

Supplementary Information (on-line only)

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Supplementary Table 1. List of probes ranked in the top/bottom 50 DNA methylation DiffScores (samples with TLSs vs. samples without TLSs, with the latter as the reference group) from 39 human primary ccRCC tumor samples.

TargetID	UCSC_REFGENE_NAME	DiffScore
cg15100067	ATIC	1301.614144
cg08299859		820.3664734
cg20667334	AGAP1	788.6686489
cg16142906	RFPL3	724.2632824
cg04006380	FIG4	642.3896341
cg16602091	LILRA3	624.3374438
cg22274273		617.5576039
cg26228351	KIF21B	553.2871514
cg03664889		491.3279515
cg01105403		477.2294851
cg05606115		462.9702089
cg17151385	SMAD3	454.0237124
cg01842321	HCG22	453.7813881
cg08795713		451.9770794
cg11282828		448.3965089
cg12016309	RNU6-2	447.691108
cg17714861	PRKAG2	444.0932293
cg02011981	CTTN	424.8132213
cg06377160	SNX19	422.5058272
cg09691340	SLC25A39	422.1295836
cg14544666	RASGEF1C	408.3021745
cg20186636	FLNC	407.6457571
cg05276972		407.537948
cg13177959	GCN1	406.5750242
cg08063850	ATP9A	405.9926602
cg06264882		403.0241351
cg11031044		385.5183399
cg25113237		384.5988929
cg04277055		381.4037882
cg25285484	ZNF597;NAA60	376.838534
cg01231985	GRHL3	376.0536623
cg14358839	VOPP1	371.2143676

cg07010314	KIF21B	365.2867175
cg24883219		362.416154
cg20361154	ZNF280B	362.4021652
cg23803868	SPPL2B	360.2530621
cg02653559	ZIC4	358.2228638
cg01003606		350.8387132
cg00584026	TMEM175	350.6765278
cg02939263	CORO2B	345.532211
cg20935165		344.5264852
cg21697769	AGAP1	341.0619601
cg08410921	HIST1H2AG;HIST1H2BJ	340.506429
cg26566189	PSORS1C1	339.8719359
cg07791065		337.642068
cg01921432	BARHL2	332.3119249
cg11660146	POU2F3	332.0592832
cg04123498		327.7876365
cg26734696	HS3ST4	325.916411
cg06584796	NFIC	324.4785328
cg01157718		-332.8399794
cg26509031		-335.8942505
cg11044162	ADAMTS9	-338.9855253
cg27288829	RAX2	-339.8445864
cg06412045		-340.5981513
cg11590144	STXBP5-AS1	-340.7638789
cg09533869	PGCP	-342.6529393
cg12391576	HLA-DPA1	-346.2588712
cg26146888	PWWP2A	-348.5197999
cg10313151		-348.5504221
cg26864661		-348.9912666
cg11834635	ZFYVE28	-351.4349366
cg16066354	APOBEC3D	-352.1586921
cg15391723	MAGI1	-356.1021777
cg20495843	AIM1	-357.8845554
cg12088417	RPTOR	-366.7466474
cg08942192	SLC25A24	-367.906286
cg18543270	EPB41L3	-368.3380409
cg27086157	RASA3	-368.6292444
cg06824156	LOXL2	-375.0022915

