and luminal characteristics of prostate cancer cells using qRT PCR. Along with OPCT-1, other classical cell lines (LNCAP, DU145 and PC3) were also profiled. As is known from the literature, LNCAP showed a prominent luminal phenotype with the expression of androgen receptor (AR), PSA (KLK3) and cytokeratin (CK)-18 and 8, with CK-5 and CK14 being completely absent. Similar to DU145 and PC3, OPCT-1 lack the expression of AR and PSA, thereby indicating their non-luminal characteristics. Although high expression of CK-5 and low expression of CK14 was uniquely detected in OPCT-1, expression of a key the basal phenotype marker, p63, was not detected, thereby indicating a transit amplifying genotype in OPCT1.*

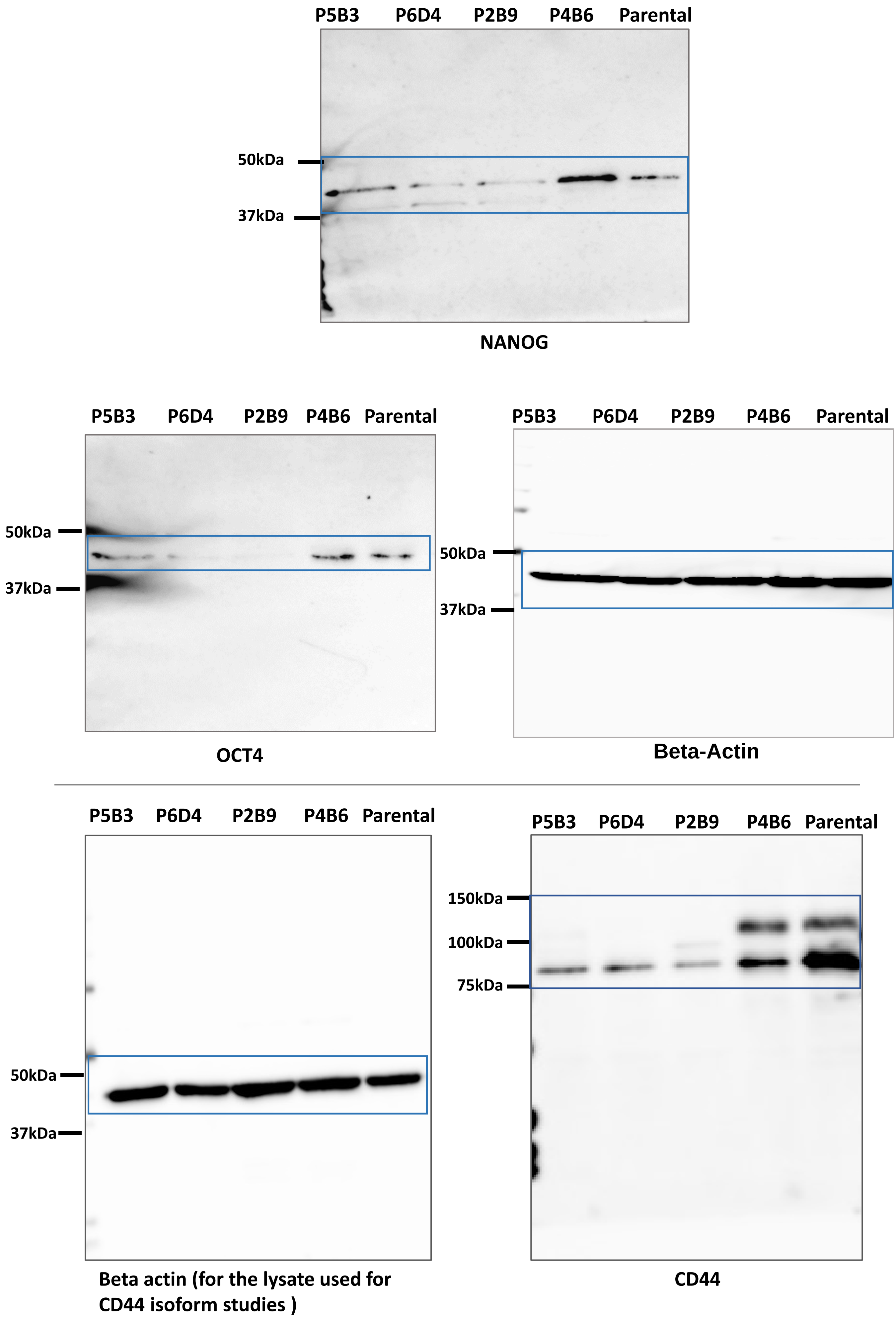
Supplementary Figure 8



Supplementary Figure 8: *Representative immunofluorescent staining of tumour sections derived from clones P5B3, P6D4, P2B9, P4B6 and parental OPCT-1 and murine kidney using a monoclonal antibody specific for murine MHC class I H-2^{Kd}. Sections were labelled with a murine monoclonal antibody against MHC class I $H2^{Kd}$ and a rabbit polyclonal antibody against murine vimentin. Primary antibody staining was detected using anti-mouse Alexa Fluor® 488 and anti-rabbit Alexa Fluor® 568 antibodies (x20 magnification). The absence of $H2^{Kd}$ staining in vimentin positive cells indicates that these cells are of human origin.*



