Supplementary material

1. Supplementary Figures

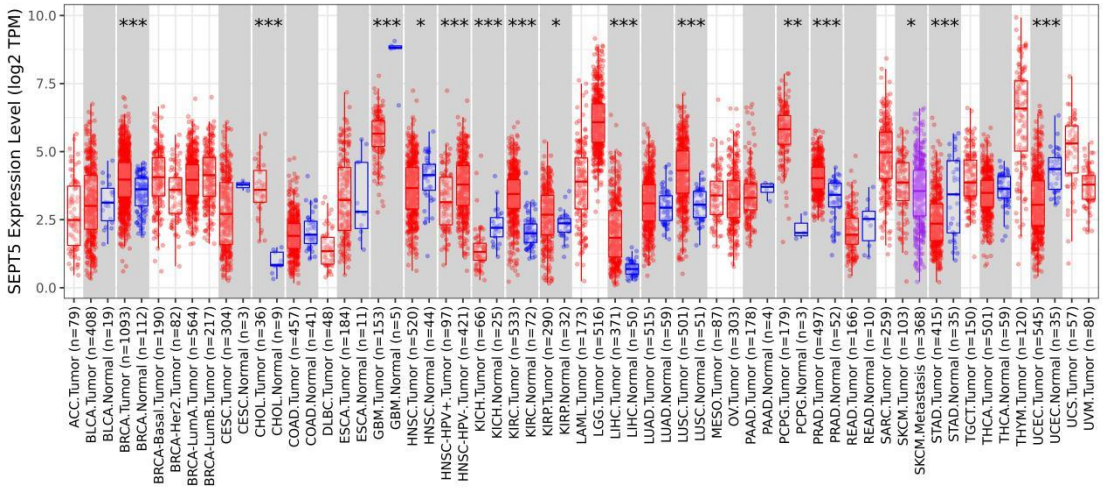

Fig. S1. Aberrant expression of SEPT5 in tumor based on TCGA dataset.

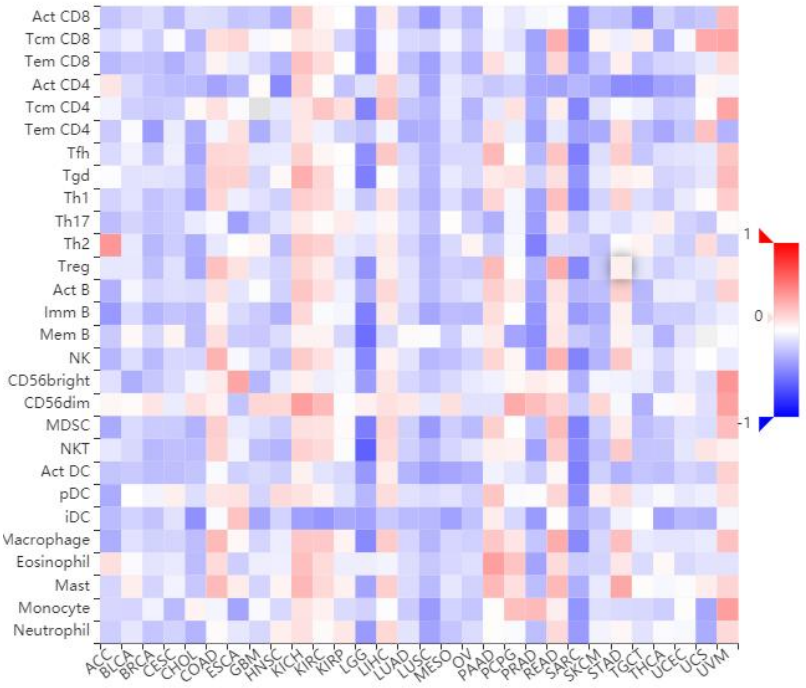

Fig. S2. Correlation between SEPT5 expression and 28 types of immune cell infiltration.