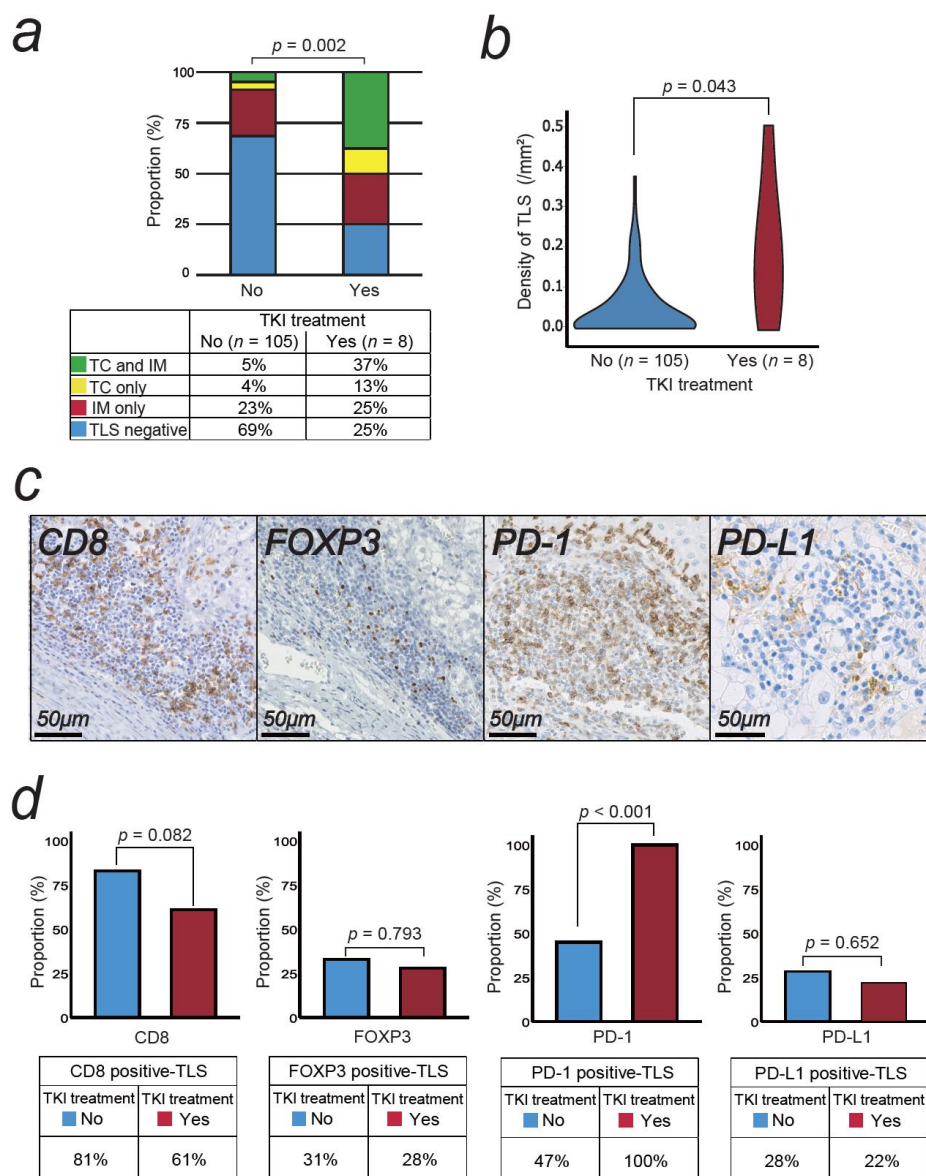
cg10536901	SLC25A24	-375.1249356
cg06568945	UBR5	-375.2251345
cg27639620	TSPAN4	-375.4455912
cg16992599		-375.5603225
cg04665049	HEATR4	-381.4605412
cg23687434		-384.7362269
cg07749655	NUBPL	-388.0252264
cg23146261		-393.7073758
cg03237181	STEAP3	-401.878064
cg04768387	PTH2R	-419.1202057
cg04312783	TDRD10	-422.1802215
cg12744031		-432.8253827
cg04054322		-433.178345
cg03462944	MIR1273H;UGDH-AS1;SMIM14	-433.5580491
cg18751375	ZNF385B	-437.7462223
cg02464073	ITGB2	-446.1953413
cg05526809	MAEA	-466.2819642
cg19303692	CARS2	-468.6063868
cg27210166	RPTOR	-478.3335249
cg14460215	APBA1	-497.410197
cg27425262	PSD4;LOC440839	-508.3051729
cg26077133	MSRA	-511.1501239
cg14851186	EFCAB1	-525.5945881
cg15852440	DNAH8	-550.1200952
cg13614887		-555.448635
cg01849514	SLC25A24	-602.9666478
cg26033510		-619.1213921
cg16771215		-712.9923852
cg02675432	PSMD14	-808.0610444
cg27024127	SCARA3	-813.6471581

Supplementary Table 2. Clinicopathological characteristics associated with TLSs in 51 human bladder tumor samples.

