

Supplementary Tables

Supplementary Table 1. Primers for real time RT-PCR in 21 selected genes

Genes	Primer and target sequence 5'-3'	RefSeq	Product (bp)	Tm, °C
<i>CDK1</i>	Forward: TTTTCAGAGCTTTGGGCACT Reverse: AGGCTTCCTGGTTTCCATTT	NM_001786	100	60
<i>CDKN2B</i>	Forward: CGGGGACTAGTGGAGAAGGT Reverse: CCCATCATCATGACCTGGAT	NM_078487	101	62
<i>CFB</i>	Forward: TCCTTCCGACTTCTCCAAGA Reverse: GAGTCTTCAGGGTGCTCCAG	NM_001710	121	58
<i>CKS2</i>	Forward: ACCGGCATGTTATGTTACCC Reverse: TGTGGTTCTGGCTCATGAAT	NM_001827	139	60
<i>CTGF</i>	Forward: CCGTACTCCCAAATCTCCA Reverse: GTAATGGCAGGCACAGGTCT	NM_001901	211	62
<i>CXCL1</i>	Forward: AGCTTGCCTCAATCCTGCATCC Reverse: TCCTTCAGGAACAGCCACCAGT	NM_001511	119	58
<i>CXCL3</i>	Forward: GCAGGGAATTCACCTCAAGA Reverse: GGTGCTCCCCTTGTTTCAGTA	NM_002090	172	60
<i>DTL</i>	Forward: TGGAGAAGGGTCTGAAATGG Reverse: GTGTCTGGGATGACGGACTT	NM_016448	125	60

<i>E2F7</i>	Forward: AAGGCCAAGCAGAAAACAGA Reverse: CAGCGACTCCAGCACATTTA	NM_203394	175	63
<i>EGR2</i>	Forward: ATTCTGAGGCCTCGCAAGTA Reverse: GCTTATGCCCAGTGTGGATT	NM_000399	148	60
<i>GADD45B</i>	Forward: ACAGTGGGGGTGTACGAGTC Reverse: GGATGAGCGTGAAGTGGATT	NM_015675	127	62
<i>IL36RN</i>	Forward: GAGCCAGTGAACATCATGGA Reverse: TGAGTCTGACAGGCTGATCG	NM_012275	160	60
<i>PHLDA1</i>	Forward: CTTCACTGTGGTGATGGCAGAG Reverse: CCTGACGATTCTTGTACTGCACC	NM_007350	110	60
<i>RGS16</i>	Forward: TCACACACCTGAGTCTCCACG Reverse: CAACCTCTCTTCCCGCTGG	NM_002928	52	60
<i>RHOU</i>	Forward: AGGCCTCTCTGCTACACCAA Reverse: TCAGGCACTGGCTTTTCTTT	NM_021205	215	60
<i>S1PR3</i>	Forward: TTGTGGTGAGCGTGTTTCATCGC Reverse: AGCACAGCCAACACGATGAACC	NM_005226	127	60
<i>SERPINE</i>	Forward:	NM_000602	183	58
1	GAGGTGCCTCTCTCTGCCCTCACCAACAT T Reverse: AGCCTGAAACTGTCTGAACATGTCG			
<i>SLAMF7</i>	Forward: GTGGACATTTGTCGGGAAAC	NM_0012825	198	58

	Reverse: GGCAAATAGCCTTGGTGTGT	89		
<i>TIMP1</i>	Forward: AATTCCGACCTCGTCATCAG	NM_003254	230	62
	Reverse: TGCAGTTTTCCAGCAATGAG			
<i>TREM1</i>	Forward: GTCTCCACTCCTGACTCTGAA	NM_018643	158	60
	Reverse: TAGGGTACAAATGACCTCAGC			
<i>GAPDH</i>	Forward: TTCTTTTGCGTCGCCAGCCGA	NM_002046	96	60
	Reverse: GTGACCAGGCGCCCAATACGA			

Supplementary Table 2. Summary and cox-coefficient of nineteen gene-based risk score classifier (TCA 19)

Activated regulators	Summary for genes	Activation Z-score	Cox_coef
S1PR3	Endothelial differentiation	3.5103	0.723
CDKN2B	Tumor suppressor	2.3091	0.1372
EGR2	Growth response	3.0036	0.2384
CTGF	Growth factor	2.5231	0.207
SERPINE1	Serine proteinase inhibitor	3.2005	0.2869
RGS16	Regulator of G-protein signaling	2.289	0.202