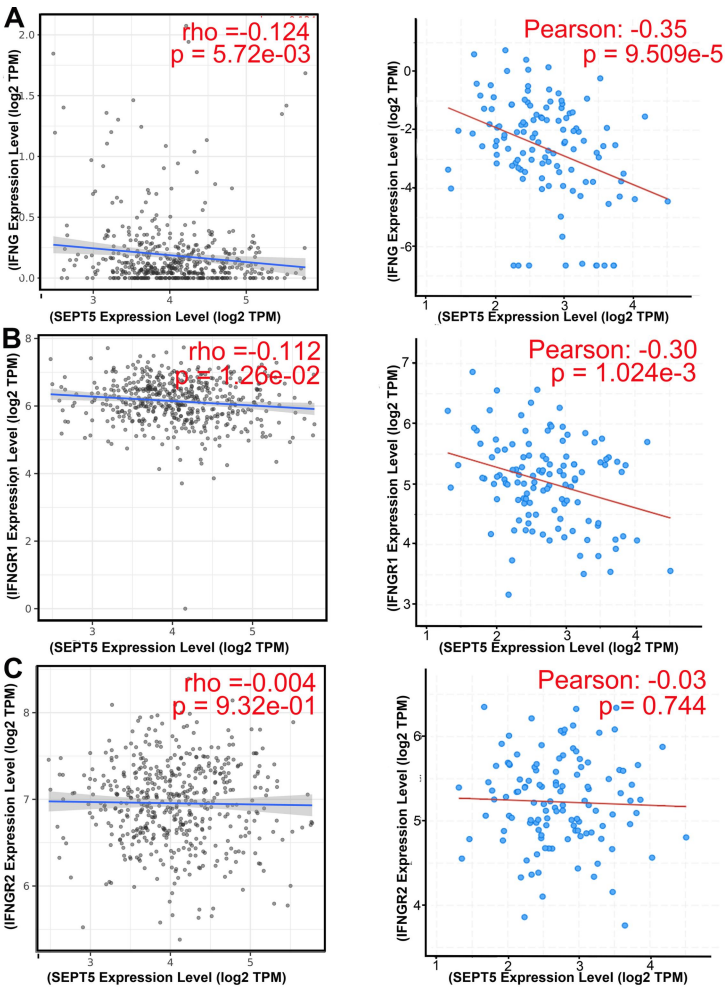

Fig. S3. SEPT5 expression may be significantly negatively correlated with IFN-γ/IFN-γR axis in PCa. The correlation between the IFNG (A), IFNGR1(B), IFNGR2 (C) expression and SEPT5 expression in PCa tissues was analyzed based on TCGA dataset and DKFZ dataset.

2. Supplementary Tables

Table. S1 Antibodies used in this study

Assay	Antibody	Company	Cat#	Dilution	Metal-labeled
WB	SEPT5	Proteintech	11631-1-AP	1:2000	
	HRP-conjugated Tubulin	Proteintech	HRP-66031	1:5000	
	IFNGR1	Proteintech	10808-1-AP	1:1000	
IHC	SEPT5	Proteintech	11631-1-AP	1:200	
	Ki67	Abcam	ab15580	1:400	
	CD8a	Sino Biological	50389-T26	1:200	
	Granzyme A	Proteintech	11288-1-AP	1:100	
	CD8	Proteintech	66868-1-Ig	1:20000	
CyTOF	CD45	Fluidigm Sciences	3089005B	1:100	89Y
	B220	Fluidigm Sciences	3144011B	1:100	144Nd
	BCMA	R&D Systems	AF593	1:200	166Er
	CD3	Fluidigm Sciences	3152004B	1:100	152Sm
	CD4	Fluidigm Sciences	3145002B	1:100	145Nd
	CD8a	Fluidigm Sciences	3168003B	1:100	168Er
	CD335	Fluidigm Sciences	3153006B	1:100	153Eu
	CD326	Fluidigm Sciences	3165014B	1:100	165Ho
	CD11b	Fluidigm Sciences	3172012B	1:100	172Yb
	Gr1	Fluidigm Sciences	3141005B	1:100	141Pr
	F4/80	Fluidigm Sciences	3146008B	1:100	146Nd

Table S2. qPCR primers used in this study.

