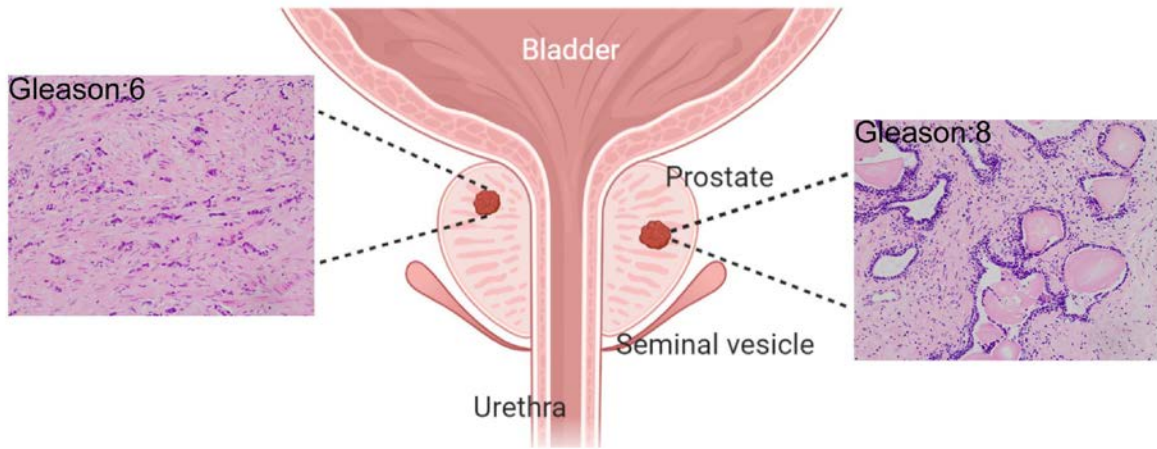


Supplementary Figure 1

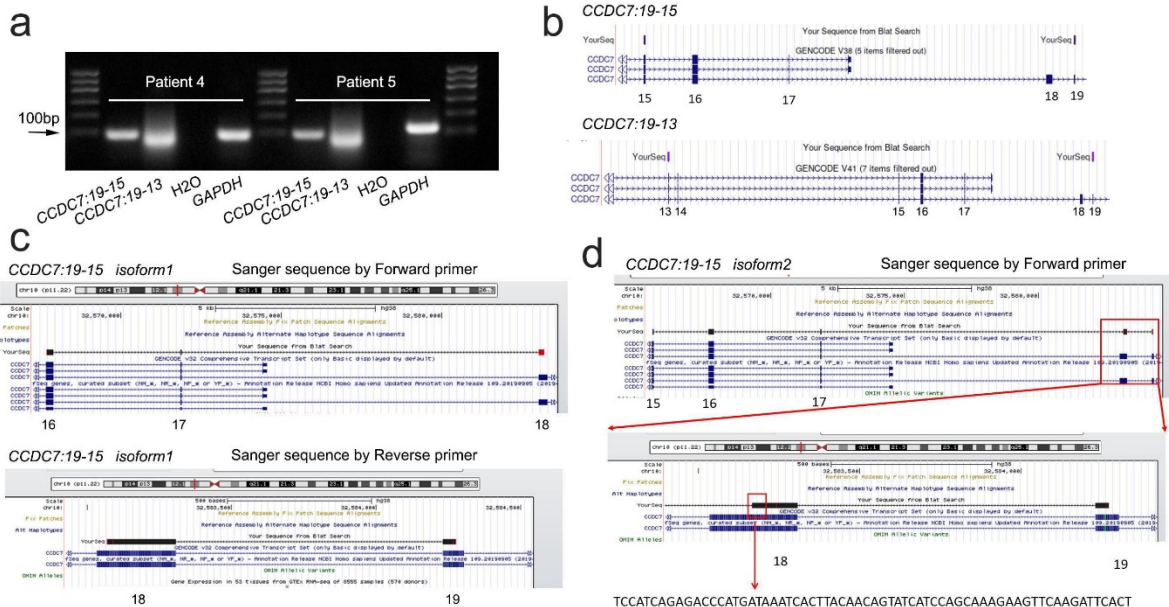
Patient



1
 2 **Supplementary Figure 1. Representative images of one of the PCa samples that met our**
 3 **selection criteria.** The two tumor sites were distributed on two different lobes. Among those
 4 sites, left site: Gleason score = 3+3, right site: Gleason score = 4+4. In total, six samples were
 5 obtained from three patients.

