

PrEC and p16 KO (Top Band) Exon 1α Sequence

Forward

PrEC	Seq_1	189	GCATGGAGCCTTCGGCTGACTGGCTGGCCACGGCCCGCGCCCGGGGTCGGGTAGAGGAGG	130
			#####	
p16 KO	Seq_2	154	GCATGGAGCCTTCGG-----GCCACGGCCCGCGCCCGGGGTCGGGTAGAGGAGG	202
PrEC	Seq_1	129	TGCGGGCGCTGCTGGAGCGGGGGCGCTGCCAACGCACCGAATAGTTACGGTCGGAGGC	70
p16 KO	Seq_2	203	TGCGGGCGCTGCTGGAGCGGGGGCGCTGCCAACGCACCGAATAGTTACGGTCGGAGGC	262

Reverse

PrEC	Seq_1	209	GGAGCCGGCGGGGGAGCAGCATGGAGCCTTCGGCTGACTGGCTGGCCACGGCCCGCGGC	150
			#####	
p16 KO	Seq_2	197	GGAGCCGGCGGGGGAGCAGCATGGAGCCTTCGG-----GCCACGGCCCGCGGC	149
PrEC	Seq_1	149	CCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGGGGGCGCTGCCAACGCACC	90
p16 KO	Seq_2	148	CCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGGGGGCGCTGCCAACGCACC	89

PrEC and p16 KO (Lower Band) Exon 1α Sequence

Forward

PrEC	Seq_1	246	AGGGGGAGAGCAGGCAGCGGGCGGGGAGCAGCATGGAGCCGGCGGGGAGCAGCA	187
			#####	
p16 KO	Seq_2	94	AGGGGGAGAGCAGGCAGCGGGCGGGG-----	122
PrEC	Seq_1	186	TGGAGCCTTCGGCTGACTGGCTGGCCACGGCCCGGGTCGGGTAGAGGAGGTGC	127
			#####	
p16 KO	Seq_2	123	-----TCGGGTAGAGGAGGTGC	139
PrEC	Seq_1	126	GGGCGCTGCTGGAGGCGGGGGCGCTGCCAACGCACCGAATAGTTACGGTCGGAGGCCGA	67
p16 KO	Seq_2	140	GGGCGCTGCTGGAGGCGGGGGCGCTGCCAACGCACCGAATAGTTACGGTCGGAGGCCGA	199

Reverse

PrEC	Seq_1	269	GCGTGCCTAGGCGGCTGCGGAGAGGGGAGAGCAGGCAGCGGGCGGGGAGCAGCAT	210
			#	
p16 KO	Seq_2	189	GCGTGCCTAGGCGGCTGCGGAGAGGGGAGAGCAGGCAGCGGGCGGGG-----	138
PrEC	Seq_1	209	GGAGCCGGCGGGGGAGCAGCATGGAGCCTTCGGCTGACTGGCTGGCCACGGCCCGCGGC	150
			#####	
p16 KO	Seq_2	137	-----	138
PrEC	Seq_1	149	CCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGGGGGCGCTGCCAACGCACC	90
			#####	
p16 KO	Seq_2	137	-----TCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGGGGGCGCTGCCAACGCACC	84

PrEC and Clone 1 Exon 2 Sequence

Forward

PrEC	Seq_1	241	CCGGGCAGCGTCGTGCACGGGTCGGGTGA-GAGTGGCGGGGTCGGCGCAGTTGGGCTCCG	299
			#	
Clone 1	Seq_2	238	CCGGGCAGCGTCGTGCACGGGTCGGGTGAAGAGTGGCGGGGTCGGCGCAGTTGGGCTCCG	297
PrEC	Seq_1	300	CGCCGTGGAGCAGCAGCAGCTCCGCCACTCGGGCGCTGCCATCATCATGACCTGCCAGA	359
Clone 1	Seq_2	298	CGCCGTGGAGCAGCAGCAGCTCCGCCACTCGGGCGCTGCCATCATCATGACCTGCCAGA	357

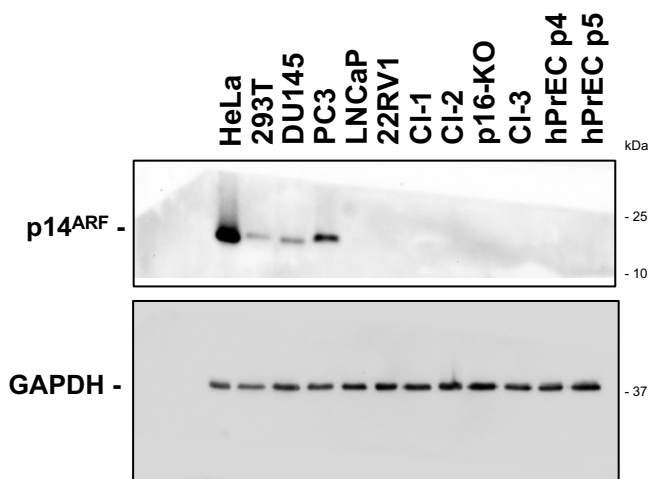
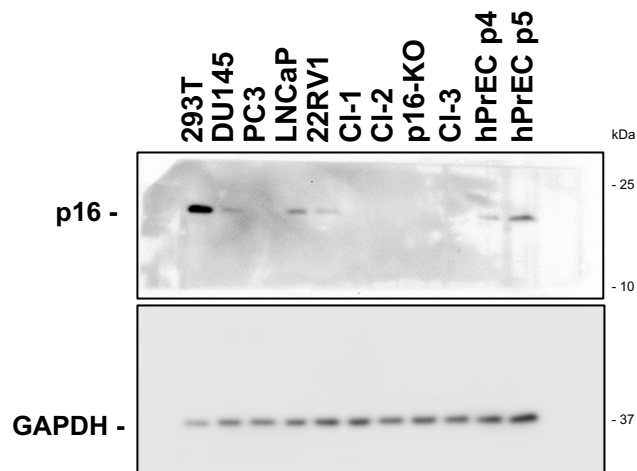
Reverse

PrEC	Seq_1	244	GGCAGCGTCGTGCACGGGTCGGGTGA-GAGTGGCGGGGTCGGCGCAGTTGGGCTCCGCGC	302
			#	
Clone 1	Seq_2	219	GGCAGCGTCGTGCACGGGTCGGGTGAAGAGTGGCGGGGTCGGCGCAGTTGGGCTCCGCGC	160
PrEC	Seq_1	303	CGTGGAGCAGCAGCAGCTCCGCCACTCGGGCGCTGCCATCATCATGACCTGCCAGAGAG	362
Clone 1	Seq_2	159	CGTGGAGCAGCAGCAGCTCCGCCACTCGGGCGCTGCCATCATCATGACCTGCCAGAGAG	100