RHOU	Ras Homolog Familiy	2.4098	0.2639
TIMP1	Metallopeptidase inhibitor	2.1989	0.2308
PHLDA1	Apoptosis-associated	2.8276	0.2128
IL36RN	IL 36 receptor antagonist	2.5561	1.0845
SLAMF7	CD2 subset	-2.5065	-0.169
E2F7	Trascription factor	-3.4225	-0.752
DTL	Drosophila	-2.5213	-0.2247
CFB	Complement factor	-1.2288	-0.1539
CDK1	Cyclin-dependent kinase	-3.5526	-0.3205
CXCL1	Chemokine ligand	-3.1846	-0.1751
CXCL3	Chemokine ligand	-3.2523	-0.1714
CKS2	CDC protein kinase regulator	-4.1204	-0.437
TREM1	Triggering receptor	1.2611	0.0826

Supplementary Table 3. Antibodies used for immunohistochemistry, western blotting and immunofluorescence staining

Immunohistochemistry			
anti-SLAMF7	rabbit	1:200	Abcam, CAMBRIDGE, UK
anti-TREM1	rabbit	1:400	LSBio, Seattle, WA, USA
anti-CD68	mouse	1:500	ab955, Abcam, CAMBRIDGE, UK
anti-CD73	mouse	1:100	ab54217, Abcam, CAMBRIDGE, UK
Western blot			
anti-SLAMF7	goat	1:1000	Santa cruz biotechnology, sc-46517
anti-TREM1	rabbit	1:1000	Abcam, ab93717
anti-actin		1:10000	Bethyl Laboratories, A300-491A
Immunofluorescence imaging; primary antibody			
anti-SLAMF7	goat	1:1000	sc-46517, Santa cruz biotechnology
anti-TREM1	rabbit		11791-1-AP, proteintech, Chicago, IL, USA
anti-CD68	mouse	1:500	ab955, Abcam, CAMBRIDGE, UK
anti-CD73	mouse	1:500	ab54217, Abcam, CAMBRIDGE, UK
Immunofluorescence imaging; secondary antibody			
Flamma 488-conjugated anti-mouse IgG		RSA1141, Bioacts, Incheon, Korea	
Flamma 648-conjugated anti-rabbit IgG		RSA1261, Bioacts, Incheon, Korea	
Flamma 648-conjugated anti-goat IgG		RSA4461, Bioacts, Incheon, Korea	

Supplementary Table 4. Baseline characteristics of the 60-patient cohort

Variables	No. of patients (%) or Values as median (IQR)
	(n = 60)
Sex, female/ male	27/ 33 (45.0/ 55.0)
Age at diagnosis, years	61 (52 - 69)
Follow-up period, month	16 (9 - 24)
ASA score	
1	7 (11.7)
2	51 (85.0)
3	2 (3.3)
Family history	
Yes	10 (16.7)
No	50 (83.3)
Tumor location	
Right	16 (26.7)
Left	28 (46.7)
Rectum	16 (26.7)
Bormann type	
I/ II	39 (65.0)
III/ IV	21 (35.0)

T category		
T3		38 (63.3)
T4		22 (36.7)
N category		
N0		4 (6.7)
N1		21 (35.0)
N2		35 (58.3)
M category		
M1a		31 (51.7)
M1b		29 (48.3)
Lymphovascular invasion		
negative		20 (33.3)
positive		40 (66.7)
Perineural invasion		
negative		25 (41.7)
positive		35 (58.3)
Circumferential resection		
margin		
negative		49 (81.7)
involvement		11 (18.3)
Adjuvant chemotherapy		
FL or capecitabine		9 (15.0)

FOLFOX	22 (36.7)
FOLFIRI	13 (21.7)
Targeted agents	16 (26.7)
Adjuvant radiotherapy	9 (15.0)

IQR, interquartile range; FL, 5-fluorouracil with leucovorin; FOLFOX, combination of 5-FU and oxaliplatin; FOLFIRI, combination of 5-FU and irinotecan.

