Supplementary Information for

A genetically defined disease model reveals that urothelial cells can initiate divergent bladder cancer phenotypes

Liang Wang[1], Bryan A. Smith[1], Nikolas G. Balanis, Brandon L. Tsai, Kim Nguyen, Michael W. Cheng, Matthew B. Obusan, Favour N. Esedebe, Saahil J. Patel, Hanwei Zhang, Peter M. Clark, Anthony E. Sisk, Jonathan W. Said, Jiaoti Huang, Thomas G. Graeber, Owen N. Witte[2], Arnold I. Chin[2], Jung Wook Park[2]
[1] co-first authors and [2] corresponding authors

Corresponding authors:
Jung Wook Park: Department of Pathology, School of Medicine, Duke University, Durham, NC 27710, USA; jungwookpark@mednet.ucla.edu;
Arnold I. Chin: Department of Urology, University of California, Los Angeles, California, CA 90095, USA; arnoldchin@mednet.ucla.edu;
Owen N. Witte: Department of Microbiology, Immunology, and Molecular Genetics, University of California-Los Angeles, Los Angeles, CA 90095, USA; owenwitte@mednet.ucla.edu;

This PDF file includes:

Figures S1 to S9
Tables S1

Other supplementary materials for this manuscript include Dataset S1-S7 in separate Excel files (Dataset S1-S7.xlsx)

www.pnas.org/cgi/doi/10.1073/pnas.1915770117

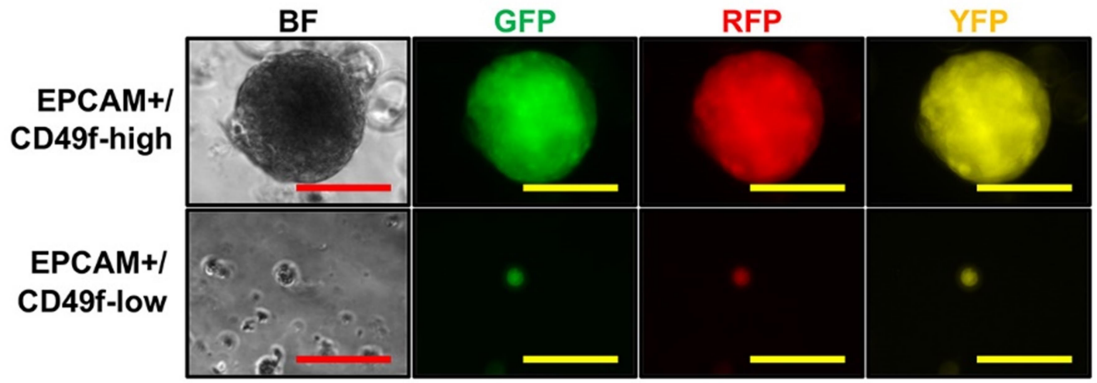


Figure S1. EPCAM+/CD49f high population but not EPCAM+/CD49f low population forms organoids.

Representative images of EPCAM+/CD49f high and EPCAM+/CD49f low population infected with PARCB lentivirus and cultured for 1 week in organoid culture system. Fluorescent pictures indicated the expression of fluorescent tags on the 3 lentivirus that carry PARCB factors. Scale=100μm.

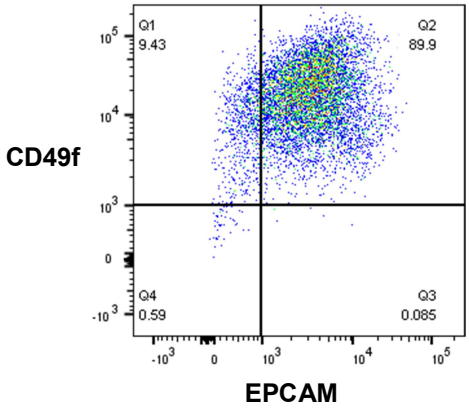


Figure S2. Commercial primary bladder epithelial cell lines express EPCAM and CD49f.

A representative figure of flow cytometry analysis using antibodies targeting EPCAM and CD49f in commercially available primary bladder epithelial cell lines (human primary bladder epithelial cell lines, ATCC). The number in each quadrant represents the percentage of positive cells.