6

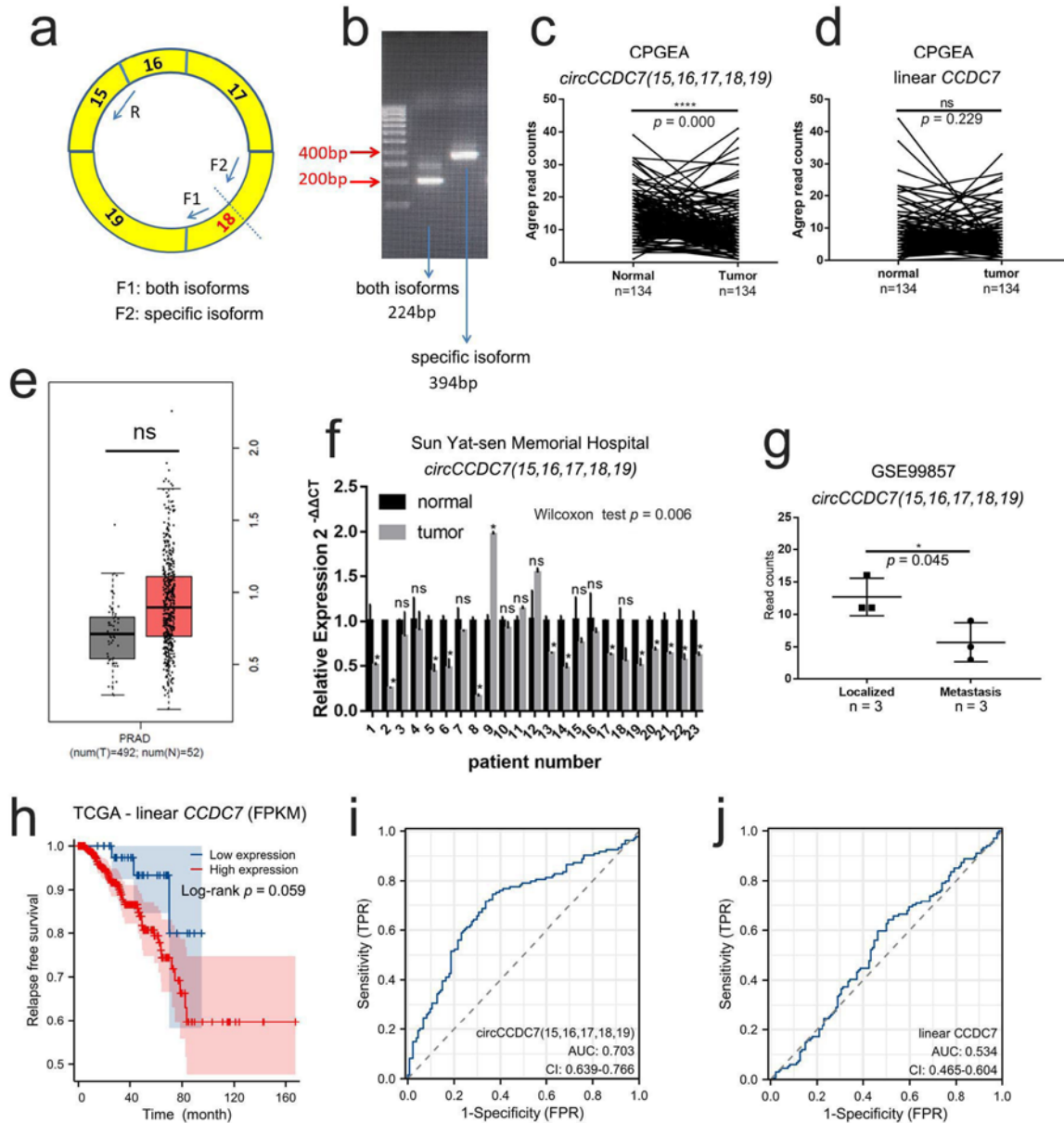
Supplementary Figure 2



7
 8 **Supplementary Figure 2. Sanger sequencing result of *CCDC7*₁₉₋₁₅ and *CCDC7*₁₉₋₁₃, and**
 9 **full length of *circCCDC7*(15,16,17,18,19).** (a) Gel image of RT-qPCR product of *CCDC7*₁₉₋₁₅
 10 and *CCDC7*₁₉₋₁₃ in another two clinical patients. Mix cDNA from tumor and adjacent normal
 11 margin was used. (b) Sanger sequence of the junction sites aligning to UCSC Genome Browser.

12 (c) Sanger sequence result of full length of *isoform1* aligning to UCSC Genome Browser. (d)
 13 Sanger sequencing result of full length *isoform2* aligning to UCSC Genome Browser.
 14