Supplementary Table 5. Quantitative real-time PCR in 10 patients with stage IV, comparing expression in normal tissue (N) and primary tumor tissue (P) for comparison of SLAMF7 and TREM1

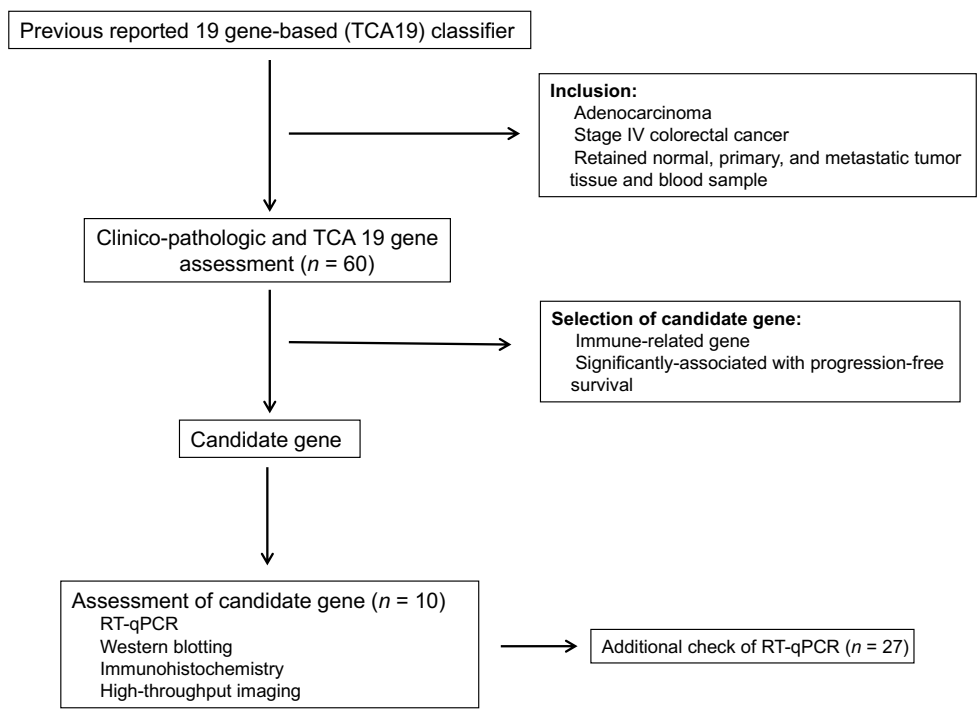
	N < P	N > P
TREM1 (n = 27)	18	9
Mean $2^{-\Delta\Delta Ct}$	34.7	0.38
SLAMF7 (n = 27)	7	20
Mean $2^{-\Delta\Delta Ct}$	20.21	0.29

Supplementary Table 6. Immunohistochemistry staining in 10 patients with stage IV colorectal cancer, comparing expression in normal tissue with primary (P) and metastatic (M) tumor tissue, and in relation to lymphovascular invasion and perineural invasion

Variables	Values as expression level in IHC (%)			
	TREM1	TREM1_	SLAMF7	SLAMF7_
	_P	M	_P	M
Expression level; normal <i>vs.</i> P or M	1 <i>vs.</i> 5.2	1 <i>vs.</i> 9.9	30 <i>vs.</i> 42	30 <i>vs.</i> 48
<i>p</i> -value	0.01	0.004	0.046	0.034
LVI compared with normal tissue, <i>p</i> -value	0.421	0.69	0.31	0.841
PNI compared with normal tissue, <i>p</i> -value	0.61	1	0.762	0.019

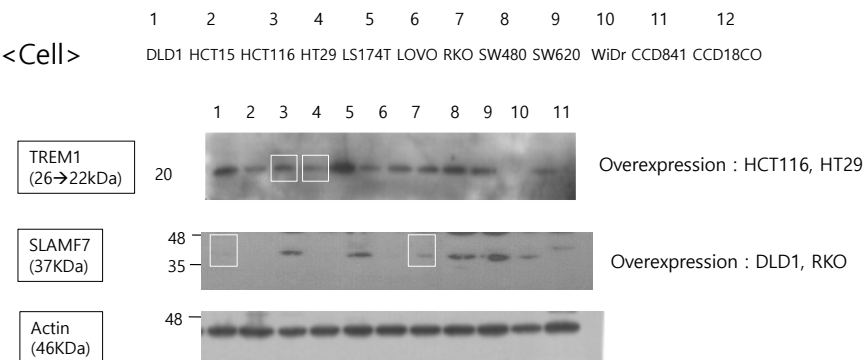
IHC, immunohistochemistry; P, primary tumor; M, metastatic tumor; LVI, lymphovascular invasion; PNI, perineural invasion; Bold, $p < 0.05$.