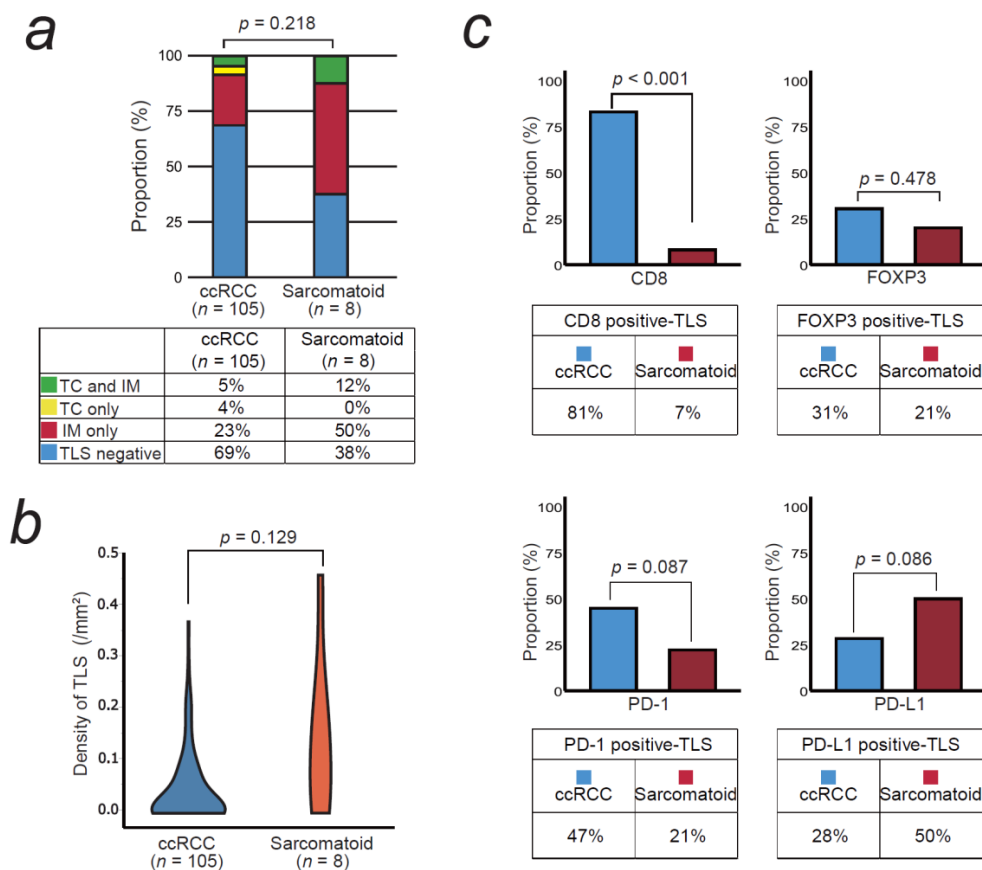
Characteristics	All patients <i>n</i> = 51	TLS negative <i>n</i> = 23	TLS positive <i>n</i> = 28	<i>p</i> value
Sex, no. (%)				0.728
Male	41 (80%)	18 (78%)	23 (82%)	
Female	10 (20%)	5 (22%)	5 (18%)	
Age at surgery, no. (%)				0.473
< 73 yr	26 (51%)	13 (57%)	13 (46%)	
≥ 73 yr	25 (49%)	10 (43%)	15 (54%)	
Pathological T stage, no. (%)				0.507
pTa/1/2	24 (47%)	12 (52%)	12 (43%)	
pT3/4	27 (53%)	11 (48%)	16 (57%)	
Tumor grade, no. (%)				0.762
Low	8 (16%)	4 (17%)	4 (14%)	
High	43 (84%)	19 (83%)	24 (86%)	
Lymphovascular invasion, no. (%)				0.802
Negative	19 (37%)	9 (39%)	10 (36%)	
Positive	32 (63%)	14 (61%)	18 (64%)	

Supplementary Table 3. Parameters associated with postoperative disease recurrence and overall mortality in bladder cancer patients after adjusting for confounding factors in the uni- and multivariate Cox regression analyses.