Supplementary Figure 3



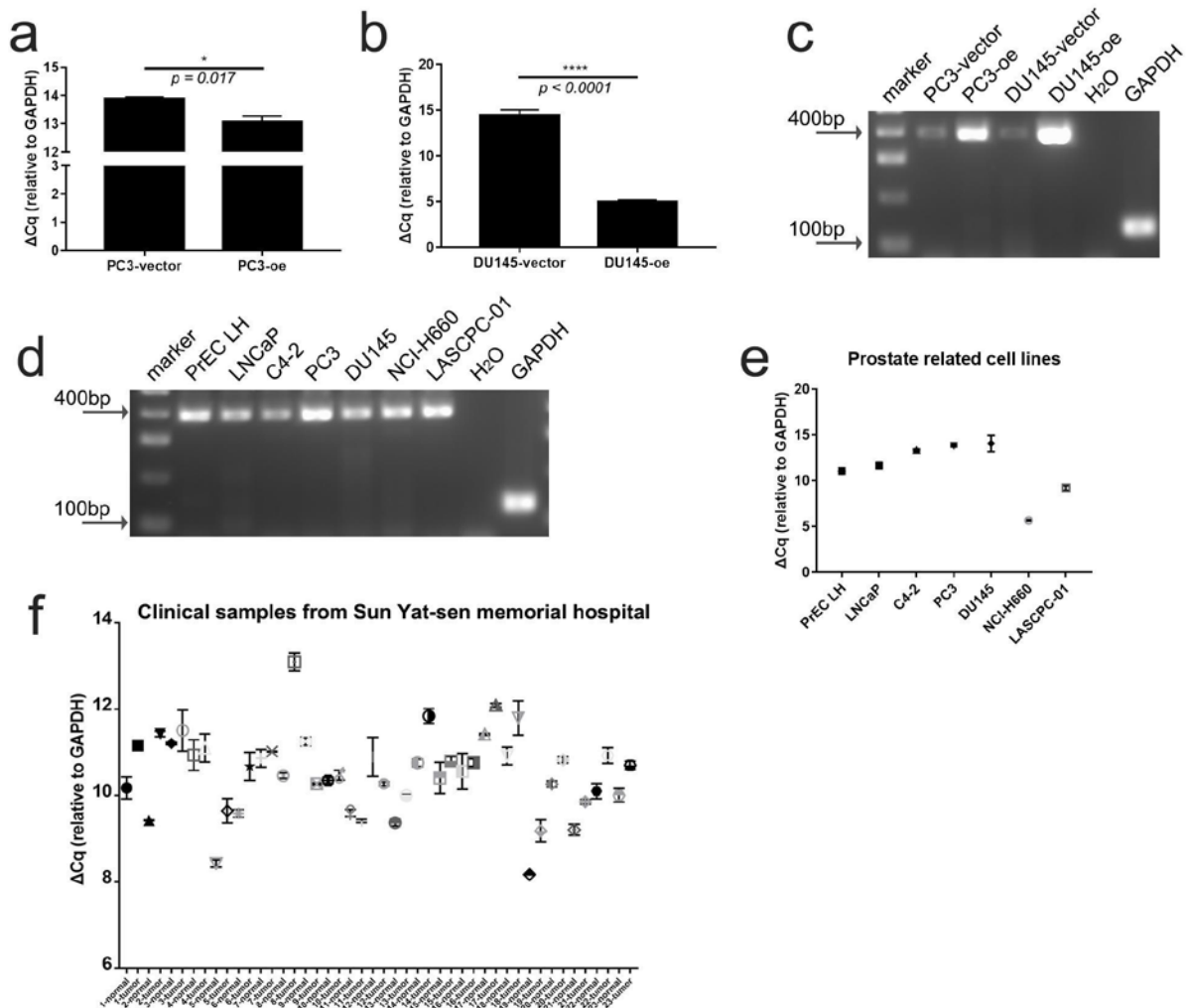
15

16 **Supplementary Figure 3. Characterization of *circCCDC7(15,16,17,18,19)*.** (a) Schematic
 17 diagram of *circCCDC7(15,16,17,18,19)* and specific primers design. (b) Gel image of RT-
 18 PCR product of two primers for both forms and specific forms. (c-d) Comparison of the
 19 expression of *circCCDC7(15,16,17,18,19)* (c) and linear *CCDC7* (d) in 134 pairs of PCa and
 20 normal margin samples from CPGEA using paired t test. (e) Expression of linear *CCDC7* from
 21 TCGA. (f) Quantitative analysis of *circCCDC7(15,16,17,18,19)* in 23 pairs of clinical samples

22 from Sun Yat-sen Memorial Hospital using paired t test. (g) Expression of
 23 *circCCDC7(15,16,17,18,19)* in metastasis dataset GSE99857 after AGREP analysis. (h)
 24 Relapse-free survival analysis of linear *CCDC7* using FPKM value TCGA. (i-j) AUC value of
 25 the ROC for normal or tumor by using the expression of *circCCDC7(15,16,17,18,19)* linear
 26 *CCDC7*. * $p < 0.05$, **** $p < 0.0001$.

27

Supplementary Figure 4



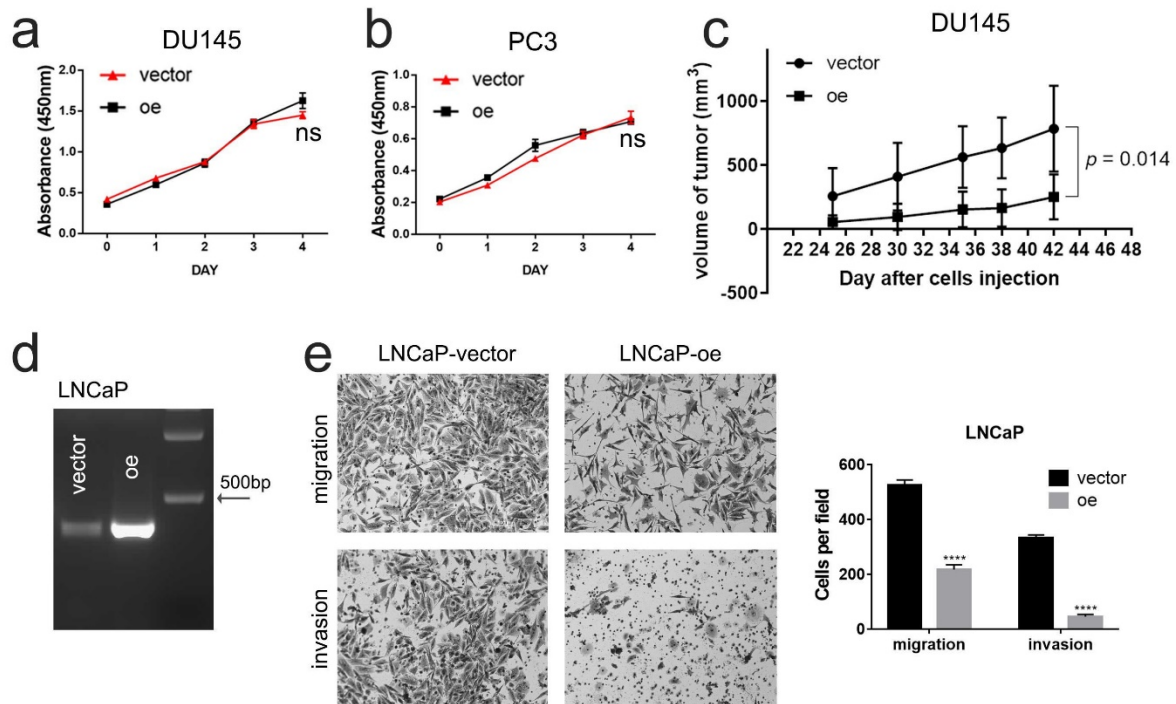
28

29 **Supplementary Figure 4. The validation of PC3 and DU145 overexpression system.** (a-b)
 30 The change of ΔCq in PC3 and DU145 after *circCCDC7(15,16,17,18,19)* overexpressed. (c)
 31 Gel image of RT-qPCR product of *circCCDC7(15,16,17,18,19)* when it was stable
 32 overexpressed in PC3 and DU145. (d) Gel image of RT-qPCR product of
 33 *circCCDC7(15,16,17,18,19)* in current wide-type prostate related cell lines. (e) The ΔCq value
 34 of *circCCDC7(15,16,17,18,19)* in current wide-type prostate related cell lines. (f) The ΔCq

