ONLINE RESOURCE

Live cell molecular analysis of primary prostate cancer organoids identifies persistent Androgen Receptor signaling

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Fluorescent Microscopy		
marker	fluorochrome	purpose
Hoechst	Hoechst 33342	Nuclear stain
EpCAM	Alexa Fluor™ 488	Epithelial marker
PSMA	PE	Prostate Specific Membrane Antigen
CD49f	Alexa Fluor™ 647	Epithelial stratification
CD45	PE	Leukocyte common antigen
CD49a	Alexa Fluor™ 647	Stromal marker
Androgen Receptor	n/a	AR-pathway marker
Donkey Anti-Rabbit IgG	PE	Secondary antibody
PSA	Cy5	Prostate Specific Antigen
Live/Dead	Image-IT™ DEAD Green™	Cell death detection

Table S1 Multi-color immune fluorescent labeling assay reagents for confocal microscopy

Flow Cytometry Assay I		
marker	fluorochrome	purpose
Live/Dead fixable	Ghost Dye™ 510	Dead exclusion; reduce autofluorescence
Fc Block™	n/a	Reduce Fc-mediated antibody binding
CD45	redFluor™ 710	Leukocyte common antigen
CD14	redFluor™ 710	Monocyte/macrophage marker
EpCAM	Brilliant Violet™ 650	Epithelial marker
TROP2	PE	Epithelial marker
CD49f	PerCP Cy5.5	Epithelial stratification
Androgen Receptor	n/a	AR-pathway marker
Donkey Anti-Rabbit IgG	Alexa Fluor™ 488	Secondary antibody
PSMA	PE-Cy7	Prostate Specific Membrane Antigen
PSA	Cy5	Prostate Specific Antigen
CD44	Brilliant UltraViolet™ 737	Stemness marker
CD56	Brilliant UltraViolet™ 650	Neuroendocrine marker

Table S2 Flow Cytometry antibodies utilized in Assay I

Flow Cytometry Assay II		
marker	fluorochrome	purpose
Live/Dead fixable	Ghost Dye™ 510	Dead exclusion; reduce autofluorescence
Fc Block™	n/a	Reduce Fc-mediated antibody binding
CD45	PE	Leukocyte common antigen
EpCAM	Alexa Fluor™ 488	Epithelial marker
CD49a	PE-Cy7	Stromal marker

Table S3 Flow Cytometry antibodies utilized in Assay II

Gene	Forward Primer (5' - 3')	Internal Probe (5' - 3')	Reverse Primer (5' - 3')
GSTP1	TTCGCTGCGCACACTTC	CGGTCCTCTTCCTGCTGTCTGTTT	CTTTCCCTCTTTCCCAGGTC
RASSF1	CCTCCAGAAACACGGGTA	TTTGCGGTCGCCGTCGTTGT	CTTCCTTCCCTCCTTCGTC
APC	TTATTACTCTCCCTCCCACCTC	TCTTGTGCTAATCCTTCTGCCCTGC	TGGCAGTTGACACGCATAG
RARB	GAAGGAGAACTTGGGATCTT	TTTCCAGGCTTGCTCGGCCAATC	AGCCTGTAATTGATCCAAATGA
LINE1	CGCAGGCCAGTGTGTGT	CCGTGCGCAAGCCGA	TCCCAGGTGAGGCAATGC