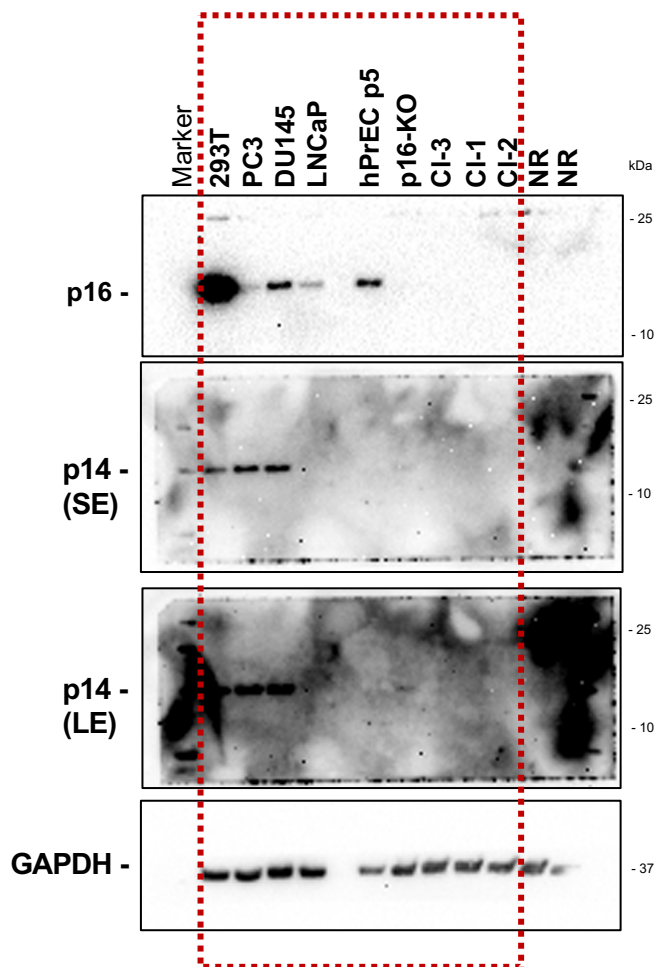
Supplementary Figure 1. Alterations determined by Sanger sequencing of the PCR-amplified products. (A) PCR-amplified products were Sanger-sequenced using the indicated primers. Lesions are shown by sequencing in both directions. **(B)** Uncropped images of the gels depicting PCR products in Fig. 2B.

a

Replicate 1 (Fig 2A)



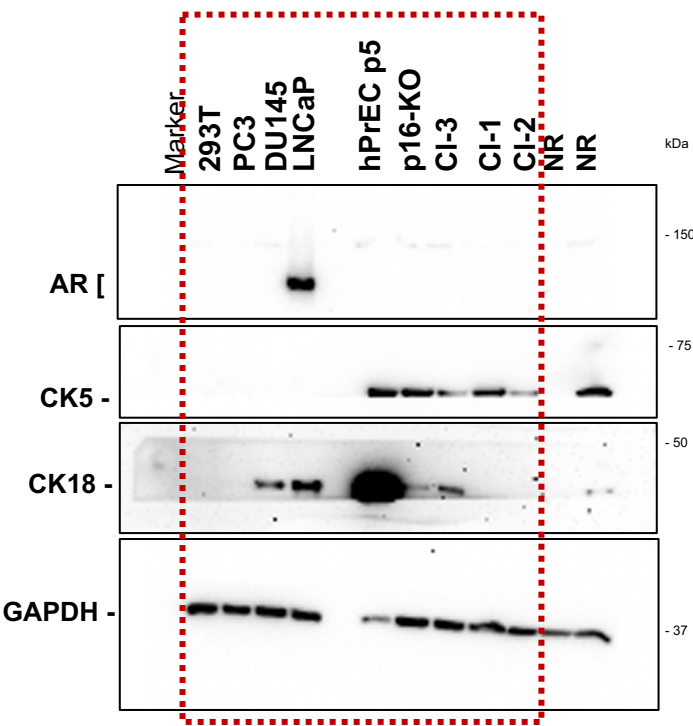
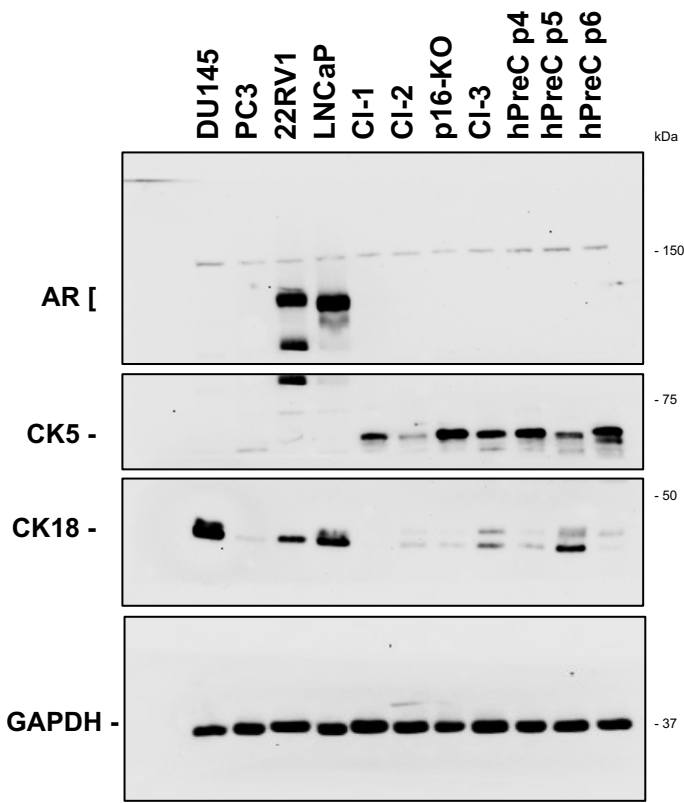
Replicate 2