35 value of *circCCDC7(15,16,17,18,19)* in 23 pairs of clinical samples from Sun Yat-sen
36 memorial hospital. * $p < 0.05$, **** $p < 0.0001$.

37

Supplementary Figure 5

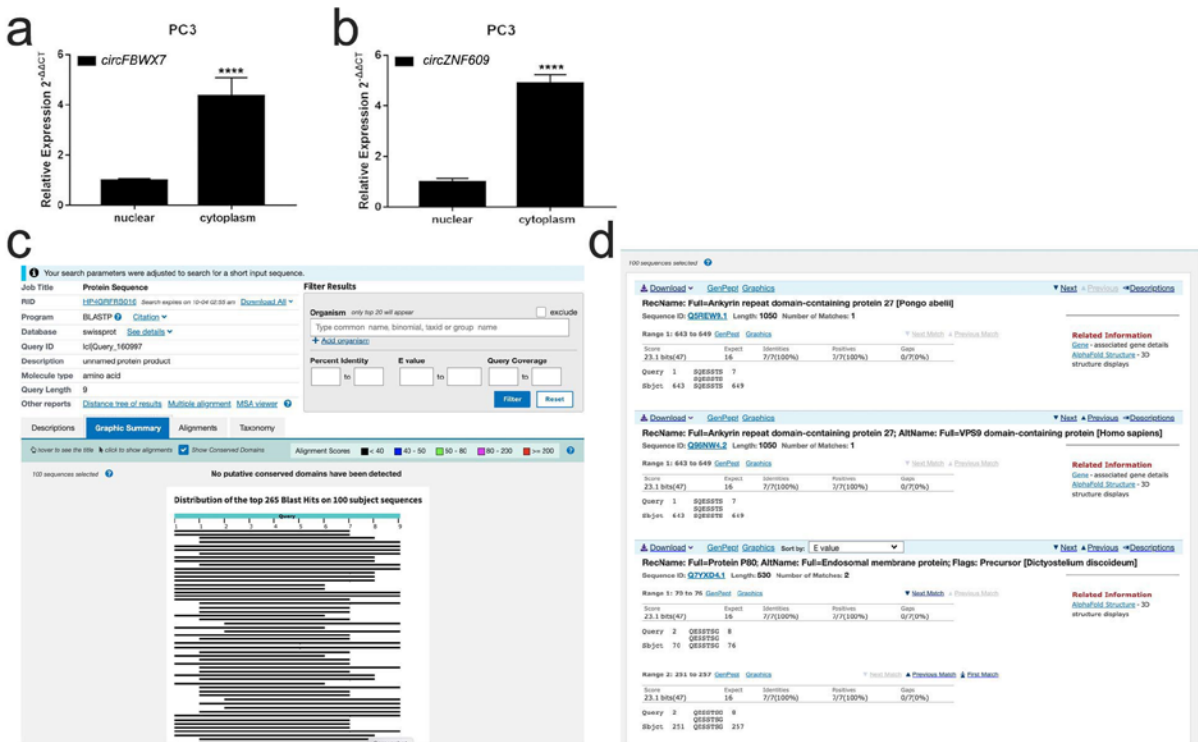


38

39 **Supplementary Figure 5. *CircCCDC7(15,16,17,18,19)* inhibits the viability, migration,**
40 **and invasion of PCa.** (a-b) CCK8 assay measuring cell proliferation in DU145 and PC3 cell
41 lines transfected with *circCCDC7(15,16,17,18,19)* overexpression plasmid or control vector.
42 (c) Tumor volume increase curves of the *circCCDC7(15,16,17,18,19)* overexpression and
43 control groups are summarized in the line chart after subcutaneous injection. (d) Gel image of
44 RT-qPCR product of *circCCDC7(15,16,17,18,19)* when LNCaP was transfected with
45 *circCCDC7(15,16,17,18,19)* plasmid. (e) Representative images of migration and invasion
46 assays using LNCaP after different plasmids transfection, and the histogram analysis of
47 migrated and invaded cell counts.