Supplementary Figure 1

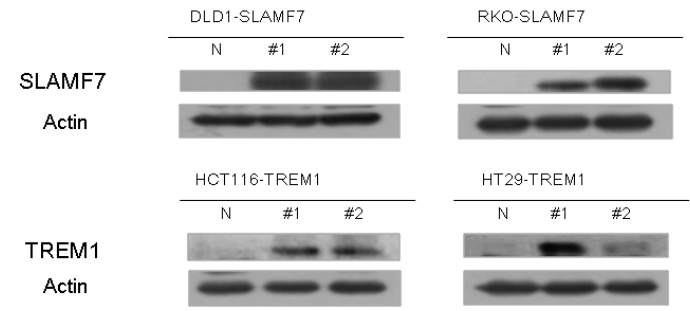


Supplementary Figure 2

A



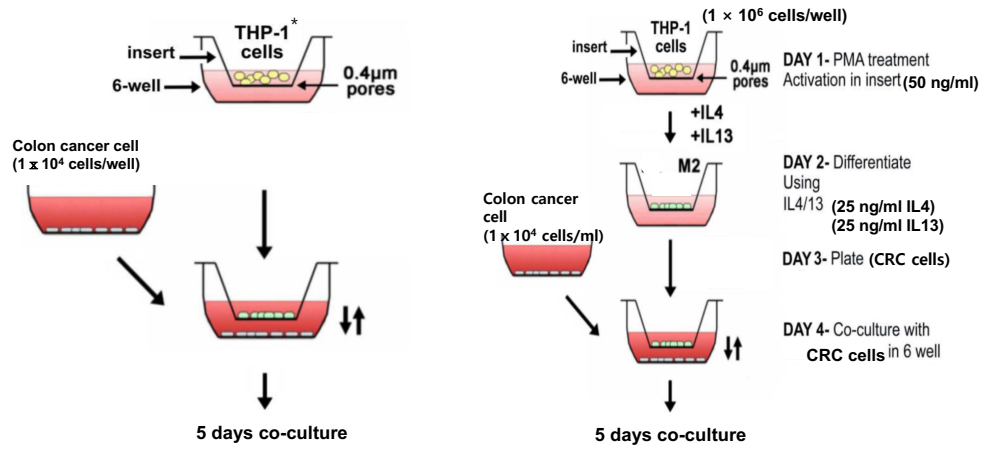
B



Supplementary Figure 3

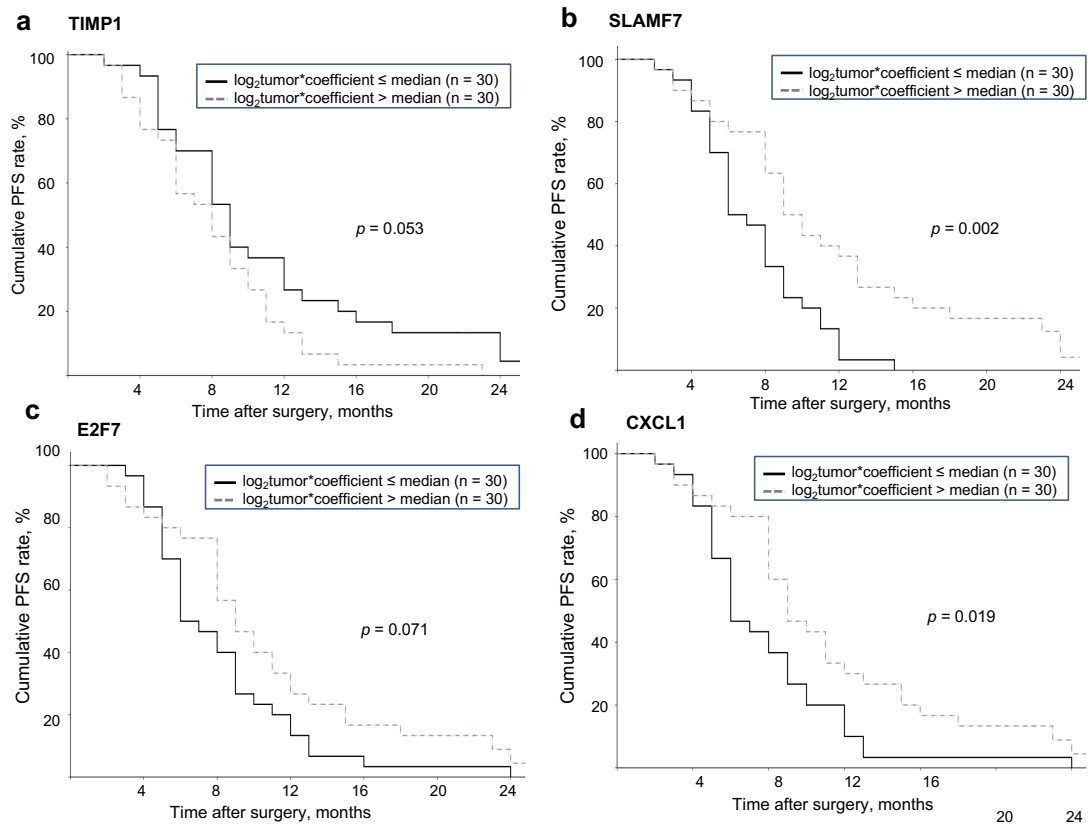
A. THP-1 (monocyte) + CRC cells co-culture