Replicate 2

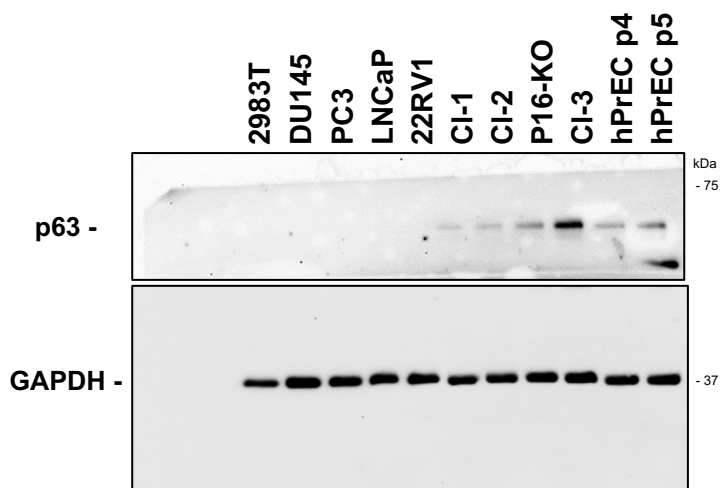
b

Replicate 1 (Fig 2B)

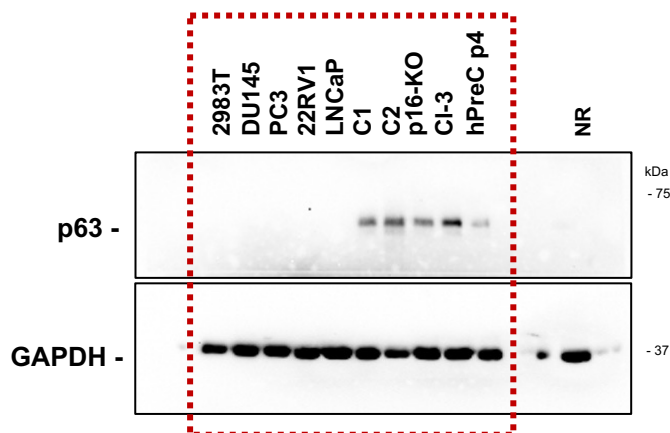


c

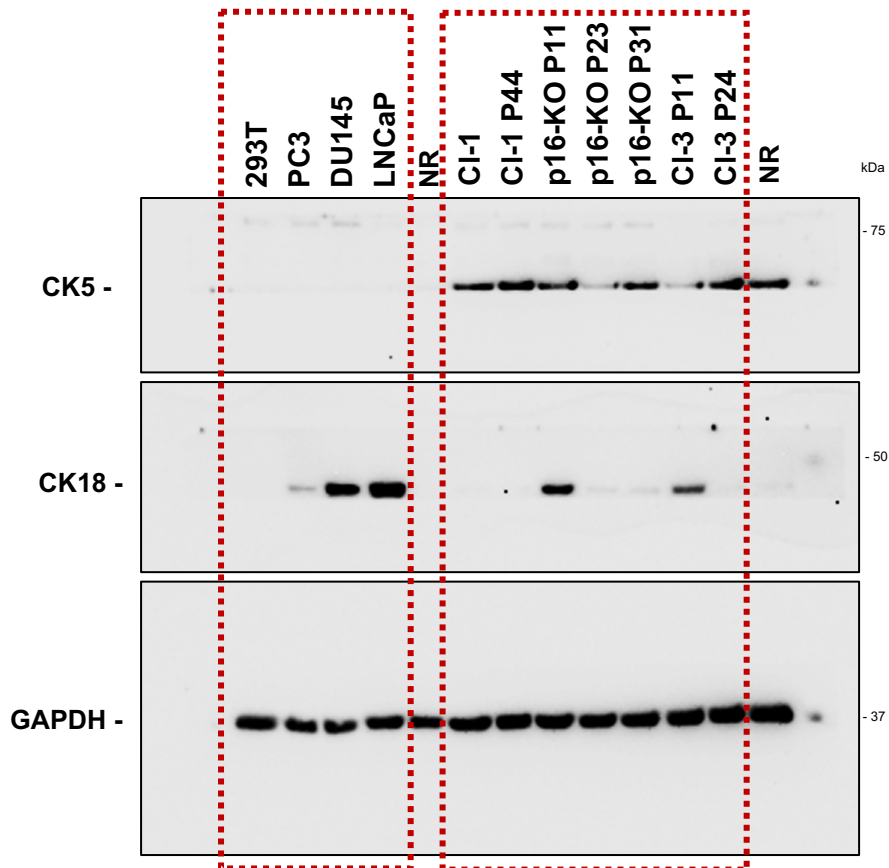
Replicate 1 (Fig 2C)



Replicate 2



d

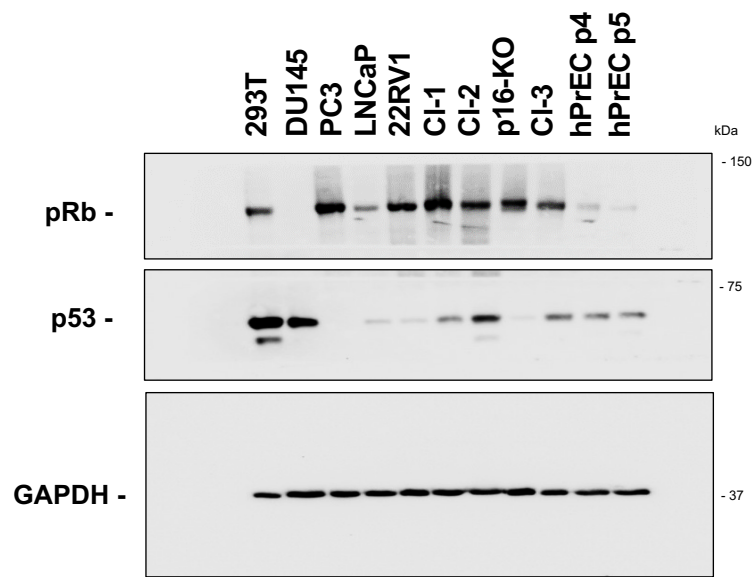


Supplementary Figure 2 c-d

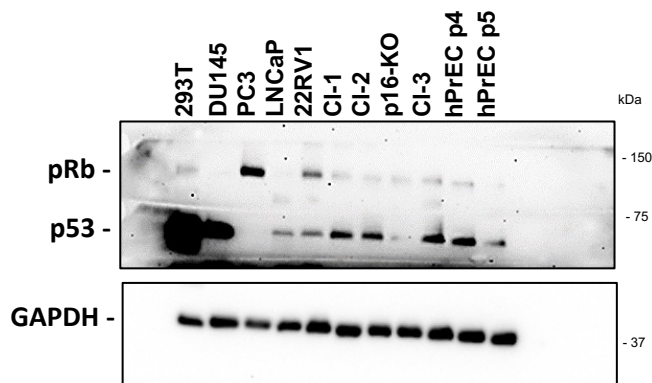
Supplementary Figure 2. Uncropped western blots for membranes used in Fig. 3 and a replicate. See Fig. 3 legend for details. Molecular markers are indicated. Membranes transferred from SDS-PAGE gels were cut horizontally in three pieces (top, middle and bottom) using protein ladders on the flanking lanes as guide. Membranes with blotted proteins in a molecular range were incubated with specific antibodies to a target protein in that molecular mass range as indicated in the figure. In replicates, relevant lanes are marked with a shaded line rectangle. Lanes with non-relevant samples are marked NR.