48

Supplementary Figure 6

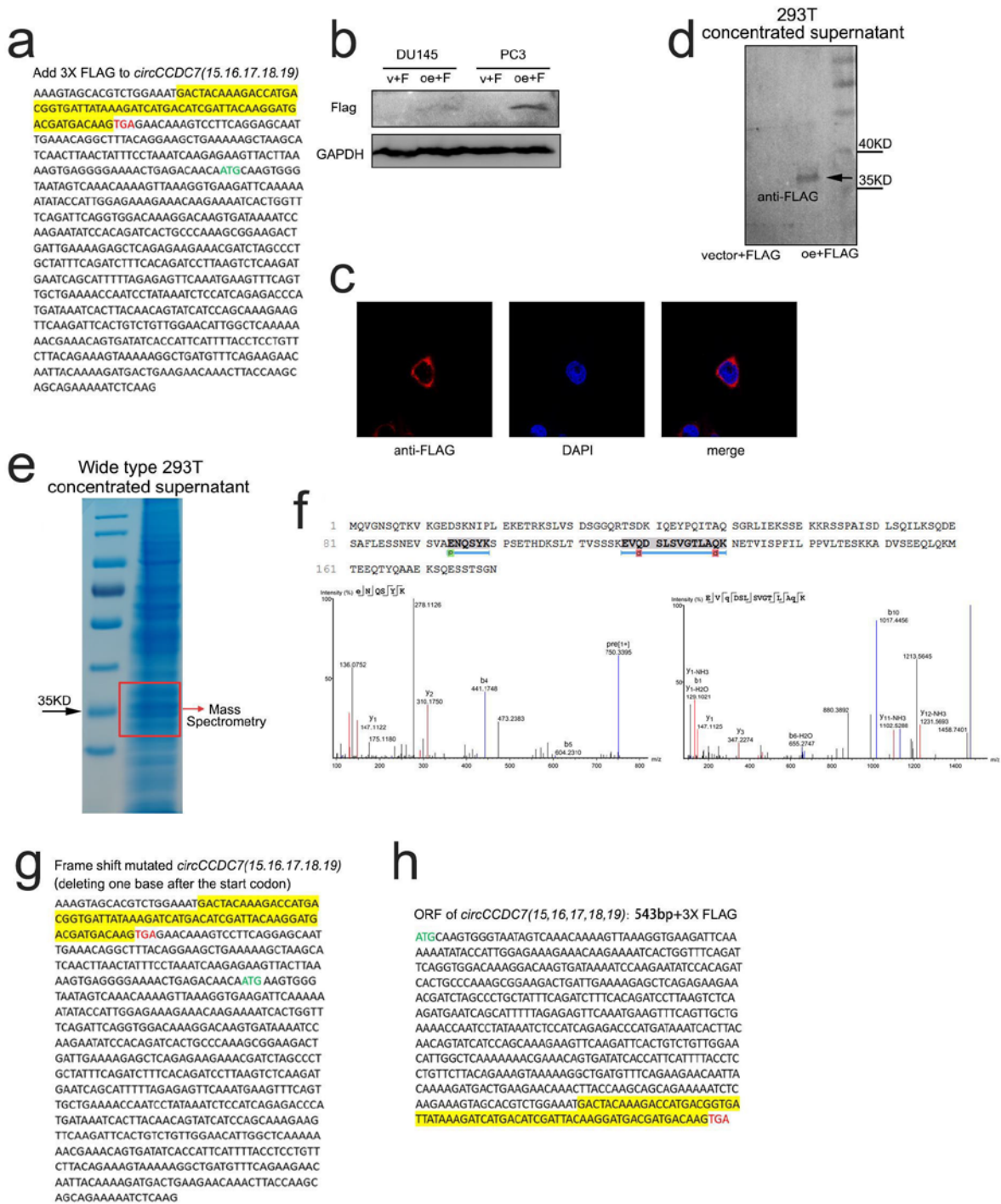


49

50 **Supplementary Figure 6. CircCCDC7-180aa cannot be derived from a linear transcript**
 51 **of another gene.** (a-b) Quantitative analysis on the expression of *circFBWX7* and *circZNF609*
 52 between nuclear and cytoplasm in PC3 cells. (c-d) The specific junction amino acids
 53 (SQESSTSGN) was used to BLASTP against UniProtKB/Swiss-Prot database
 54 (https://www.uniprot.org) and found no exact match.