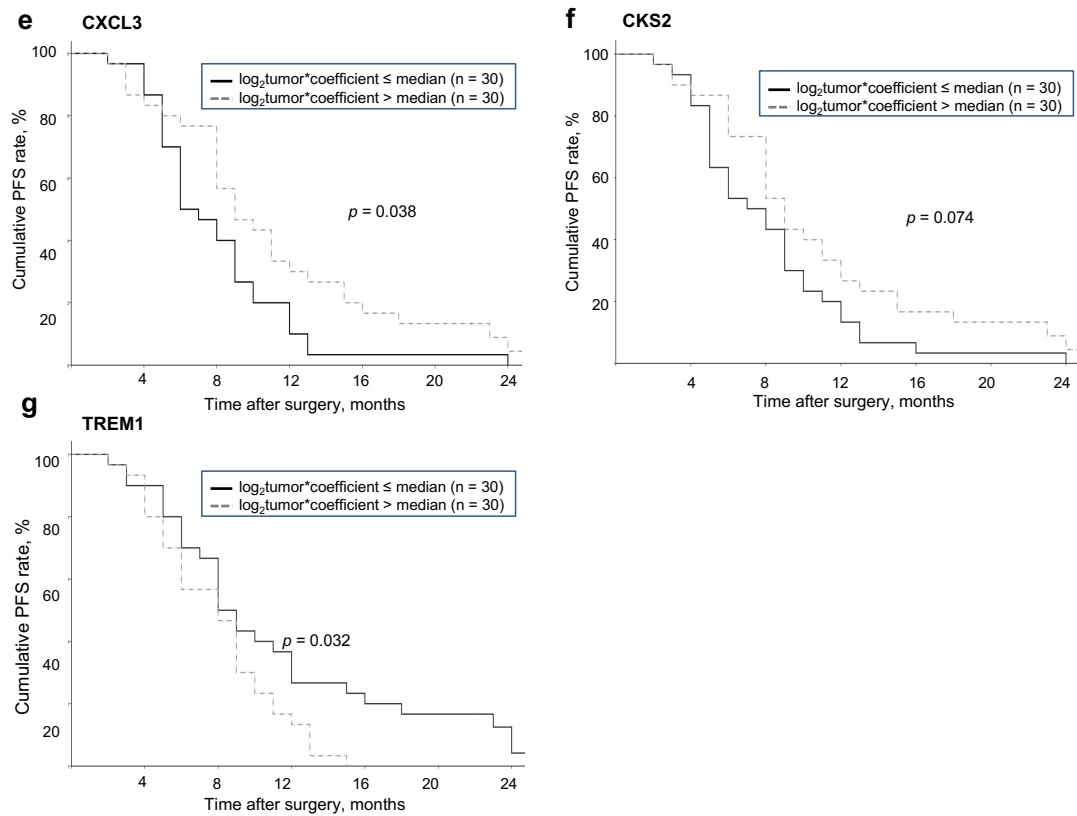
B. THP-1 (M2-macrophage) + CRC cells co-culture