Table S4 Primer and probe sequences used in qRT-PCR and pre-amplification of enriched methylated DNA
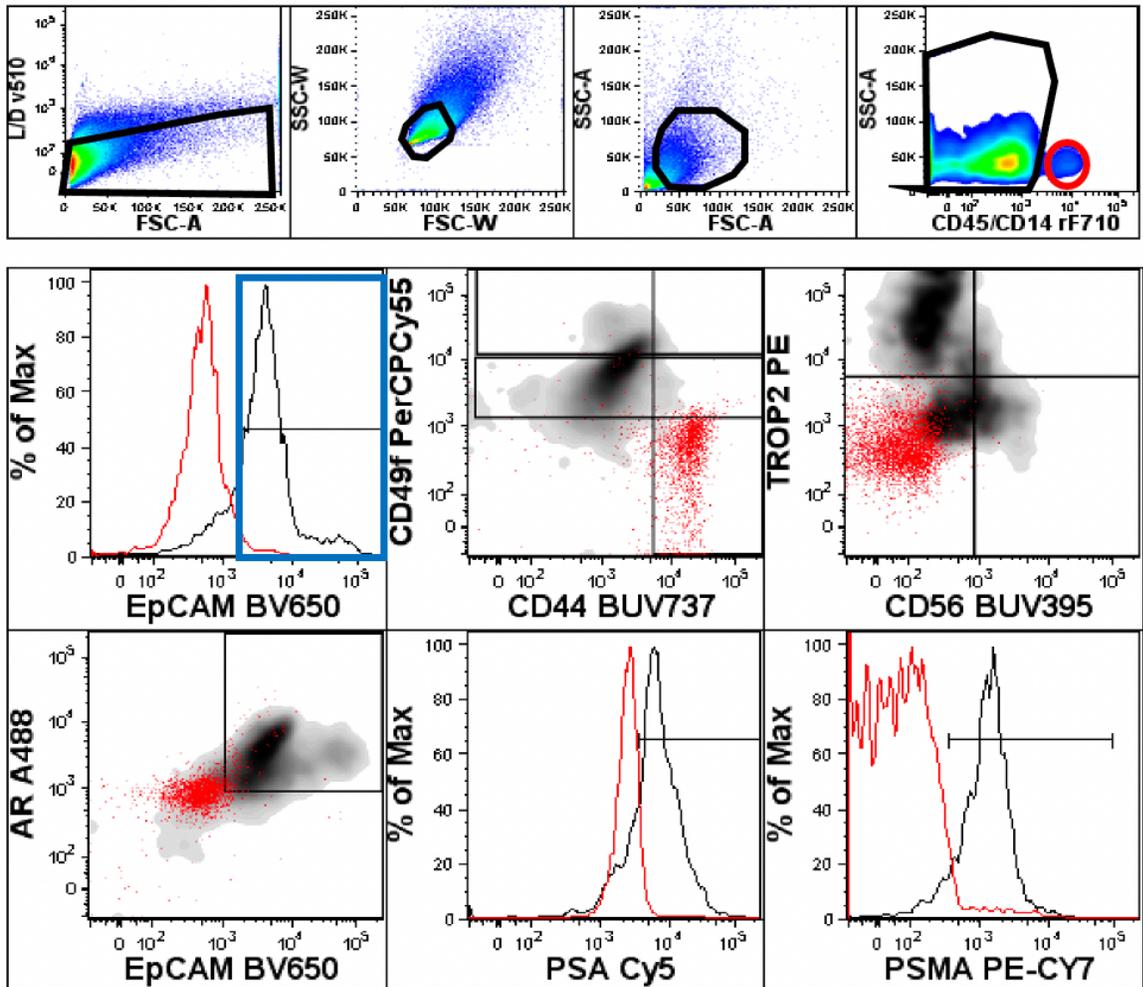
Figure S1 Flow Cytometry Gating Strategy. The live single epithelial cell content was extracted after excluding dead cells (live/dead stain negative; top row first plot), doublets (pulse Width low; top row second plot) and small cells/debris (top row third plot). EpCAM expression was then analyzed after exclusion of $CD45^+$ and $CD14^+$ immune cells (top right, red circle). The $CD45^+/CD14^+$ cells served as an Internal Negative Control (red histogram overlay) to establish EpCAM expression in the $CD45^-/CD14^-$ fraction (black