Variables	Disease-free survival			Overall survival		
	Univariate	Multivariate		Univariate	Multivariate	
	<i>p</i> value	HR (95% CI)	<i>p</i> value	<i>p</i> value	HR (95% CI)	<i>p</i> value
Gender (male vs female)	0.022	5.48 (1.70-17.7)	0.004	0.050		
Age at surgery (\geq 73 yr vs < 73 yr)	0.469			0.466		
Pathological T stage (pT3/4 vs pTa/1/2)	0.014	1.94 (1.08-3.46)	0.028	0.016		
Tumor grade (high vs low)	0.877			0.916		
Lymphovascular invasion (positive vs negative)	0.009			0.014	10.7 (1.31-87.5)	0.027
TLS (positive vs negative)	0.043	0.25 (0.09-0.71)	0.009	0.072		

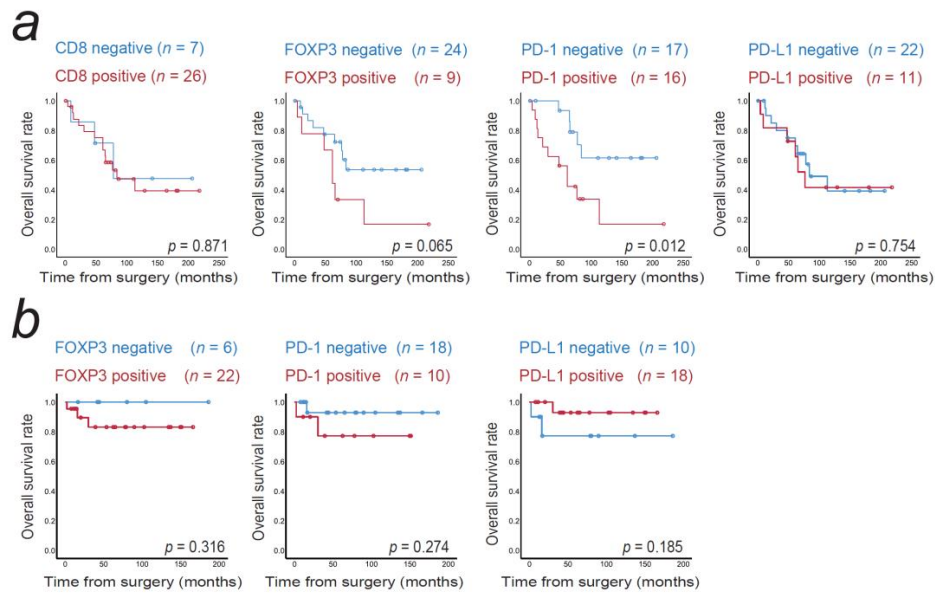


Supplementary Fig 1. Differences in TLS Features under Anti-angiogenic Treatments. **a** Differences in the TLS distribution in ccRCC tumors between COHORT A and COHORT B (with/without TKI treatment prior to surgery). The p value was obtained from the chi-square test. **b** Comparison of the tumor area occupied by TLSs in ccRCC tumors between COHORT A and COHORT B (with/without TKI treatment prior to surgery). The p value was obtained from the Mann-Whitney U test. **c** Representative image of a TLS in ccRCC with infiltration of CD8⁺/FOXP3⁺/PD-1⁺/PD-L1⁺ cells. **d** Differences in TLS status according to the infiltration of CD8⁺/FOXP3⁺/PD-1⁺/PD-L1⁺ cells in ccRCC tumors between COHORT A and COHORT B (with/without TKI treatment prior to surgery). The p value was obtained from the chi-square test.



Supplementary Fig 2. Differences in TLS Features in ccRCC Tumors with Sarcomatoid Components. a

Differences in the TLS distribution in ccRCC tumors between COHORT A and COHORT C (without/with sarcomatoid components). The p value was obtained from the chi-square test. **b** Comparison of the tumor area occupied by TLSs in ccRCC tumors between COHORT A and COHORT C (without/with sarcomatoid components). The p value was obtained from the Mann-Whitney U test. **c** Differences in TLS status according to the infiltration of CD8⁺/FOXP3⁺/PD-1⁺/PD-L1⁺ cells in ccRCC tumors between COHORT A and COHORT B (without/with sarcomatoid components). The p value was obtained from the chi-square test.



Supplementary Fig 3. Prognostic differences in tumors with CD8-, FOXP3-, PD-1-, and PD-L1-positive TLSs.

a-b Kaplan–Meier survival curves for overall survival rates in 105 ccRCC patients (**a**) and 51 bladder cancer patients (**b**) following surgery according to the CD8, FOXP3, PD-1, and PD-L1 status of the TLSs. The p value was obtained from the log-rank test.