Species	Name		Sequence(5' - 3')
Mus musculus	**SEPT5**	Forward	GAAAGGTTTCGACTTCACGCT
		Reverse	CCGGTCCTTATACAGGTCGGT
	Actin	Forward	CGTTGACATCCGTAAAGACC
		Reverse	AACAGTCCGCCTAGAAGCAC
	CCL5	Forward	GCTGCTTTGCCTACCTCTCC
		Reverse	TCGAGTGACAAACACGACTGC
	CXCL5	Forward	TCCAGCTCGCCATTCATGC
		Reverse	TTGCGGCTATGACTGAGGAAG
	CXCL9	Forward	TCCTTTTGGGCATCATCTTCC
		Reverse	TTTGTAGTGGATCGTGCCTCG
	CXCL10	Forward	CCAAGTGCTGCCGTCATTTTC
		Reverse	GGCTCGCAGGGATGATTTCAA
	IFNGR1	Forward	CTTGAACCCTGTCGTATGCTGG
		Reverse	TTGGTGCAGGAATCAGTCCAGG
Homo sapiens	**SEPT5**	Forward	CGCATCAGCCAGACGGTAG
		Reverse	CCGCTCTCATCACGGAAGT
	Actin	Forward	CATGTACGTTGCTATCCAGGC
		Reverse	CTCCTTAATGTCACGCACGAT
	CCL5	Forward	TGTACTCCCGAACCCATTTC
		Reverse	TACACCAGTGGCAAGTGCTC
	CXCL5	Forward	AGCTGCGTTGCGTTTGTTTAC
		Reverse	TGGCGAACACTTGCAGATTAC
	CXCL9	Forward	CCAGTAGTGAGAAAGGGTCGC
		Reverse	AGGGCTTGGGGCAAATTGTT
	CXCL10	Forward	GTGGCATTCAAGGAGTACCTC
		Reverse	TGATGGCCTTCGATTCTGGATT
	IFNGR1	Forward	GAGACGAGCAGGAAGTCGAT
		Reverse	CATCTTCCTTCTGCGTGAGT

Table S3. Cell_abundance between SEPT5 low group and SEPT5 high group based on TCGA dataset

Cell type	Cell_abundance	
	SET5_low	SET5_high
Act_CD8	-0.004 ± 0.02082	-0.0241 ± 0.02246
Tcm_CD8	**0.076 ± 0.01521**	**-0.0671 ± 0.01583**
Tem_CD8	**0.115 ± 0.02451**	**-0.0835 ± 0.02514**
Act_CD4	**0.0833 ± 0.02362**	**-0.1086 ± 0.02216**
Tcm_CD4	**0.0706 ± 0.01506**	**-0.061 ± 0.01537**
Tem_CD4	**0.065 ± 0.01352**	**-0.0604 ± 0.01322**
Tfh	**0.065 ± 0.01775**	**-0.061 ± 0.0179**
Tgd	**0.0277 ± 0.01216**	**-0.0293 ± 0.01332**
Th1	**0.0983 ± 0.01833**	**-0.714 ± 0.01814**
Th17	**0.099 ± 0.01494**	**-0.0603 ± 0.0158**
Th2	**0.1268 ± 0.01607**	**-0.0918 ± 0.01696**
Treg	**0.0829 ± 0.02545**	**-0.0835 ± 0.02419**
Act_B	**0.1214 ± 0.02723**	**-0.1331 ± 0.02605**
Imm_B	**0.129 ± 0.02488**	**-0.1397 ± 0.02494**
Mem_B	**0.1312 ± 0.02043**	**-0.0978 ± 0.01952**
NK	**0.1156 ± 0.01675**	**-0.0785 ± 0.01675**
CD56bright	-0.0046 ± 0.0117	0.0085 ± 0.01162
CD56dim	**-0.0662 ± 0.0164**	**0.0629 ± 0.01563**
MDSC	**0.0791 ± 0.02815**	**-0.082 ± 0.02901**
NKT	**0.097 ± 0.01828**	**-0.808 ± 0.01907**
Act_DC	**0.097 ± 0.01828**	**-0.0808 ± 0.01907**
pDC	0.0124 ± 0.01055	0.005 ± 0.01047
iDC	**0.0876 ± 0.01358**	**-0.0641 ± 0.01327**
Macrophage	**0.0606 ± 0.01936**	**-0.0451 ± 0.01974**
Eosinophil	**0.1015 ± 0.0218**	**-0.0755 ± 0.01979**
Mast	**0.0846 ± 0.02175**	**-0.0497 ± 0.02274**
Monocyte	**-0.0622 ± 0.01598**	**0.0384 ± 0.01469**
Neutrophil	**0.0702 ± 0.02055**	**-0.0536 ± 0.01872**