55

Supplementary Figure 7

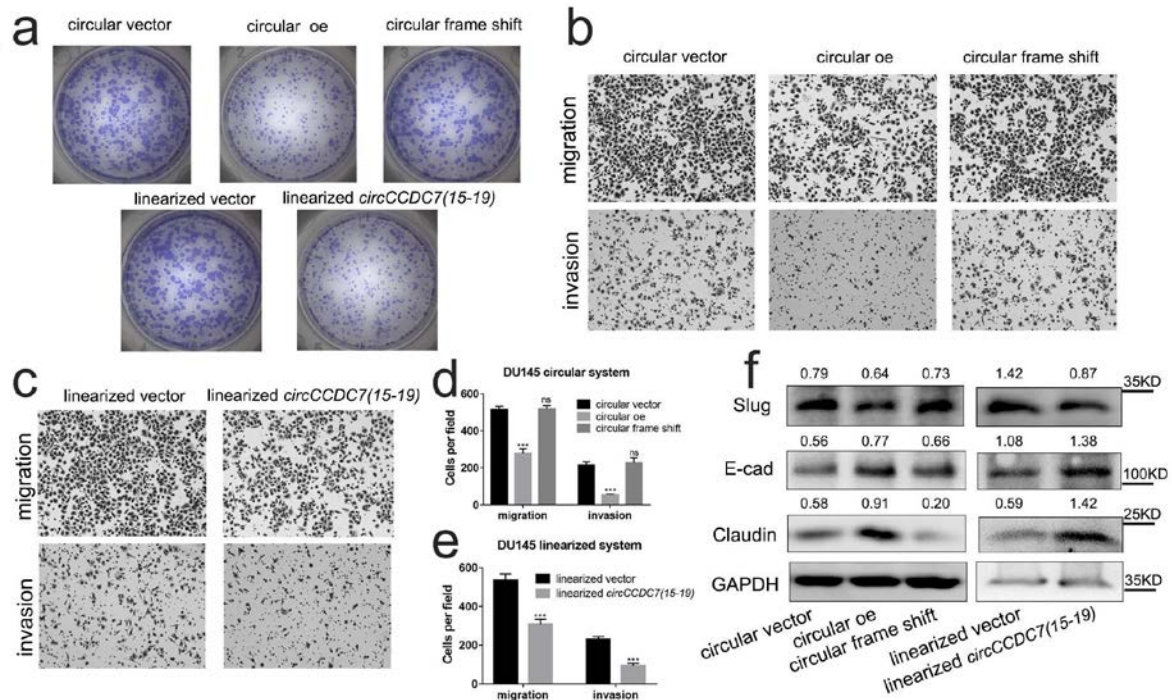


56

57 **Supplementary Figure 7. *CircCCDC7(15,16,17,18,19)* encodes a protein which can be**
 58 **secreted.** (a) 3× FLAG was added just before the stop codon (yellow highlights). (b) FLAG tag
 59 antibody was used to detect *circCCDC7*-180aa expression in PC3 and DU145 cells after
 60 transfection. (c) Immunofluorescence assay demonstrated that *circCCDC7*-180aa was mainly
 61 located around nucleus in du145 cell line which *circCCDC7(15,16,17,18,19)* was stable

62 overexpressed. (d) The supernatant from 293T cells was lyophilized, following by western blot
 63 to measure the secreted protein. (e-f) The supernatant from 293T cells was used to do IP and
 64 mass spectrometry, which identified the peptides of circCCDC7-180aa. (g-h) The full length
 65 of frame shift mutation plasmid and linearized *circCCDC7(15,16,17,18,19)* plasmid.
 66

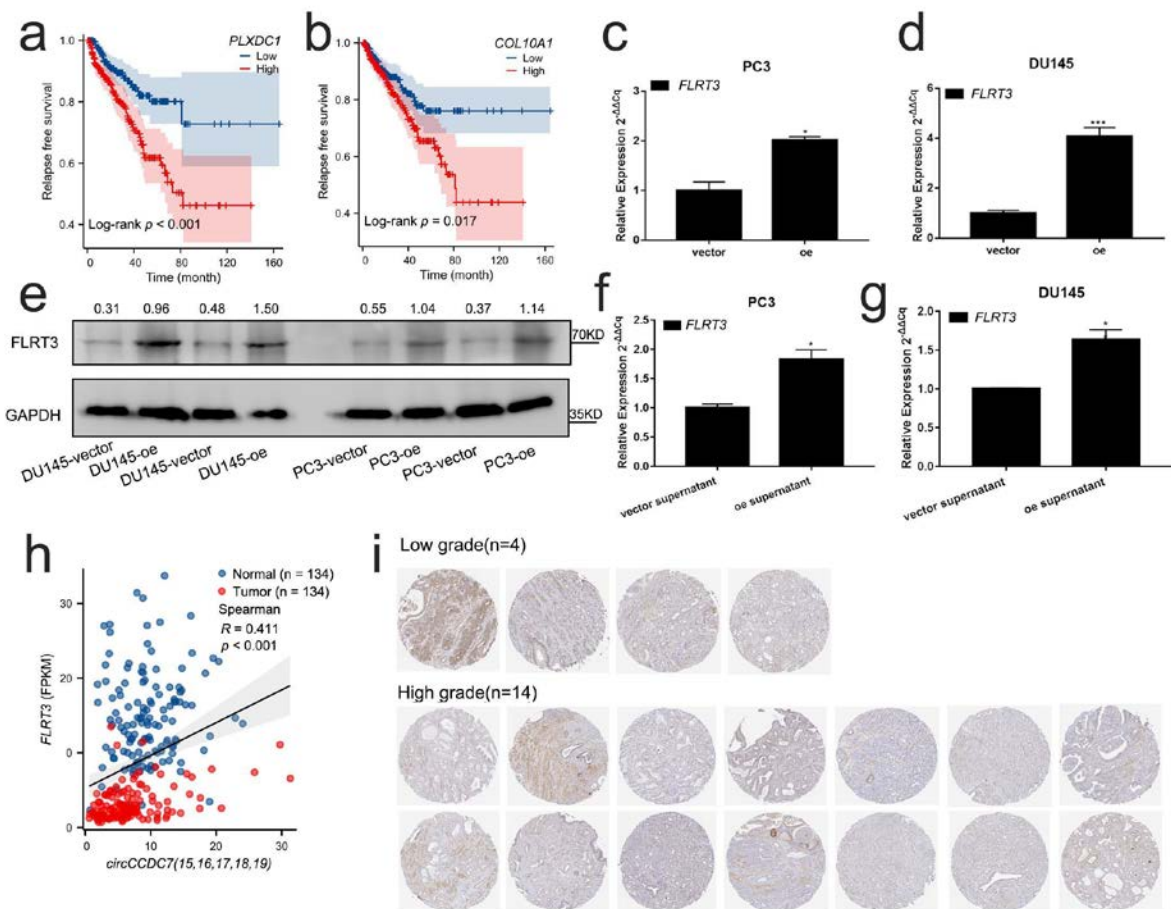
Supplementary Figure 8



67
 68 **Supplementary Figure 8. *CircCCDC7(15,16,17,18,19)* suppresses the cell viability,**
 69 **migration, and invasion of DU145 by its encoded protein.** (a) Colony formation assay
 70 measuring cell viability in DU145 cells when different plasmids were transfected. (b-c)
 71 Representative images of migration and invasion assays using DU145 after different plasmids
 72 transfection. (d-e) Histogram analysis of migrated and invaded cell counts. (f) Representative
 73 image of the western blotting analysis of Slug, E-cad, and Claudin protein levels after different
 74 plasmids transfection. *** $p < 0.001$.