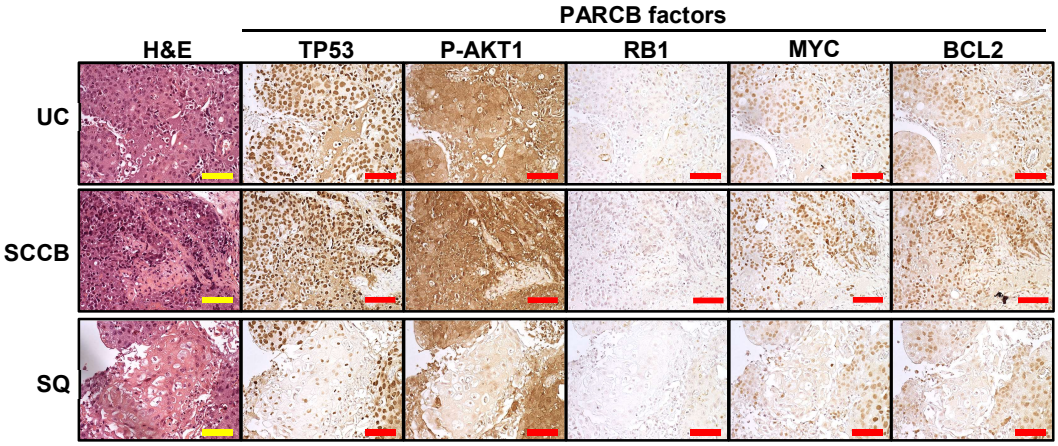


Figure S3. PARCB tumors express the five oncogenic factors.
Representative H&E stained images and IHC images with antibodies against p53, phospho-Akt1, Rb1, Myc, and Bcl2 in the UC, SCCB and SQ portion of an individual PARCB tumors. All phenotypes in PARCB tumors express the PARCB factors. UC: urothelial carcinoma, SQ: squamous carcinoma. Scale=100μm

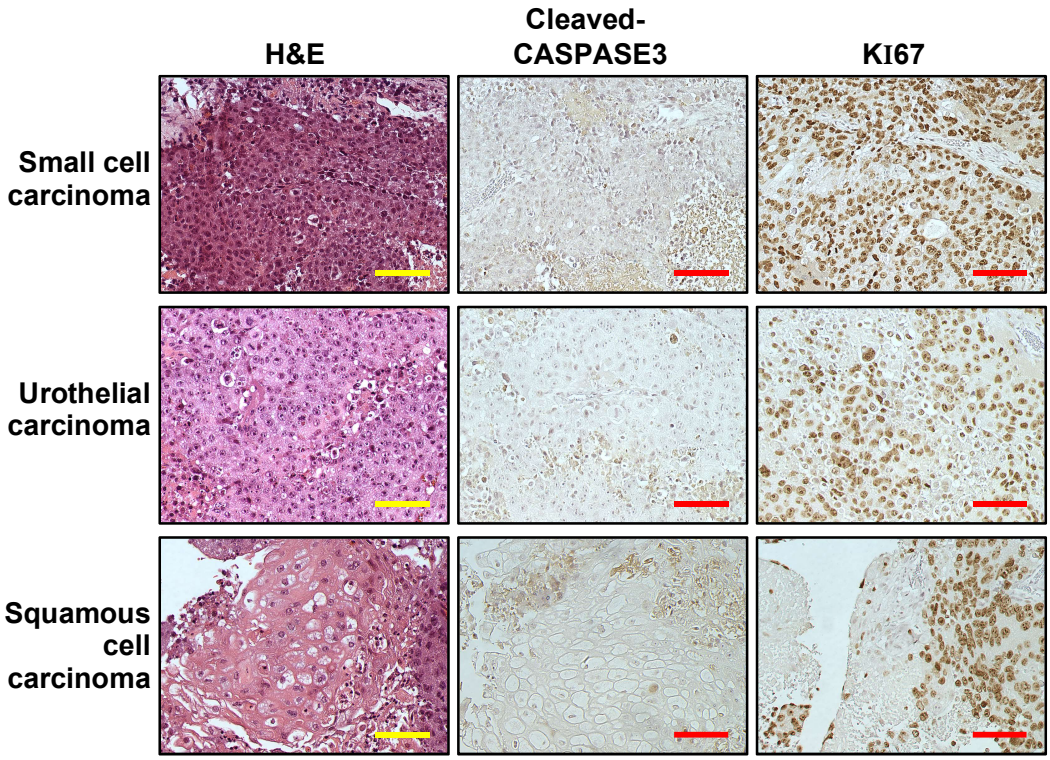


Figure S4. The SCCB portion of PARCB tumors expresses cell apoptosis and cell proliferation markers

Representative H&E and IHC images using antibodies against cell apoptosis marker cleaved-CASPASE3 and cell proliferation marker KI67 in non-SCCB and SCCB portions of PARCB tumors. KI67 and cleaved-CASPASE3 are strongly expressed in the SCCB portion of PARCB tumor, but are weakly expressed or negative in non-SCCB portions of PARCB tumor. Scale= 100μm.