Table S4. Correlation analysis between SEPT5 expression and chemokine-related gene expression based on TCGA dataset

infiltrates	rho	p value
CCL1	0.015977186	0.722086048
CCL2	**-0.204376803**	**4.27E-06**
CCL3	-0.068212787	0.128466639
CCL4	**-0.143145713**	**0.001360468**
CCL5	**-0.234116824**	**1.26E-07**
CCL7	**-0.129669961**	**0.003747382**
CCL8	-0.084497647	0.059527838
CCL11	-0.082055452	0.067305707
CCL13	**-0.088463349**	**0.048489957**
CCL14	**-0.145979336**	**0.001086963**
CCL15	-0.064404097	0.15125439
CCL16	-0.012228713	0.785453398
CCL17	**-0.139425697**	**0.001815648**
CCL18	-0.072704525	0.105117208
CCL19	**-0.166191004**	**0.000195066**
CCL20	**-0.247769253**	**2.11E-08**
CCL21	**-0.136146866**	**0.002328384**
CCL22	**-0.213979971**	**1.44E-06**
CCL23	**-0.129244522**	**0.003863609**
CCL24	-0.028597049	0.524323003
CCL24	-0.028597049	0.524323003
CCL25	**0.089406234**	**0.046134235**
CCL26	0.0485633	0.279408381
CCL27	0.055548734	0.215915883
CCL28	**-0.237089442**	**8.60E-08**
CXCL1	**-0.237999767**	**7.65E-08**
CXCL2	**-0.235817348**	**1.01E-07**
CXCL3	**-0.24882309**	**1.83E-08**
CXCL5	**-0.367121668**	**2.46E-17**
CXCL6	**-0.346495371**	**1.71E-15**
CXCL9	**-0.098625075**	**0.027754034**
CXCL10	**-0.104057865**	**0.020199614**
CXCL11	-0.060134572	0.180310909
CXCL12	**-0.205399332**	**3.81E-06**
CXCL13	**-0.202369621**	**5.32E-06**
CXCL14	-0.047713813	0.287914873
CXCL16	**-0.128477216**	**0.004081516**
CXCL17	**-0.292130185**	**2.96E-11**
CX3CL1	**-0.26506952**	**1.88E-09**