histogram overlay (second row left). The $EPCAM^+$ cells (blue box on histogram) were then projected for analysis of epithelial and AR-pathway related features as shown in density plots (second row, middle, right) and on histograms (third row middle, right). AR expression is shown on the total $CD45^-/CD14^-$ fraction on the density plot on bottom left. The red populations on both density plots and histograms represent the $CD45^+/CD14^+$ INC control to confirm gating thresholds
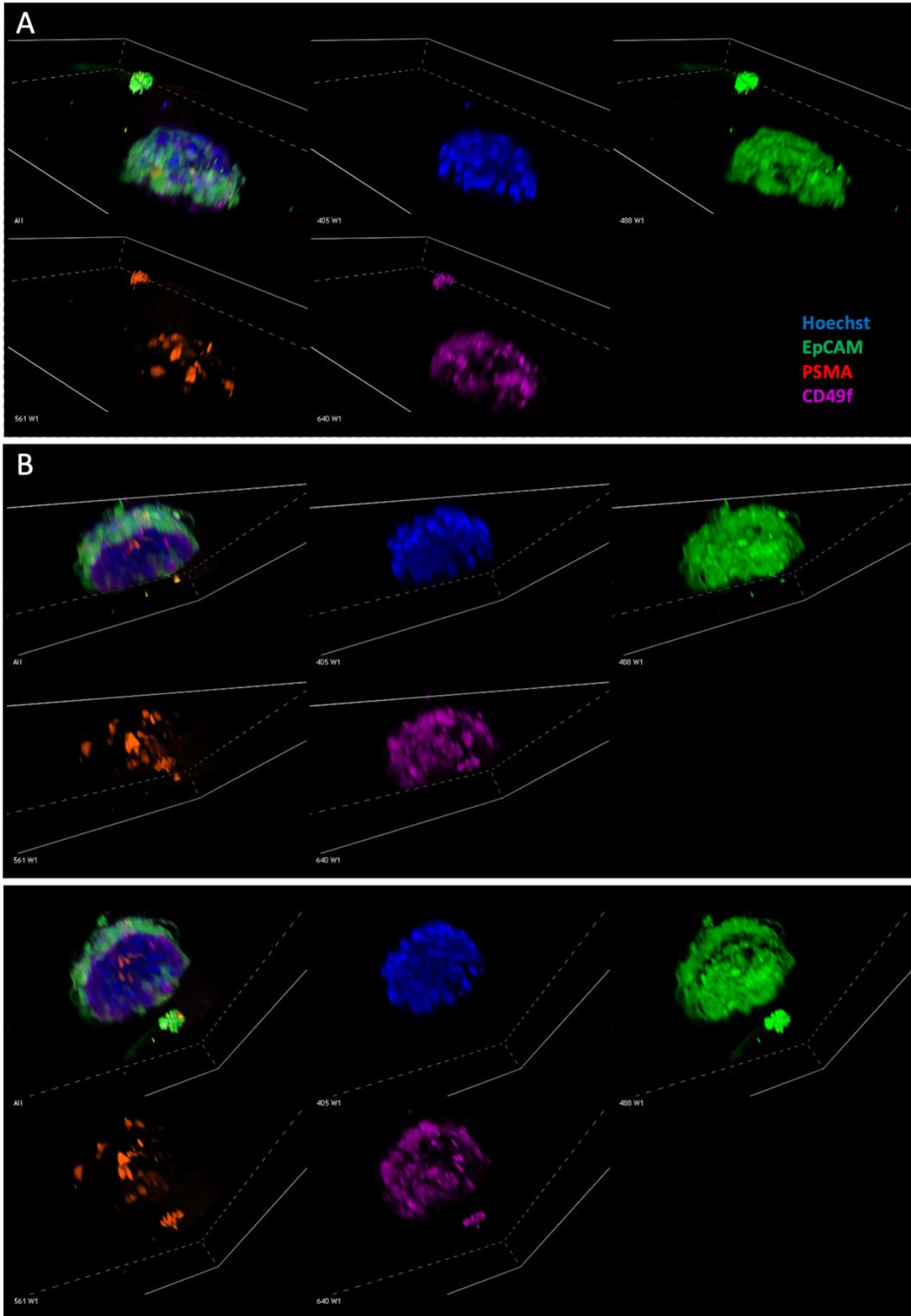
Figure S2. Three-dimensional images of prostate PDCO. Condensed three-dimensional images of PDCO shown in three different angles (a, b,c, respectively). Hoechst (blue), EpCAM (green), PSMA (red) and CD49f (purple).