75

Supplementary Figure 9



76

77 **Supplementary Figure 9. *FLRT3* is a potential downstream of *circCCDC7(15,16,17,18,19)*.**

78 (a-b) Relapse-free survival analysis of PLXDC1 (a) and COL10A1 (b) in TCGA. (c-d)

79 Quantitative analysis expression of *FLRT3* in PC3 and DU145 when

80 *circCCDC7(15,16,17,18,19)* was overexpressed. (e) Representative image of the western

81 blotting analysis of *FLRT3* protein levels when *circCCDC7(15,16,17,18,19)* was

82 overexpressed in DU145 and PC3. The grayscale ratio of *FLRT3* to GAPDH was listed on top

83 of the lanes. (f-g) Quantitative analysis expression of *FLRT3* in wide-type PC3 and DU145

84 when treated with supernatant from *circCCDC7(15,16,17,18,19)* overexpressed 293T cells. (h)

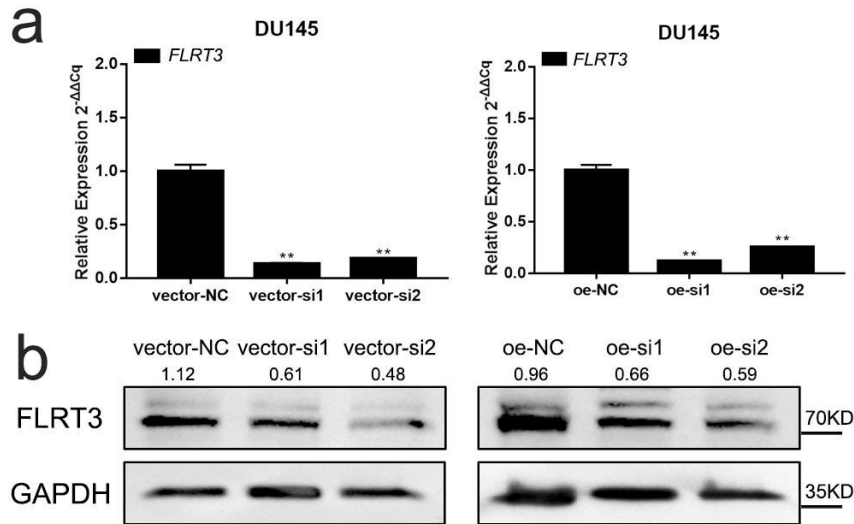
85 The correlation between *circCCDC7(15,16,17,18,19)* and *FLRT3* in CPGEA dataset. (i)

86 Photomicrographs of the immunohistochemical staining of *FLRT3* in the Proteintlas. * $p <$

87 0.05, *** $p < 0.001$.

88

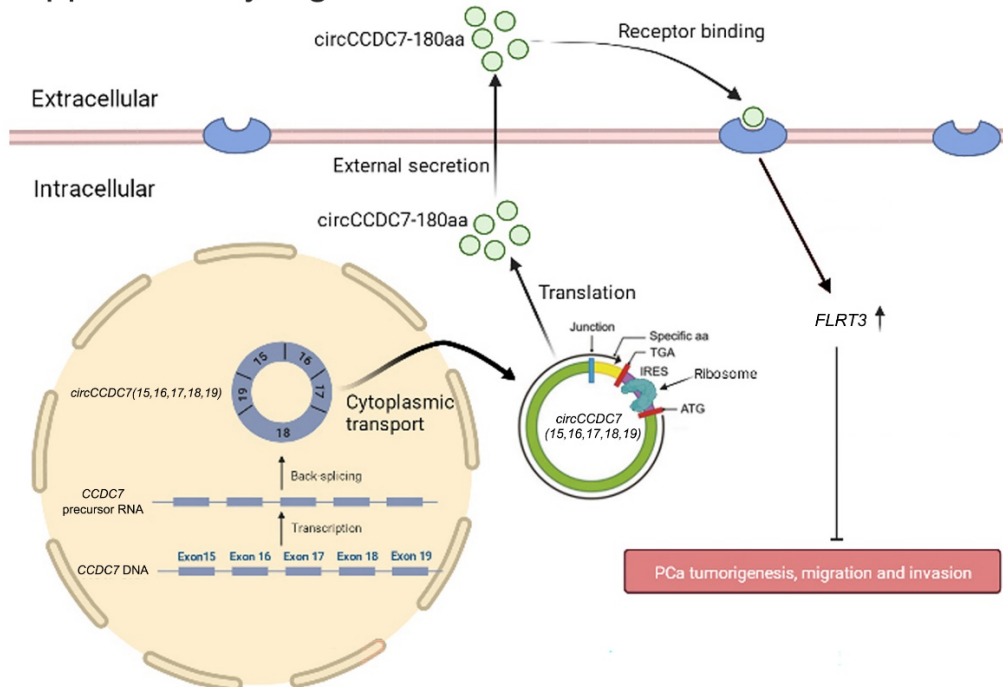
Supplementary Figure 10



89

90 **Supplementary Figure 10.** RT-qPCR (a) and western blot (b) analysis of FLRT3 expression
 91 levels in *FLRT3*-knockdown cells with *circCCDC7(15,16,17,18,19)* overexpressing combined
 92 with *FLRT3* knocking down. The grayscale ratio of FLRT3 to GAPDH was listed on top of the
 93 lanes. ** $p < 0.01$.

Supplementary Figure 11



94

95 **Supplementary Figure 11.** Schematic diagram of *circCCDC7(15,16,17,18,19)* influencing
 96 prostate cancer.