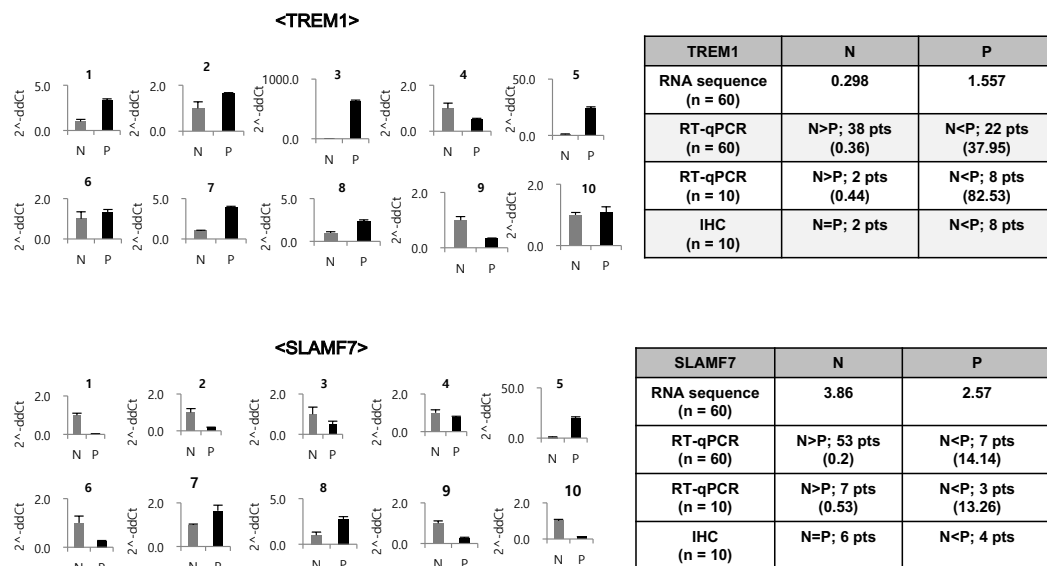
* THP-1 cells: monocytes or/ LPS (1ug/ml) activated (5 × 10⁴ cells/well)