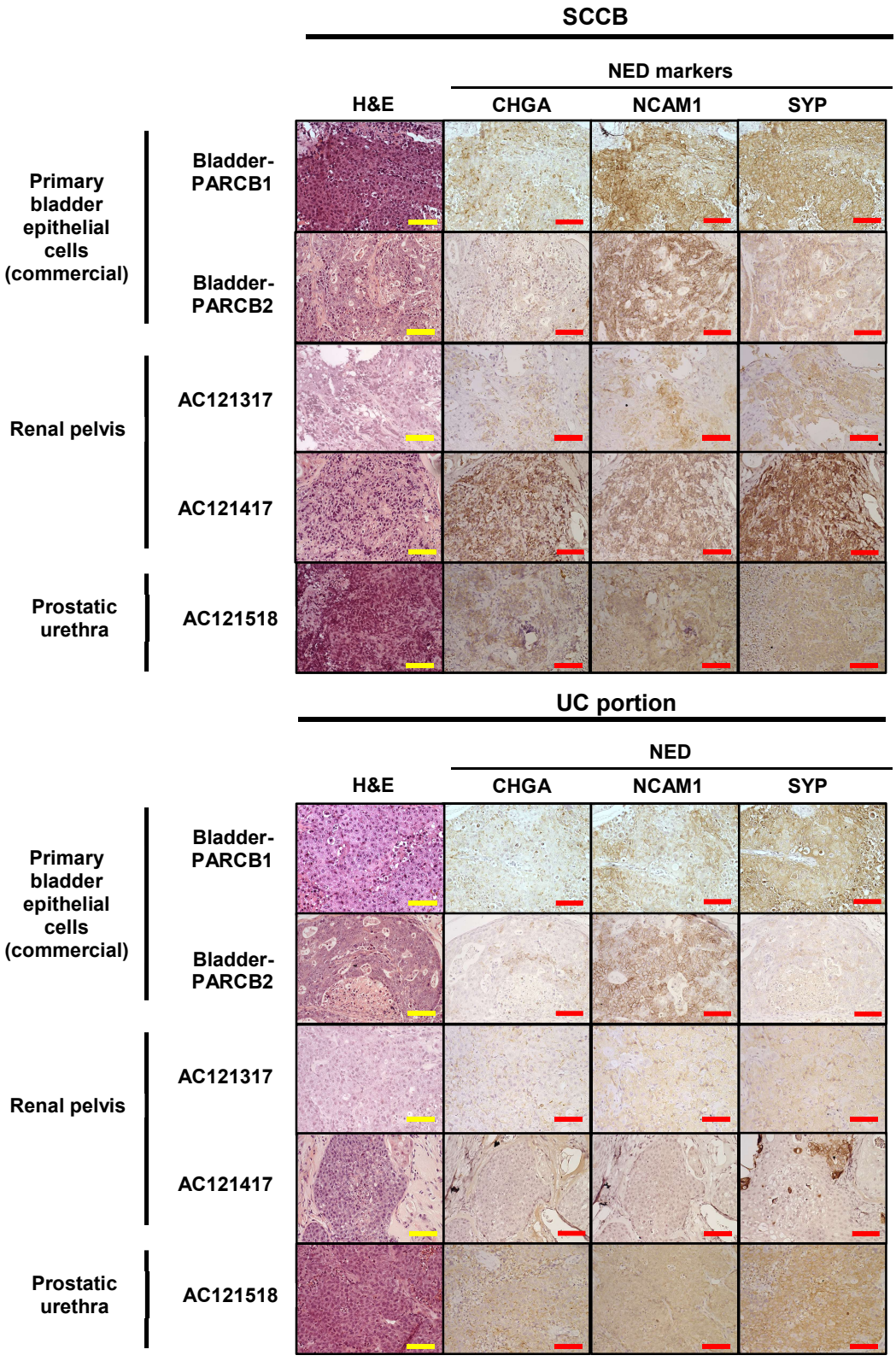

SCCB
NED markers
H&E
CHGA
NCAM1
SYP
Primary bladder epithelial cells (commercial)
Bladder-PARCB1
Bladder-PARCB2
Renal pelvis
AC121317
AC121417
Prostatic urethra
AC121518
UC portion
NED
H&E
CHGA
NCAM1
SYP
Primary bladder epithelial cells (commercial)
Bladder-PARCB1
Bladder-PARCB2
Renal pelvis
AC121317
AC121417
Prostatic urethra
AC121518