97 **Supplementary Table 1. Prognostic value of *circCCDC7(15,16,17,18,19)* expression for**
 98 **the survival by Cox proportional hazards model.**

Variable	HR (95% CI)	<i>p</i>
Univariable analysis		
Age (≥ 70 vs. < 70)	1.404 (0.717-2.751)	0.323
Gleason score (< 7 vs. ≥ 7)	0.039 (0.000-3.856)	0.166
Tumor stage (T2 vs. T3–T4)	0.561 (0.282-1.114)	0.099
Lymph node stage (N0 vs. N1)	0.369 (0.166-0.820)	0.014*
PSA (< 20 vs. ≥ 20)	0.250 (0.113-0.552)	0.001***
Bone metastasis (Yes vs. No)	0.335 (0.117-0.957)	0.041*
Distant metastasis (M0 vs. M1)	0.553 (0.192-1.597)	0.274
Seminal Vesical (Yes vs. No)	0.338 (0.164-0.695)	0.003**
Surgical Margin (Yes vs. No)	0.540 (0.275-1.062)	0.074
<i>circCCDC7</i> (Low vs. High)	2.612 (1.070-6.378)	0.035*
Multivariable analysis		
Gleason score (< 7 vs. ≥ 7)	0.000 (0.000-)	0.977
Lymph node stage (N0 vs. N1)	0.500 (0.211-1.187)	0.116
PSA (< 20 vs. ≥ 20)	0.445 (0.186-1.063)	0.068
Bone metastasis (Yes vs. No)	0.341 (0.106-1.091)	0.070
Seminal Vesical (Yes vs. No)	0.433 (0.192-0.979)	0.044*
<i>circCCDC7</i> (Low vs. High)	2.855 (1.132-7.354)	0.026*

99 Cut off value of *circCCDC7(15,16,17,18,19)* score: 10% low expression vs 90% high expression; PSA,
 100 prostate-specific antigen; T, tumor; N, node; M, metastasis. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

101 Note: Univariable Cox regression using the log transformed *circCCDC7(15,16,17,18,19)* did not result in a
 102 significant result.

103

104 **Supplementary Table 2. Correlation of *FLRT3* expression with clinico-pathologic**
 105 **characteristics of PCa patients in TCGA database.**

Clinical features	Total patients, <i>n</i>	Low, <i>n</i> (%)	High, <i>n</i> (%)	<i>P</i>
Age, years				0.745
≤65	354	175 (49.4%)	179(50.6%)	
>65	143	73 (51%)	70 (49%)	
Gleason score				0.012*
≤7	292	132 (45.2%)	160 (54.8%)	
>7	205	116 (56.6%)	89 (43.4%)	
Serum PSA levels, ng/ml				0.13
≤4	413	198 (47.9%)	215 (52.1%)	
>4	27	17 (63%)	10 (37%)	
T stage				0.364
T1-T2	187	89 (47.6%)	98 (52.4%)	
T3-T4	303	157 (51.8%)	146 (48.2%)	
Lymph node metastasis				0.178
N0	346	168 (48.6%)	178 (51.4%)	
N1	79	45 (57%)	34 (43%)	
Distant metastasis				0.258
M0	454	229 (50.4%)	225 (49.6%)	
M1	3	3 (100%)	0 (0%)	

106 Cut off value of *FLRT3* score: medium; PSA, prostate-specific antigen; T, tumor; N, node; M,
 107 metastasis. **P* < 0.05.

108

109

110 **Supplementary Table 3. Calculation of IHC score of FLRT3 in Proteinatlas.**

Patient	Grade	intensity	Quantity	Score
1	Low	2	3	6
2	Low	2	3	6
3	Low	2	2	4
4	Low	2	2	4
5	High	1	3	3
6	High	1	1	1
7	High	1	2	2
8	High	2	3	6
9	High	0	0	0
10	High	1	1	1
11	High	1	2	2
12	High	1	3	3
13	High	1	2	2
14	High	2	3	6
15	High	0	0	0
16	High	1	1	1
17	High	2	2	4
18	High	2	2	4

111

112 **Supplementary Table 4. The sequences of primers used in this study.**

NO.	Target	Forward (5'-3')	Reverse (5'-3')
1	Fusion <i>CCDC7: 19-15</i>	ACAAACTTACCAAGCAGCAG	AGCCTGTTTCAATTGCTCCT
2	Fusion <i>CCDC7: 19-13</i>	ACAAACTTACCAAGCAGCAG	CACAGCTTTCTTTGTTTCGAT
3	Linear <i>CCDC7</i>	TCCGTACAACGTCAAGAAGG	AAAGCTTGGACCAGCCCTAT
4	Divergent <i>CCDC7: 19-15</i>	AATCTCAAGAAAGTAGCACGTC	CTGCTGCTTGGTAAGTTTGTT
5	All isoforms of <i>circCCDC7(15,16,17,18,19)</i>	CAAGATTCACTGTCTGTTGG	AGCCTGTTTCAATTGCTCCT
6	Specific isoform of <i>circCCDC7(15,16,17,18,19)</i>	AACGATCTAGCCCTGCTATT	AGCCTGTTTCAATTGCTCCT
7	<i>circFBWX7</i>	CAGTGTCACGAACTCCAGTA	CACATTCCTCTGACCCAGTA
8	<i>circITCH</i>	AAGGAGCAATGCAGCAGTTT	AGTCACAACACTACTTCTTCAACCCAT
9	<i>FLRT3</i>	ACAGTGTATCCTGCCCAAGG	AGCCAGCTGAGTCTCAAAGC
10	<i>GAPDH</i>	CAAGGCTGAGAACGGGAAG	TGAAGACGCCAGTGGACTC

113

114