a

Replicate 1 (Fig 3A)

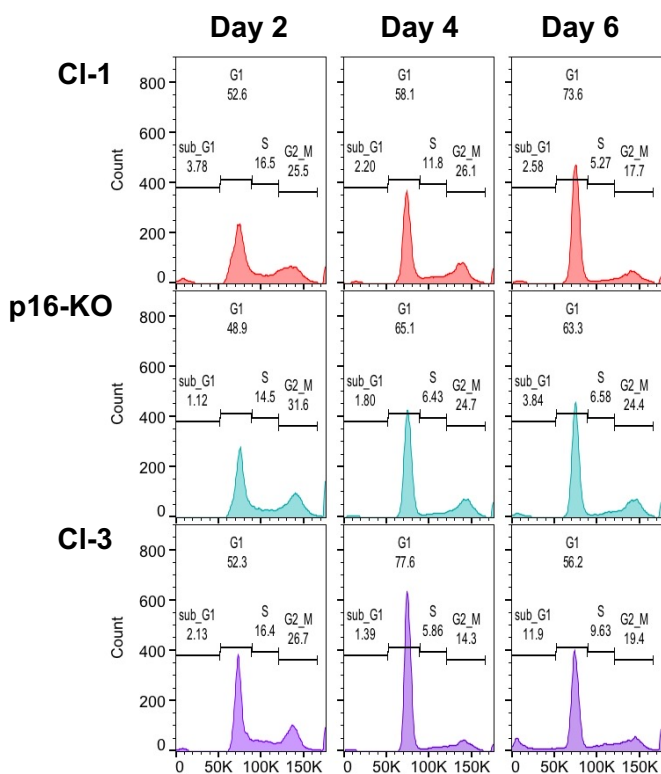


Replicate 2

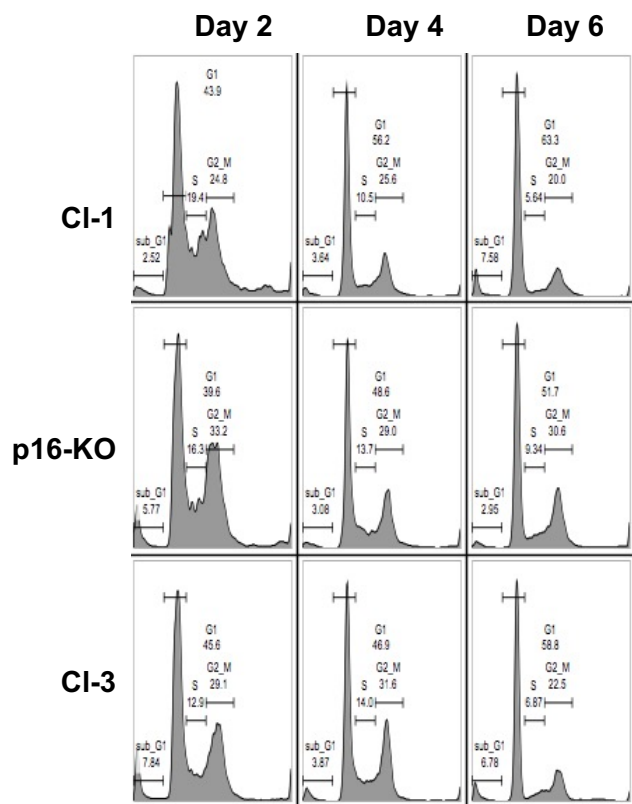


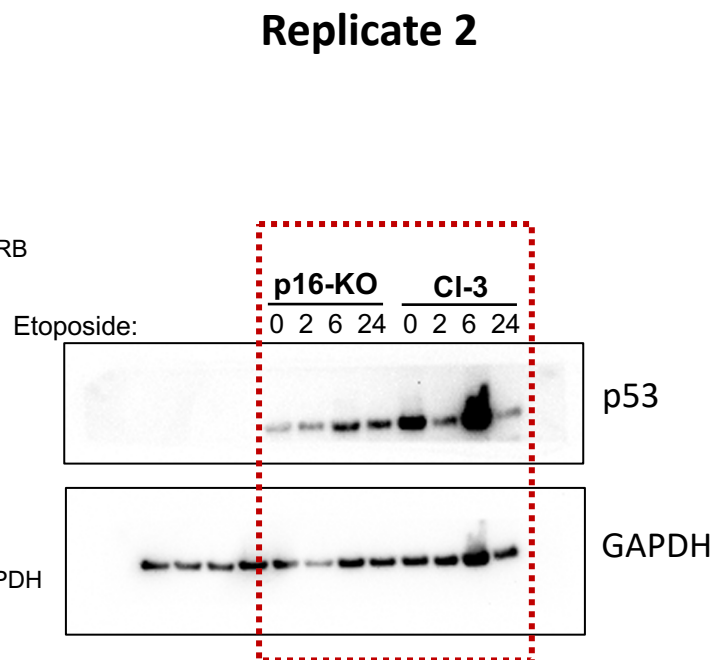
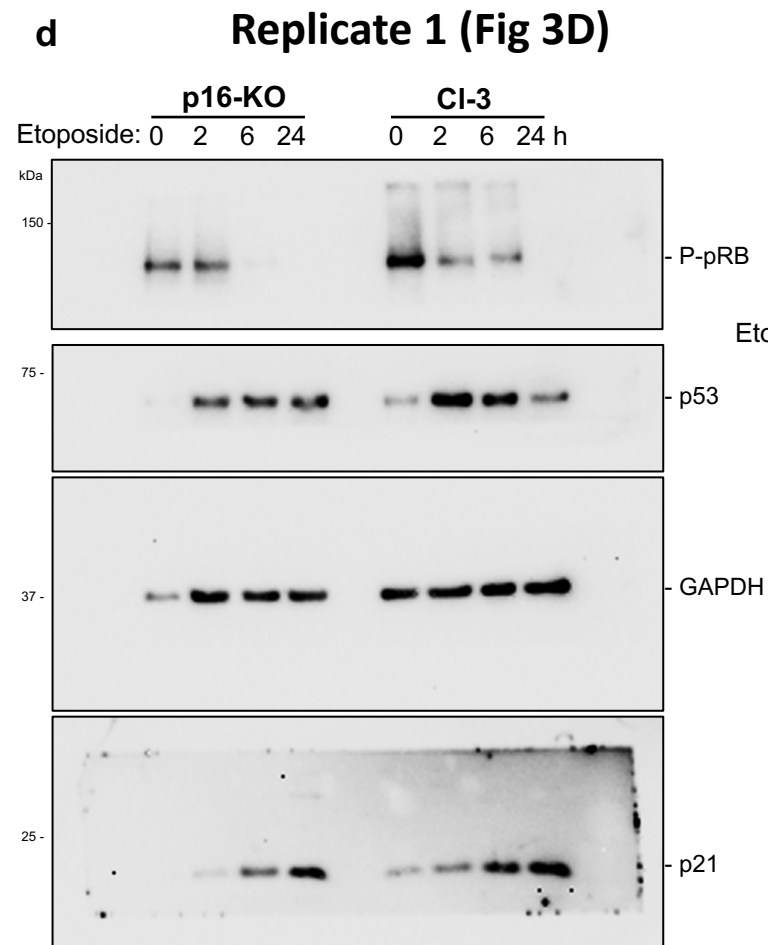
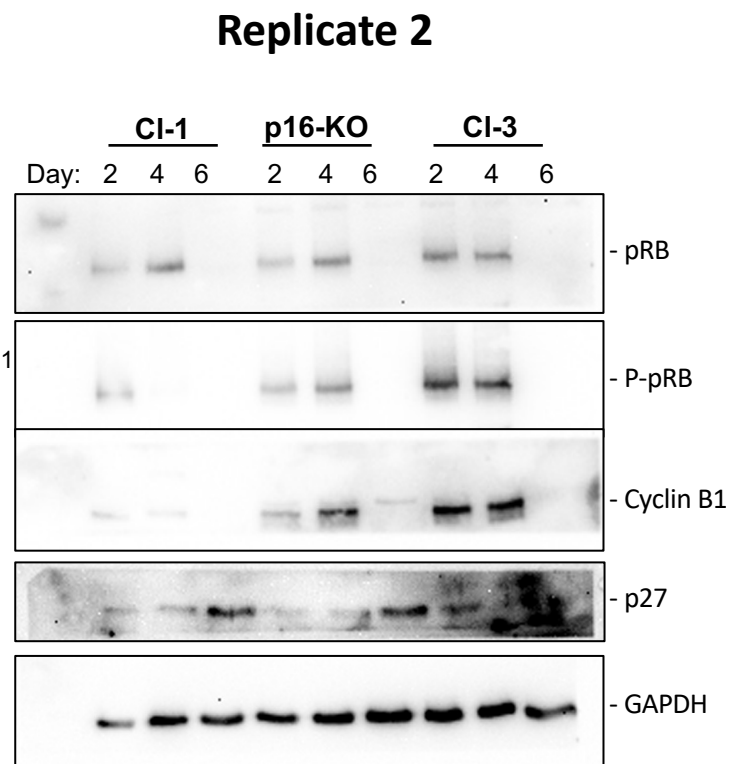
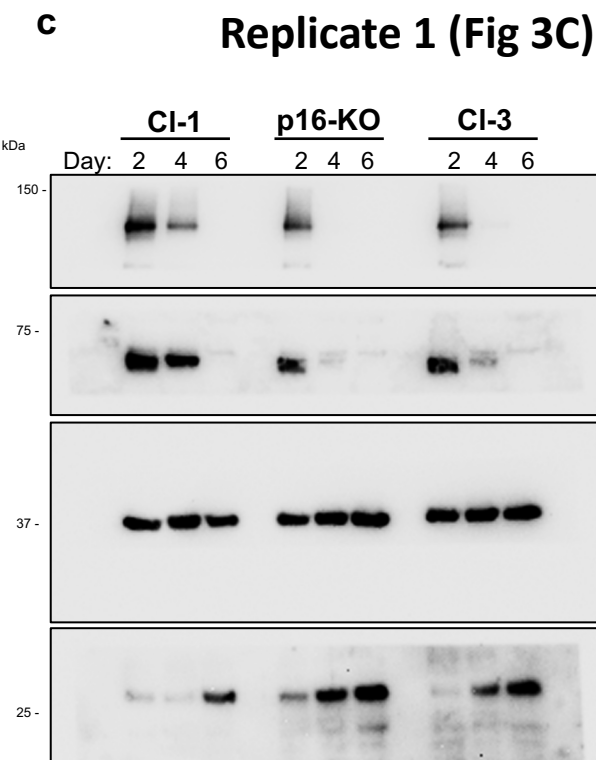
b

Replicate 1 (Fig 3B)