Figure S5. PARCB tumors from different sites of human urinary tract exhibit SCCB and UC phenotypes

Representative H&E and IHC images using antibodies against neuroendocrine differentiation markers NCAM1, SYP and CHGA in PARCB tumors derived from bladder, renal pelvis, or prostatic urethra urothelial cells. Each PARCB tumor has both SCCB and UC phenotypes. Scale= 100μm.

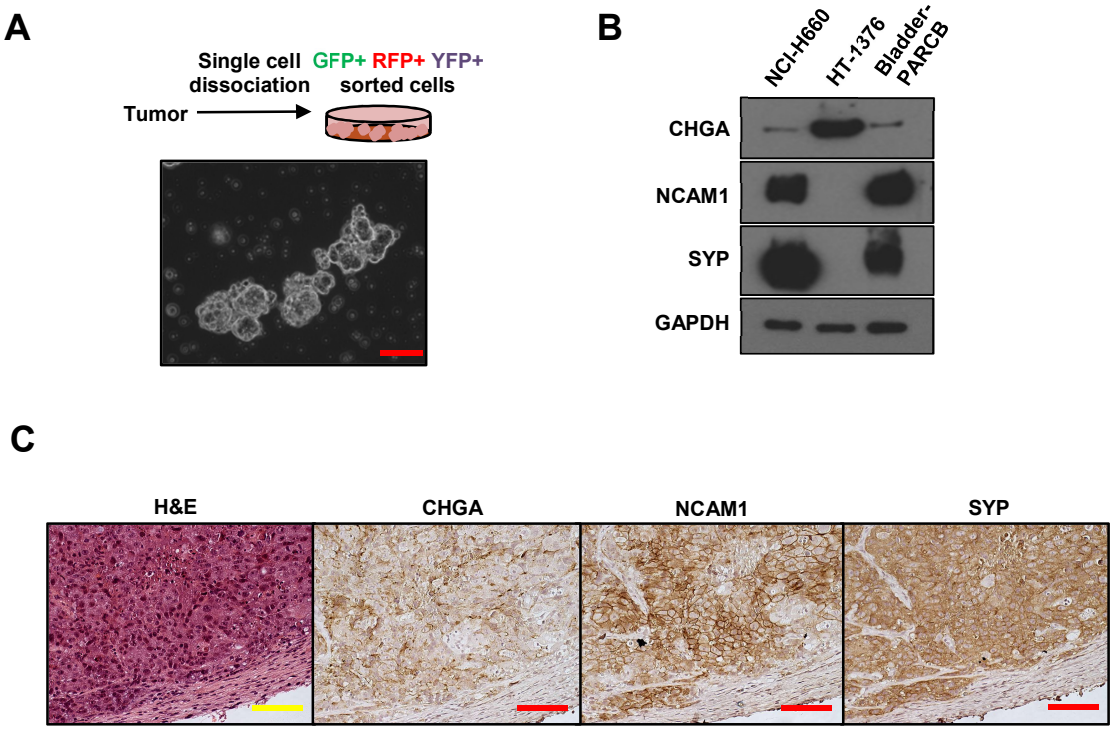


Figure S6. Bladder-PARCB cell lines express NED markers.
(A)Schematic work flow of generating Bladder-PARCB cell lines. PARCB tumor were dissociated into single cell suspension. The fluorescent positive cells were isolated using flow cytometry and cultured into bladder-PARCB cell lines. The bright field image shows the morphology of cultured bladder-PARCB cells. Scale=100μm.
(B) Representative immunoblotting of neuroendocrine prostate cancer cell line NCI-H660, urothelial carcinoma cell line HT1376 and Bladder-PARCB cell lines for NED marker SYP, CHGA, NCAM1, and internal control GAPDH. Proteins from bladder-PARCB1 cell and HT1376 cells were harvested for immunoblotting assay using antibodies targeting NED markers and GAPDH. Bladder-PARCB cells express SYP, CHGA and NCAM1 at the protein level. GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase.
(C) Representative H&E and IHC images of detecting SYP, CHGA, and NCAM1 in tumors derived from Bladder-PARCB cells. Bladder-PARCB cells were cultured and subcutaneously injected into NSG mice. Tumors were harvested, processed, and probed for NED markers using IHC. Bladder-PARCB cell derived tumor exhibit SCCB phenotype and express NED markers. Scale=100μm.