Table S5. Correlation analysis between SEPT5 expression and cytokine-related gene expression based on TCGA dataset

infiltrates	rho	p value
IL1A	-0.051261379	0.253528582
IL1B	**-0.18990156**	**1.99E-05**
IL2	**-0.092237493**	**0.039630529**
IL3	0.060205925	0.179793535
IL4	0.044112995	0.325889888
IL5	-0.013208842	0.76872775
IL6	**-0.178510246**	**6.18E-05**
IL7	**-0.319684483**	**2.70E-13**
IL8	**-0.257158617**	**5.80E-09**
IL9	-0.031261827	0.486395477
IL10	**-0.164458794**	**0.000227849**
IL11	**0.112313257**	**0.012141172**
IL12A	**-0.145143708**	**0.001161822**
IL12B	**-0.119733346**	**0.007475749**
IL13	-0.019306572	0.667340283
IL15	**-0.212543824**	**1.70E-06**
IL16	**-0.21725399**	**9.85E-07**
IL17A	**-0.212738572**	**1.66E-06**
IL17B	-0.069461903	0.121602845
IL17C	0.026266678	0.558687027
IL17D	**0.169343758**	**0.000146445**
IL17F	**-0.151920628**	**0.000670192**
IL18	**-0.227929763**	**2.73E-07**
IL19	-0.079838595	0.075070021
IL20	**-0.132989237**	**0.002943891**
IL21	-0.051310818	0.253070428
IL22	**-0.124249756**	**0.005493993**
IL23A	**-0.179367583**	**5.69E-05**
IL28A	-0.001150015	0.97957706
IL28B	0.049140268	0.273728915
IL24	**-0.166592149**	**0.000188132**
IL25	**-0.130273179**	**0.003588015**
IL26	-0.085004581	0.058010297
IL27	-0.004960855	0.91206956
IL29	-0.04000638	0.372987047
IL31	**0.097932484**	**0.028873012**
IL32	**-0.10747164**	**0.016428534**
IL33	**-0.327294379**	**6.74E-14**

IL34	-0.08412544	0.060662846
TGFA	**-0.263539029**	**2.34E-09**
TGFB1	-0.022659851	0.613931618
TGFB2	**-0.31905439**	**3.02E-13**
TGFB3	**-0.238476954**	**7.19E-08**
CSF1	**-0.231401835**	**1.77E-07**
CSF2	-0.06002218	0.181128079
CSF3	**-0.180194381**	**5.25E-05**
TNF	**-0.186658672**	**2.77E-05**
IFNG	**-0.123674714**	**0.005716833**

Table S6. The expression of cytokine gene beween SEPT5-vector group and SEPT5-KD group according to RNA-seq results