P5B3
P6D4
P2B9
P4B6
Parental
150kDa
100kDa
N-Cadherin
75kDa
50kDa
Vimentin
E-cadherin
250kDa
Fibronectin
ZEB1
37kDa
25kDa
SNAIL
SLUG
SOX2



Supplementary Figure 9: Unprocessed original scans of Western blots

***Supplementary Table 1**: Demonstrating the results reporting the expression of E-Cadherin, Vimentin and CD44 by 51 OPCT-1 clones screened by immunofluorescence*

Clone	*E-Cadherin*	*Vimentin*	*Cellular co-expression*	*CD44*
P1C7	+++	+++	Yes	+++
P1D2	+++	++	Yes	+++
P1D4	+++	++	Yes	+++
P1E6	+++	+++	Yes	+++
P1G3	+++	++	Yes	+++
P2B9	+++	+++	Yes	+++
P2D8	+++	++	Yes	+++
P2E7	+++	+	Yes	+++
P2E9	+++	+	Yes	+++
P2F3	+++	+	Yes	+++
P2G3	+++	+	Yes	+++
P3B11	+++	+	Yes	+++
P3D3	+++	+	Yes	+++
P3D10	+++	+	No	+++
P3D11	+++	++	Yes	+++
P3E5	+++	-	n/a	+++
P3G3	+++	++	Yes	+++
P3G10	+++	++	Yes	+++
P3H8	+++	+++	Yes	+++
P4B6	+++	+++	Yes	+++
P4C3	+++	+	Yes	+++
P4C7	+++	+	Yes	+++
P4E10	+++	+	Yes	+++
P4F2	+++	+	Yes	+++
P4F4	+++	+++	Yes	+++
P4G6	+++	+++	Yes	+++
P4G10	+++	++	Yes	+++
P5A5	+++	+	Yes	+++
P5A6	+++	+	Yes	+++
P5B3	+++	-	n/a	++
P5B7	+++	+++	Yes	+++
P5C3	+++	+	Yes	+++
P5D4	+++	+++	Yes	+++
P5D6	+++	+	No	+++
P5E4	+++	+	Yes	+++
P5E8	+++	+	Yes	+++
P5F8	+++	+++	Yes	+++
P5H8	+++	++	Yes	+++
P5H9	+++	++	Yes	+++

Clone	*E-Cadherin*	*Vimentin*	*Cellular Co-expression*	*CD44*
P5F2	+++	+++	*Yes*	+++
P5F3	+++	++	*Yes*	+++
P6D4	+++	+	*Yes*	+++
P6E6	+++	+++	*Yes*	+++
P6H5	+++	++	*Yes*	+++
P6C7	+++	++	*Yes*	+++
P6D1	+++	++	*Yes*	+++
P6D2	+++	+++	*Yes*	+++
P6E10	+++	++	*Yes*	+++
P6F12	+++	+	*No*	+++
P5A2	+++	++	*Yes*	+++
P5G11	+++	+	*Yes*	+++

+++ expressed by ˜ 10-100% of cells ++ expressed by ˜ 1-10% of cells + expressed by ˜ <1% of cells – no positive cells observed
Yes = individual cells expressing both markers present No = only single-positive cells observed

Supplementary Table 2: List of primers used for the qRT PCR characterisation

Primer name	Sequence (5'-3')
CDH1-F	TGCCCAGAAAATGAAAAAGG
CDH1-R	GTGTATGTGGCAATGCGTTC
CDH2-F	ACAGTGGCCACCTACAAAGG
CDH2-R	CCGAGATGGGGTTGATAATG
FN1-F	CAGTGGGAGACCTCGAGAAG
FN1-R	TCCCTCGGAACATCAGAAAC
VIM-F	GAGAACTTTGCCGTTGAAGC
VIM-R	GCTTCCTGTAGGTGGCAATC
SNAI1-F	CCTCCCTGTCAGATGAGGAC
SNAI1-R	CCAGGCTGAGGTATTCCTTG
TWIST-F	GGAGTCCGCAGTCTTACGAG
TWIST-R	TCTGGAGGACCTGGTAGAGG
SNAI2-F	GGGGAGAAGCCTTTTTCTTG
SNAI2-R	TCCTCATGTTTGTGCAGGAG
FOXC2-F	GCCTAAGGACCTGGTGAAGC
FOXC2-R	TTGACGAAGCACTCGTTGAG
ZEB1- F	GGCATACACCTACTCAACTACGG
ZEB1- R	TGGGCGGTGTAGAATCAGAGTC
OCT4- F	GATCACCCTGGGATATACAC
OCT4- R	GCTTTGCATATCTCCTGAAG
SOX2-F	ATAATAACAATCATCGGCGG
SOX2-R	AAAAAGAGAGAGGCAAACTG
NANOG- F	CCAGAACCAGAGAATGAAATC
NANOG- R	TGGTGGTAGGAAGAGTAAAG
HPRT-F	TGACACTGGCAAAACAATGCA
HPRT-R	GGTCCTTTTCACCAGCAAGCT