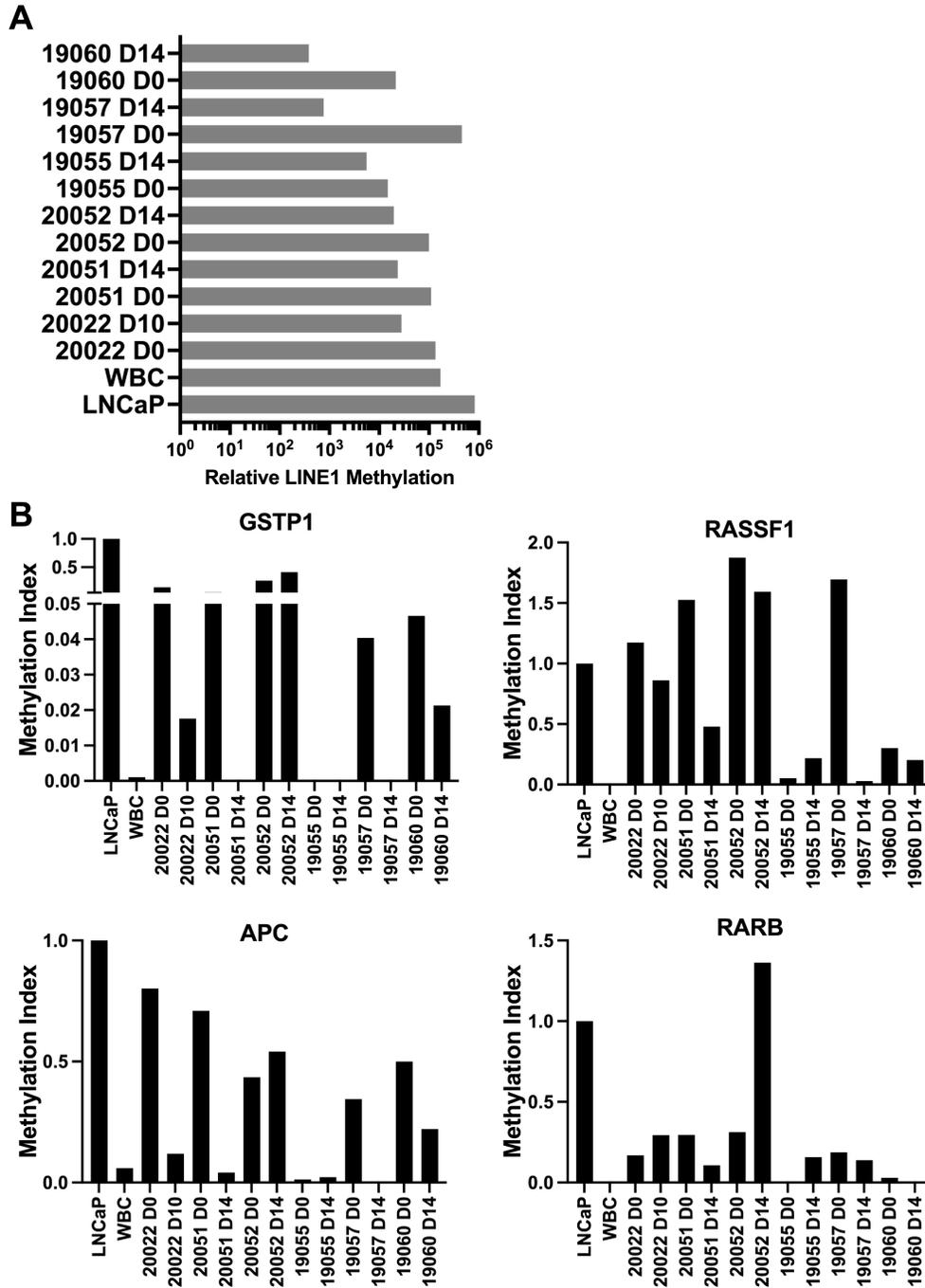
Figure S3 (a) Relative *LINE1* methylation in primary biopsy (D0) and matched PDCO cultures (D10 or D14) after 10-14 days of *ex vivo* culture. LNCaP and WBC *LINE1* methylation is also included. (b) Methylation Index for *GSPT1*, *RASSF1*, *APC*, and *RARB* in primary biopsy (D0) and matched PDCO cultures (D10 or D14) after 10-14 days of culture. Data is the same as Figure 5 and represents individual core punches from radical prostatectomy specimens from the same donor. LNCaP and WBC represent positive and negative controls for promoter methylation at the genes, respectively