Replicate 2

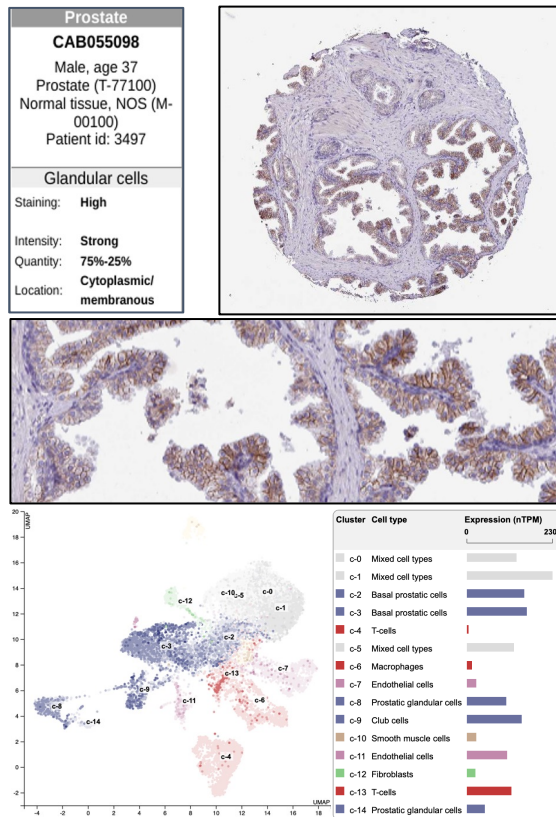




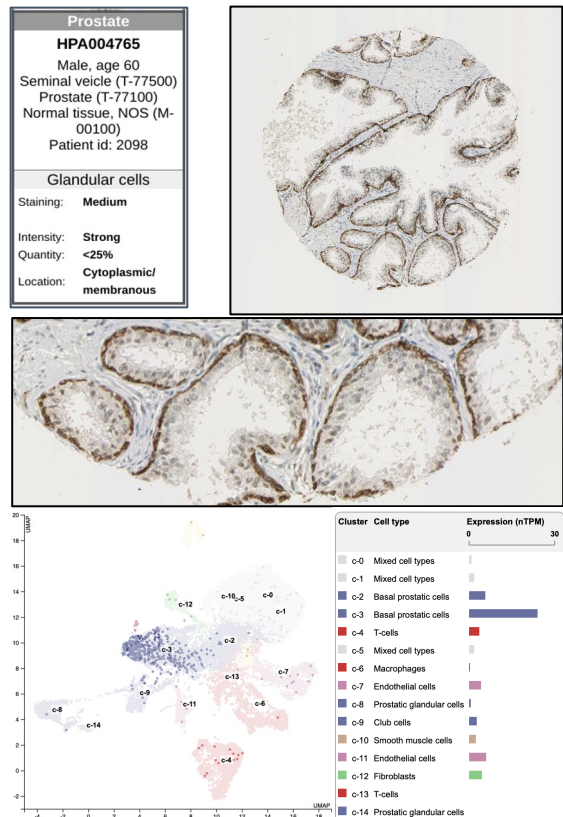
Supplementary Figure 3c-d

Supplementary Figure 3. Uncropped western blots for membranes used in Fig. 4 and replicates and replicate for DNA content cell cycle analysis. See Fig. 3 legend for details. Molecular markers are indicated. Membranes transferred from SDS-PAGE gels were cut horizontally in three pieces (top, middle and bottom) using protein ladders on the flanking lanes as guide. Membranes with blotted proteins in a molecular range were incubated with specific antibodies to a target protein in that molecular mass range. In replicate 2 for Fig. 3D, relevant lanes are marked with a shaded line rectangle.