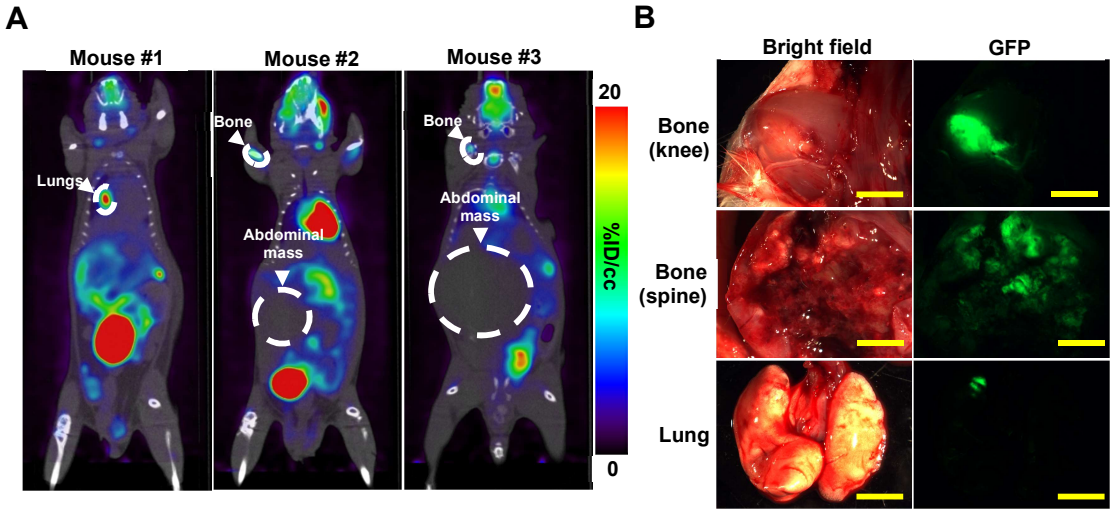
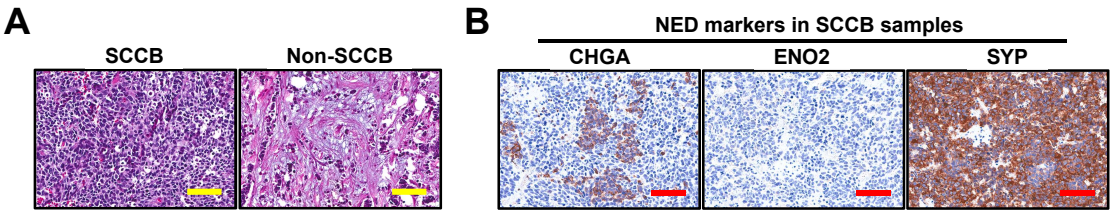


Figure S7 Bladder-PARCB1 cells are metastatic in *NSG* mice.
(A) Representative positron emission tomography (PET) image showing bladder-PARCB1 cells metastatic sites in *NSG* mice 4 weeks after tail vein injection. Potential metastatic sites were highlighted and examined using autopsy.
(B) Representative bright filed and fluorescent images of bladder-PARCB1 metastatic sites in immune-deficient mice. GFP pictures indicate tumors are from bladder-PARCB1 cells, but not from mouse cells. Scale=5mm



C

SCCB				Non-SCCB		
Sample ID	Sex	Stage	NED markers percentage	Sample ID	Sex	Stage
SCCB1	M	T2	> 95%	Non-SCCB1	M	T3N0Mx
SCCB2	M	T2	> 95%	Non-SCCB2	F	T2
SCCB3	F	T2	> 95%	Non-SCCB3	M	T3N1Mx
SCCB4	M	T3N1Mx	100%	Non-SCCB4	F	T3N3Mx
SCCB5	F	T3N1Mx	> 95%	Non-SCCB5	F	T2
SCCB6	M	T2N0Mx	> 95%	Non-SCCB6	F	T2
SCCB7	F	T2	80%	Non-SCCB7	M	T3N1Mx
SCCB8	M	T3	90%	Non-SCCB8	M	T4N2Mx
SCCB9	M	T2	100%	Non-SCCB9	M	T3N0Mx
				Non-SCCB10	F	T2