Supplementary Figure 4

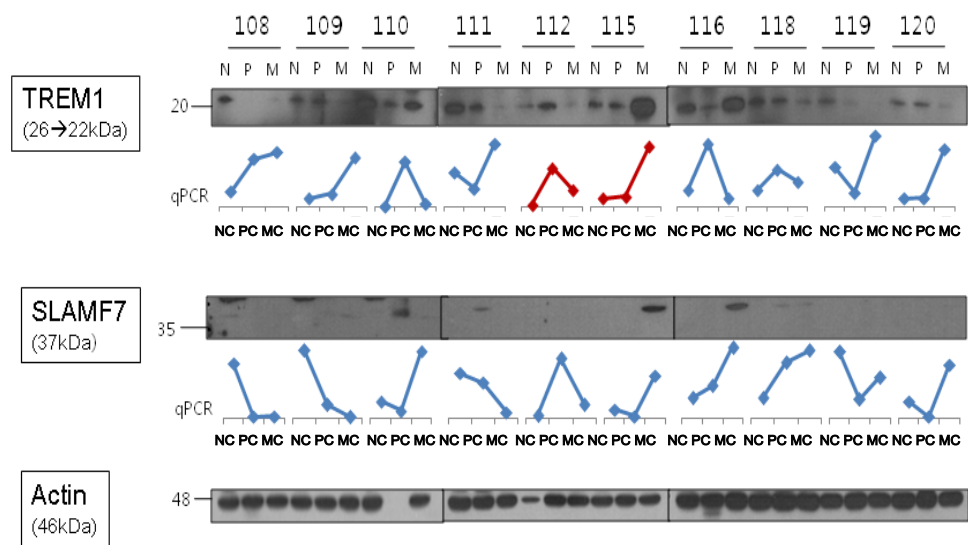




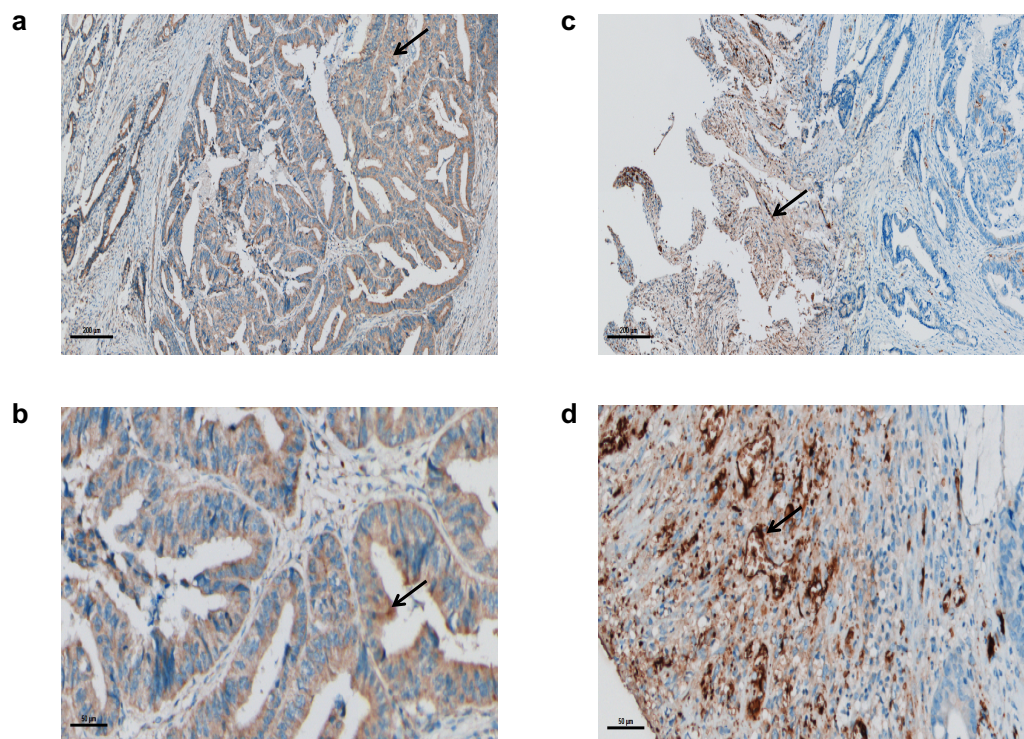
Supplementary Figure 5



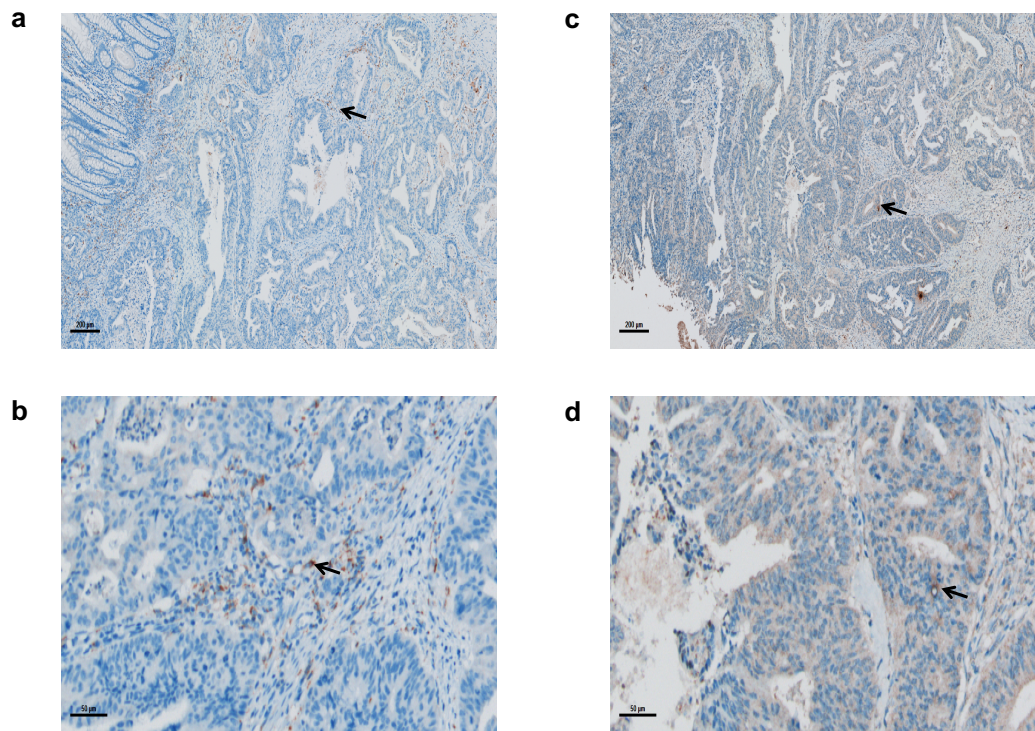
Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8



Supplementary Figure 9