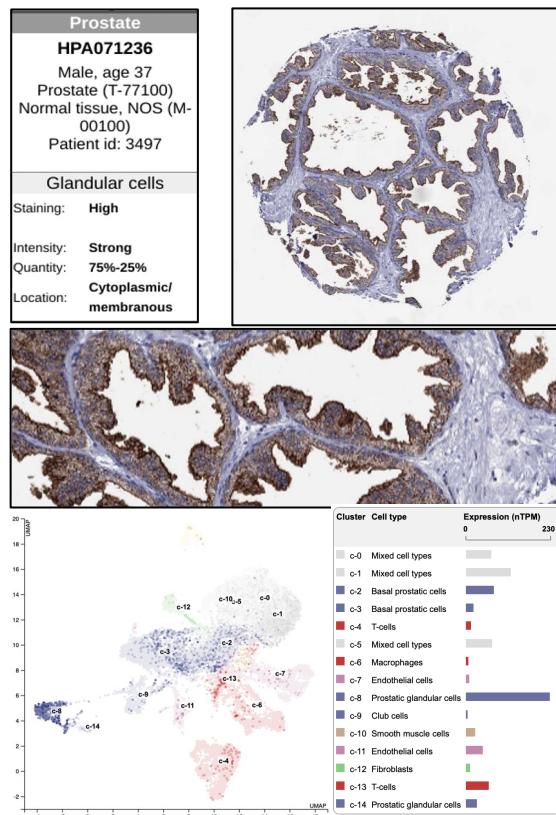
CD326-Epithelial



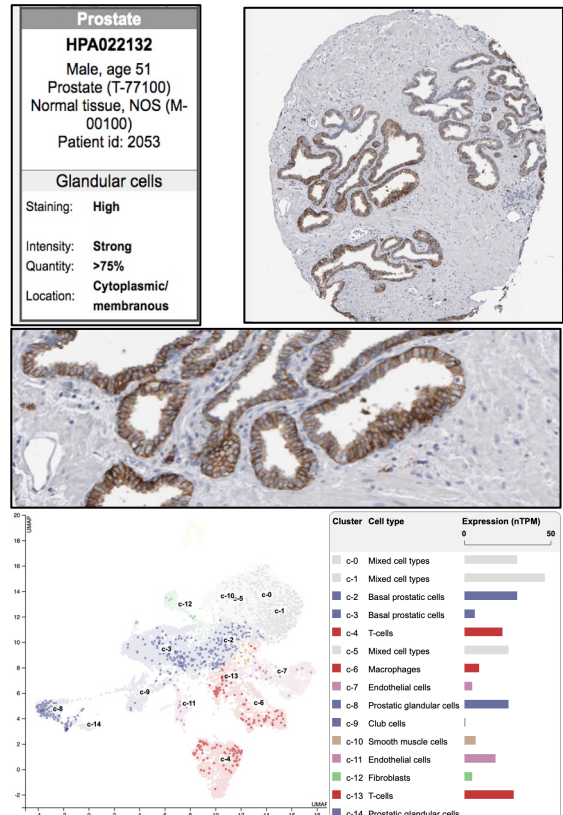
CD271-Basal



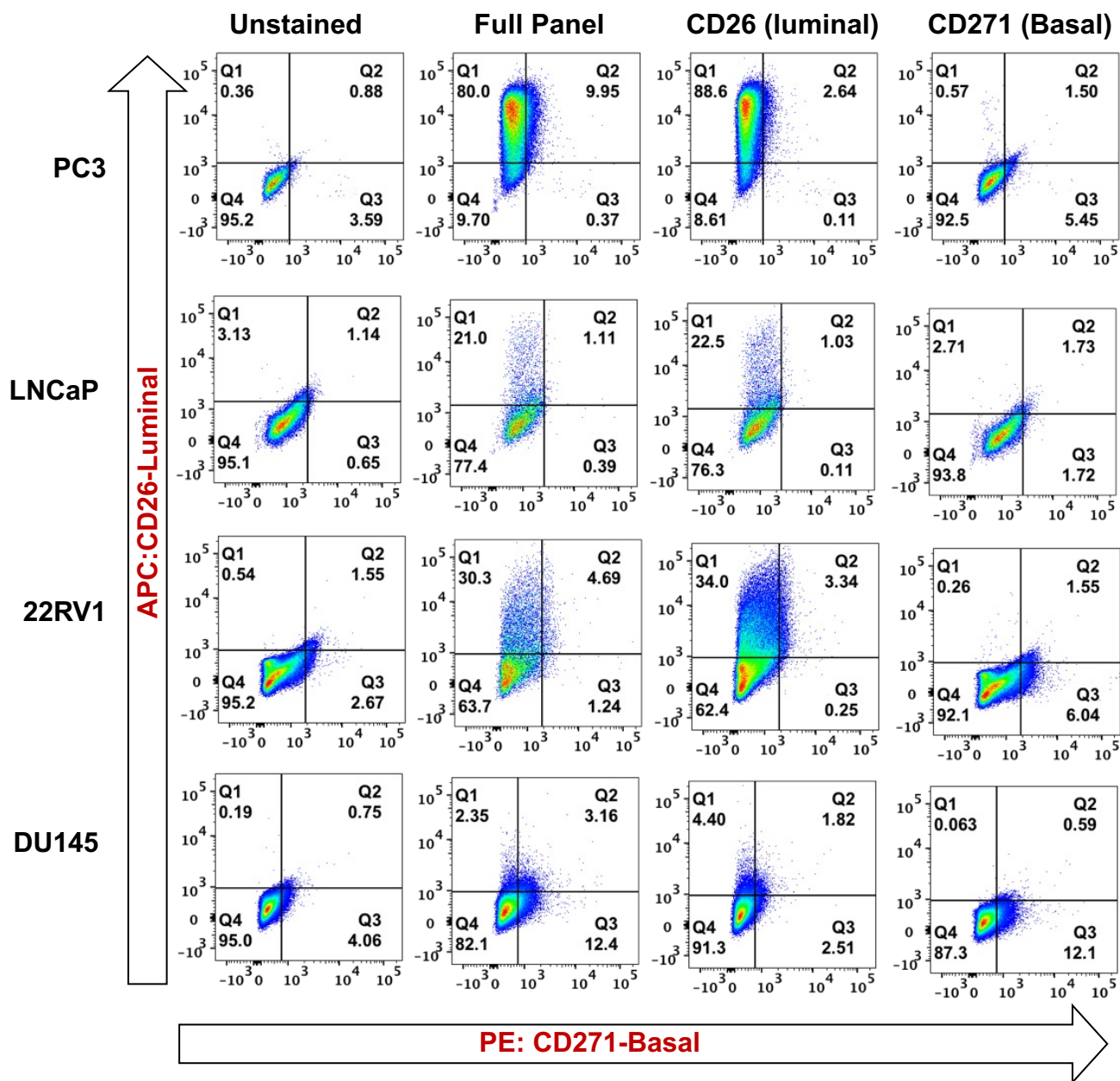
CD26-Luminal



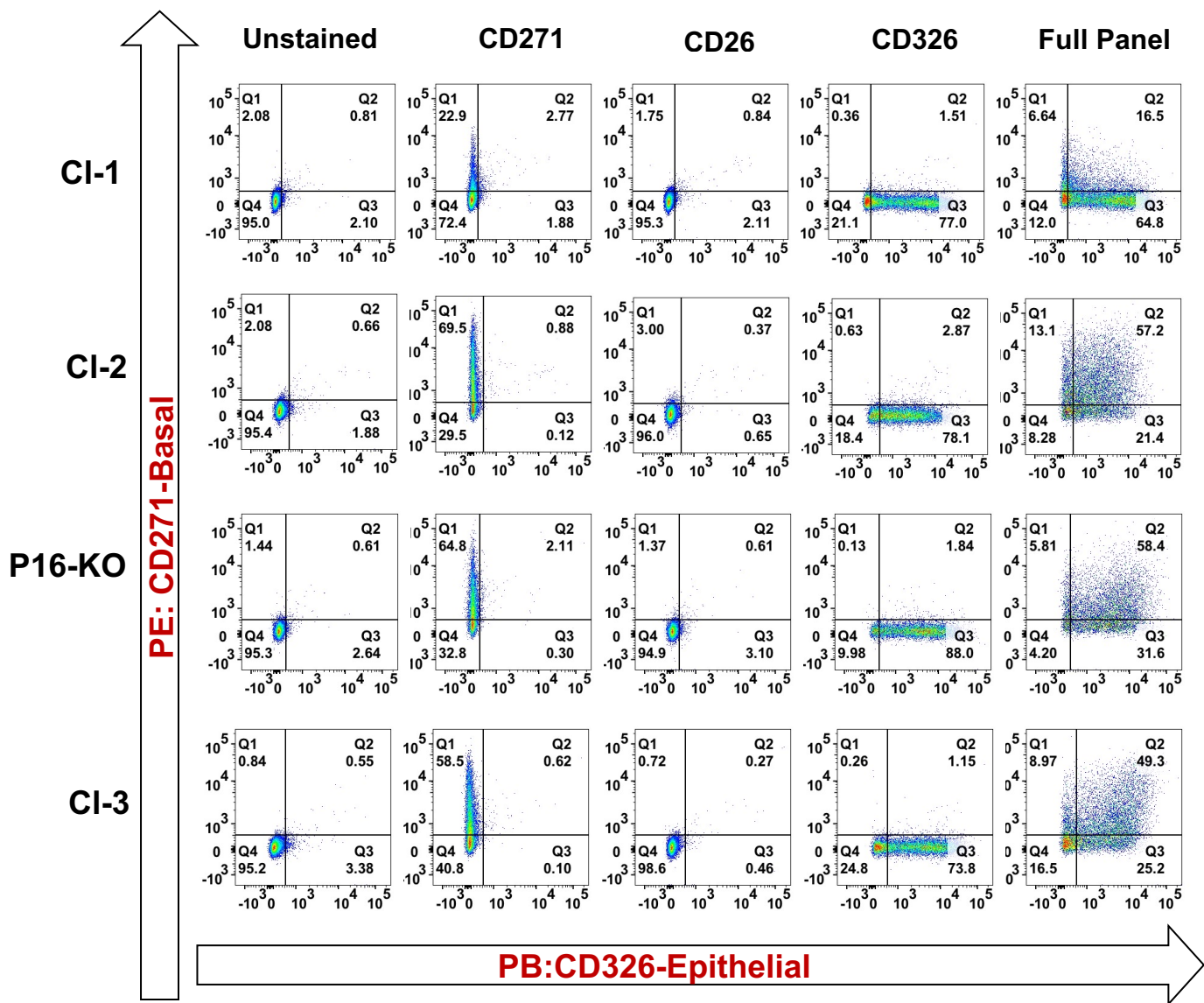
CD38-Luminal



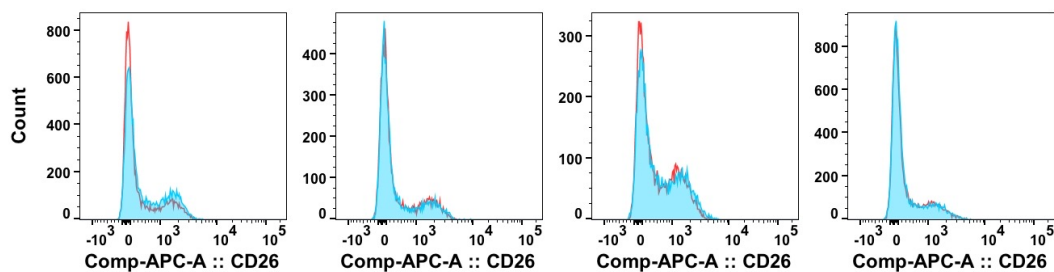
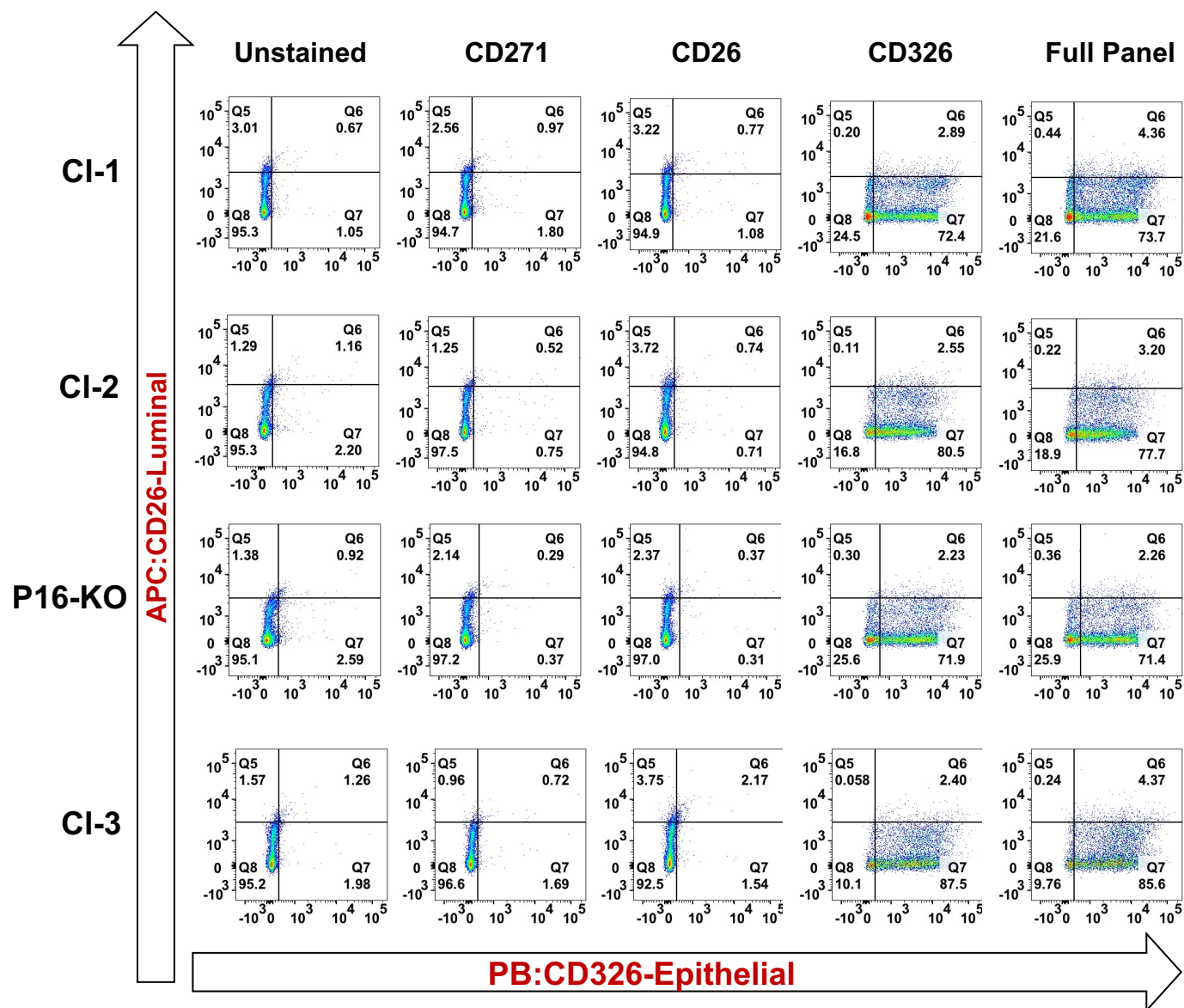
Supplementary Figure 4. Prostate tissue section microarrays immunohistochemistry (IHC) of markers used for immunophenotyping and prostate cell populations mapped using scRNA-Seq data positive for the expression of these markers (*Human Protein Atlas* database). Tissue cores show protein expression patterns in prostate acini determined by staining with specific antibodies via IHC. Patient information and the intensity of the stain are indicated on the left. The expression of cell surface markers is shown in individual cells