Figure S8. A new cohort of SCCB is established using formalin-fixed paraffin embedded samples.
(A) Representative histological pictures of SCCB and non-SCCB samples from the UCLA-BLCA cohort. Scale=100μm
(B) Representative image of IHC staining using antibodies against NED markers CHGA, ENO2, and SYP in SCCB samples from the UCLA-BLCA cohort. Scale=100μm, ENO2: enolase 2.
(C) Table of sample information in the UCLA-BLCA cohort. NED markers percentage is a combined positivity of CHGA, SYP and ENO2 in tumor cells. T: tumor stage; N: node; M: metastasis.

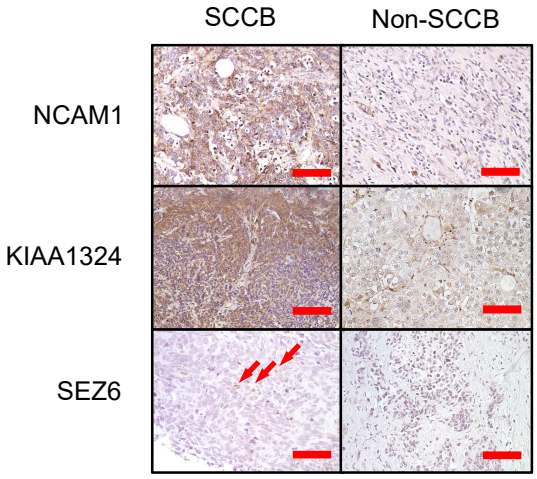


Figure S9. NCAM1, KIAA1324 and SEZ6 are expressed in clinical SCCB samples. Representative IHC images using antibodies against KIAA1324, NCAM1 and SEZ6 in SCCB and non-SCCB samples from the UCLA-BLCA cohort. Red arrow shows the SEZ6 positive cells in SCCB samples. NCAM1 expression level is higher in SCCB than in Non-SCCB samples. The expression of KIAA1324 are highly variable in both SCCB and non-SCCB samples. SEZ6 is only expressed in some cells in SCCB samples, but not in non-SCCB samples. Scale= 100μm.

Table S1. IHC profile of PD-L1 in the UCLA-BLCA cohort

SCCB	Tumor		TIL	
Sample ID	Positive Area	Intensity	Positive Area	Intensity
SCCB1	0.50%	1.3	5.00%	3
SCCB2	2.50%	1.3	7.50%	2.5
SCCB3	2.50%	1.4	50.00%	3.5
SCCB4	0.50%	0.8	0.00%	0
SCCB5	0.50%	0.8	5.00%	3.1
SCCB6	5.00%	3.1	50.00%	3.5
SCCB7	0.50%	0.5	6.00%	3.1
SCCB8	0.00%	0	2.50%	0.9
SCCB9	3.50%	3.1	55.00%	3.5
Non-SCCB	**Tumor**		**TIL**	
Sample ID	Positive Area	Intensity	Positive Area	Intensity
Non-SCCB1	78.80%	3	15.00%	3
Non-SCCB2	0.50%	0.8	3.00%	2.6
Non-SCCB3	2.00%	1.3	7.50%	3
Non-SCCB4	41.30%	2.6	6.30%	2.6
Non-SCCB5	0.50%	0.8	3.00%	2.5
Non-SCCB6	0.00%	0	0.50%	0.8
Non-SCCB7	12.50%	0.9	0.00%	0.8
Non-SCCB8	0.00%	0	5.00%	2.5
Non-SCCB9	50.00%	2.6	25.00%	3
Non-SCCB10	2.50%	0.8	3.50%	2.5

Dataset 01 (separate file).

Sheet 1:
Gene sets enriched in SCCB (1-1) or non-SCCB (1-2) samples.

Sheet 2:
List of genes used to calculate pan-small cell carcinoma gene signature score in the UCLA-BLCA cohort.

Sheet 3:
Output of VIPER analysis (filtered by P-value < 0.05)

Sheet 4:
List of CSP genes in the Uniprot database

Sheet 5:
List of phenotype associated CSPs (P-value <0.05) in the format of DEseq2 output using the UCLA-BLCA cohort.

Sheet 6:
Log2 fold change and P-value of CSPs in DEseq2 analysis using UCLA-BLCA or TCGA-BLCA datasets