Symbol	FPKM		p value
	Vector	SEPT5 knockdown	
IL1A	**0.76 ± 0.18**	**15.74 ± 2.68**	**0. 03**
IL1B	**292.39 ± 17.18**	**1802.79 ± 43.02**	**0**
IL1F10	0.01 ± 0.01	0.1 ± 0.05	0. 179
IL6	**37.54 ± 2.77**	**153.82 ± 10.99**	**0. 001**
IL7	**0.17 ± 0.04**	**0.76 ± 0.18**	**0. 032**
IL11	6.75 ± 0.71	7.82 ± 0.19	0. 218
IL12A	**0.45 ± 0.12**	**1.61 ± 0.1**	**0. 002**
IL15	**3.68 ± 0.32**	**5.17 ± 0.37**	**0. 039**
IL16	0.01 ± 0.01	0.02 ± 0.01	0. 326
IL17C	0.12 ± 0.06	0.06 ± 0.05	0. 483
IL17D	11.18 ± 0.87	11.23 ± 0.36	0. 958
IL18	**40.61 ± 1.35**	**58.99 ± 2**	**0. 002**
IL19	**0 ± 0**	**0.25 ± 0.08**	**0**
IL20	0.01 ± 0.01	0.4 ± 0.13	0. 089
IL23A	**24.82 ± 0.77**	**24.51 ± 1**	**0. 039**
IL24	**0.66 ± 0.16**	**13.16 ± 0.43**	**0. 001**
IL27	**0 ± 0**	**0.04 ± 0.02**	**0**
IL32	75.83 ± 5.21	124.47 ± 3.87	0. 158
IL33	**0.01 ± 0.01**	**0.04 ± 0.01**	**0. 002**
IL34	0.05 ± 0.05	0.04 ± 0.02	0. 116
IL36A	0.02 ± 0.02	0 ± 0	0. 904
IL36B	0.11 ± 0.09	0 ± 0	0. 374
IL36G	**0.19 ± 0.04**	**0.57 ± 0.04**	**0. 002**
IL37	**11.85 ± 0.15**	**113.2 ± 4.95**	**0**
TNF	**0.65 ± 0.06**	**4.23 ± 0.12**	**0**
CSF1	**2.68 ± 0.13**	**5.09 ± 0.58**	**0. 016**
CSF2	**29.11 ± 0.72**	**136.55 ± 4.3**	**0**
CSF3	13.5 ± 1.46	14.32 ± 1.07	0. 674
VEGFA	38.12 ± 2.15	43.34 ± 2.64	0. 2
VEGFB	**28.69 ± 1.12**	**41.53 ± 1.44**	**0. 002**
VEGFC	**15.65 ± 0.73**	**22.84 ± 0.35**	**0. 001**
TGFA	**12.38 ± 1.37**	**19.68 ± 0.45**	**0. 007**
TGFB1	32.45 ± 0.58	37.97 ± 1.33	0. 059
TGFB1I1	**4.96 ± 0.61**	**8.27 ± 0.74**	**0. 026**
TGFB2	**8.16 ± 0.52**	**15.45 ± 1.22**	**0. 005**
TGFB3	1.62 ± 0.15	1.4 ± 0.06	0. 249

Table S7. The expression of chemokine gene beween SEPT5-vector group and SEPT5-KD group according to RNA-seq results

Symbol	FPKM		
	Vector	SEPT5 knockdown	p value
CCL2	0.02 ± 0.02	0.13 ± 0.13	0.488
CCL3	0 ± 0	0.13 ± 0.05	0.058
CCL3L1	**0 ± 0**	**0.2 ± 0.06**	**0.031**
CCL4	0 ± 0	0.06 ± 0.06	0.374
CCL5	**0.47 ± 0.1**	**54.97 ± 2.19**	**0.002**
CCL17	0 ± 0	0.08 ± 0.04	0.152
CCL22	**0.03 ± 0.02**	**1.91 ± 0.14**	**0.005**
CCL24	0.51 ± 0.21	0.45 ± 0.06	0.081
CCL25	**0.02 ± 0.02**	**0.12 ± 0.02**	**0.025**
CCL26	4.67 ± 0.67	4.17 ± 0.46	0.574
CCL28	4.38 ± 0.35	4.97 ± 0.44	0.347
CX3CL1	0 ± 0	0.03 ± 0.01	0.114
CXCL1	302.82 ± 19.49	313.95 ± 24.62	0.741
CXCL2	**12.81 ± 0.81**	**15.22 ± 0.67**	**0.082**
CXCL3	**110.21 ± 5.14**	**54.42 ± 3.39**	**0.001**
CXCL5	**121.17 ± 5.56**	**199.96 ± 11.03**	**0.003**
CXCL6	46.94 ± 2.79	50.75 ± 0.95	0.266
CXCL8	**443.41 ± 26.78**	**723.56 ± 31.02**	**0.002**
CXCL9	**0 ± 0**	**0.19 ± 0.03**	**0.002**
CXCL10	**0.04 ± 0.02**	**5.51 ± 0.5**	**0.000**
CXCL11	**0.05 ± 0.03**	**3.79 ± 0.74**	**0.037**
CXCL12	0 ± 0	0.03 ± 0.03	0.423
CXCL16	**39.13 ± 0.66**	**46.98 ± 1.53**	**0.009**
CXCL17	0.11 ± 0.07	0.01 ± 0.01	0.263
XCL1	0 ± 0	0.010.01	0.374