positioned using UMAPs based on scRNA-Seq data available on the *Human Protein Atlas* site. **(A)** EPCAM (CD326) expression in prostate tissue and primary cells from the prostate of a patient sample. **(B)** Nerve Growth Factor Receptor (NGFR)(CD271) expression in prostate tissue and primary cells from the prostate. **(C)** Dipeptidyl peptidase 4 (DPP4) (CD26) expression in prostate tissue and primary cells from the prostate. **(D)** CD38 molecule (CD38) expression in prostate tissue and primary cells from the prostate.



Supplementary Fig. 5b



Supplementary
Fig. 5c



CI-1

CI-2

P16-KO

CI-3

**Supplementary
Fig. 5d**

Supplementary Figure 5. FACS data for Fig 5 including unstained controls. (A)

Supplementary FACS data for Fig 5A. Expression of CD326 (Epithelial) on the x-axis and CD26 (Luminal), CD271 (Basal) and CD38 (luminal) on the y-axis in primary hPrEC stained with the respective antibodies. **(B).** Supplementary FACS data for Fig 5B. Expression of CD271 (basal) on the x-axis and CD26 (Luminal) on the y-axis in PCa cell lines stained with the respective antibodies. **(C).** Supplementary FACS data for Fig 5C. Expression of CD326 (epithelial) on the x-axis and CD271 (basal) on the y-axis in immortalized PrEC–derived cell clones stained with the respective antibodies. **(D, upper panel)** Supplementary FACS data for Fig 5D. Expression of CD326 (epithelial) on the x-axis and CD26 (Luminal) on the y-axis in immortalized PrEC–derived cell clones stained with the respective antibodies. **(C, lower panel)** Overlay of histograms comparing the intensities of the APC-CD26 channel in the unstained and full panels. All data were gated and analyzed in FlowJo.