

## **Supplementary Information**

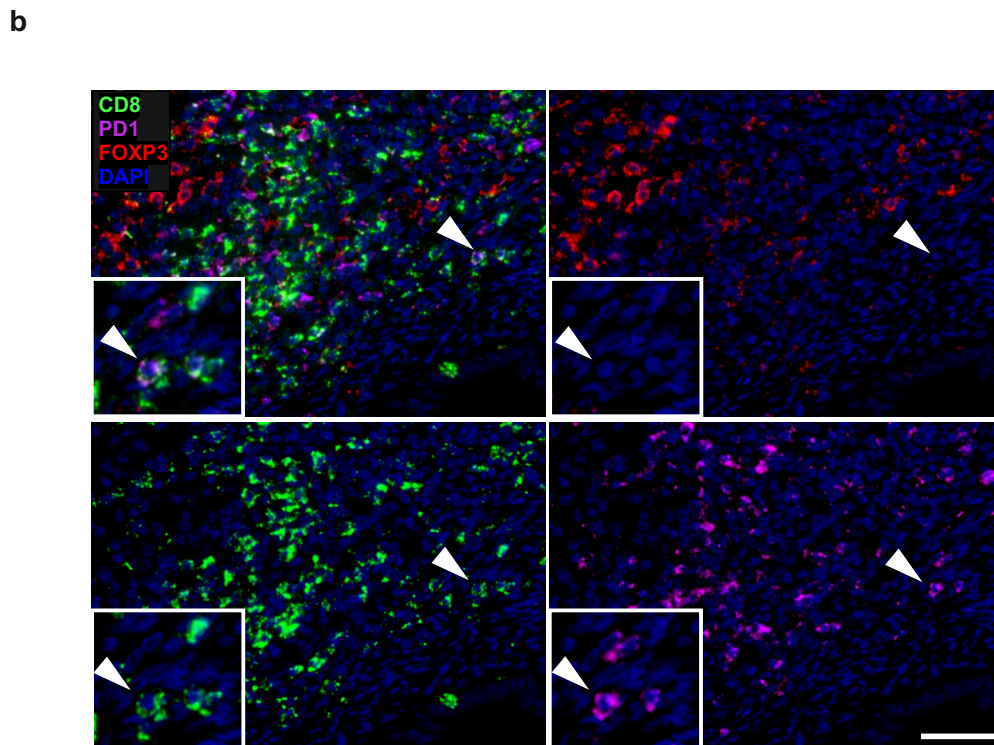
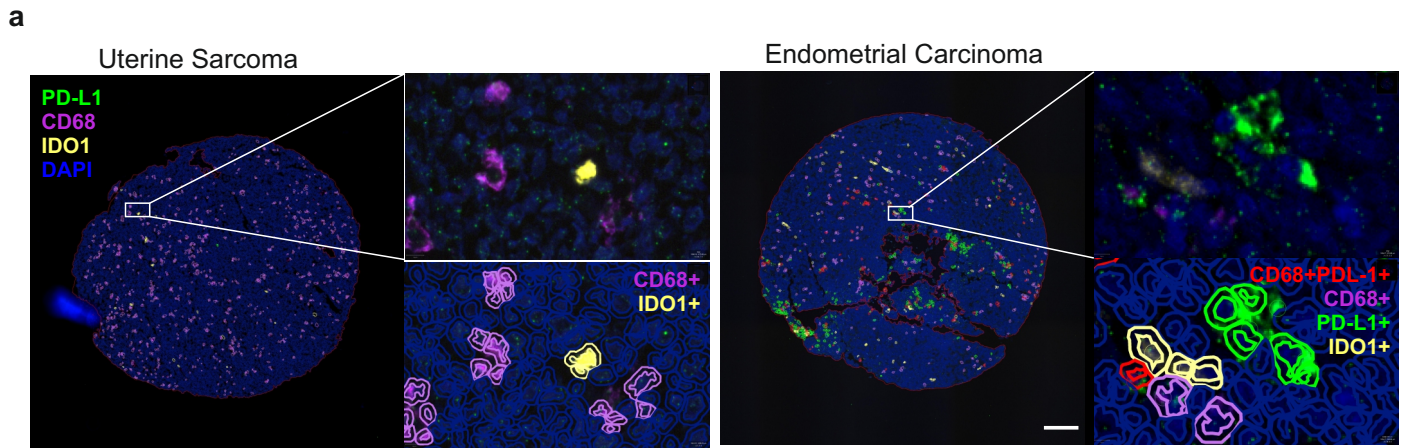
**FOXP3+ T cells in uterine sarcomas are associated with favorable prognosis, low extracellular matrix expression and reduced YAP activation**

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	Leiomyosarcoma	Low grade endometrial stromal sarcoma	Undifferentiated uterine sarcoma	YWHAE-FAM22 translocated sarcoma	Leiomyoma
<b>Number of cases</b>	13	16	26	3	13
<b>Age at diagnosis</b> (mean, years)	61.4	54.4	62.2	59.1	
<b>Stage</b>					
1	6	12	10	3	
2	0	3	3	0	
3	0	1	4	0	
4	4	0	2	0	
N/A	3	0	7	0	
<b>Time to last follow up</b> (months)	59.8	96.0	41.6	87.9	
<b>Status at last follow up</b>					
Alive	2	13	5	1	
Deceased	11	3	21	2	

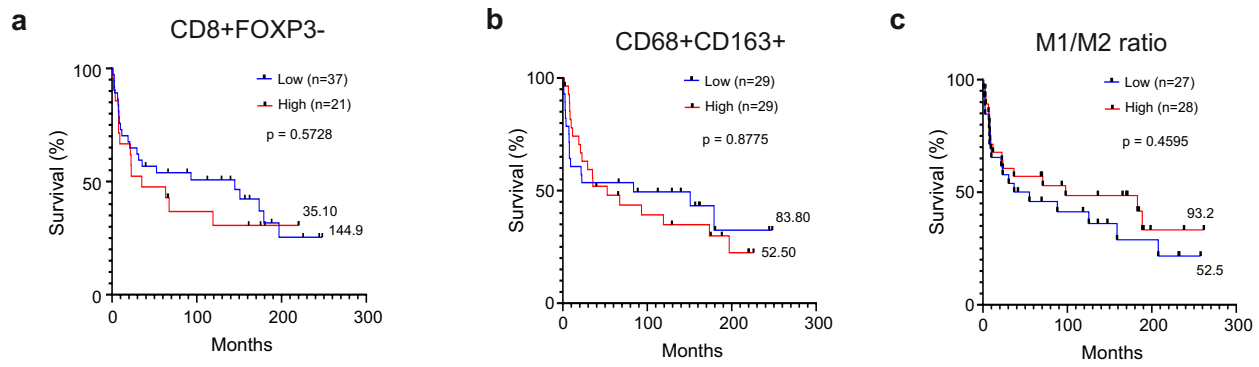
### Supplementary Table 1

Clinical Data of Cohort including age at diagnosis, disease stage at diagnosis, and survival.



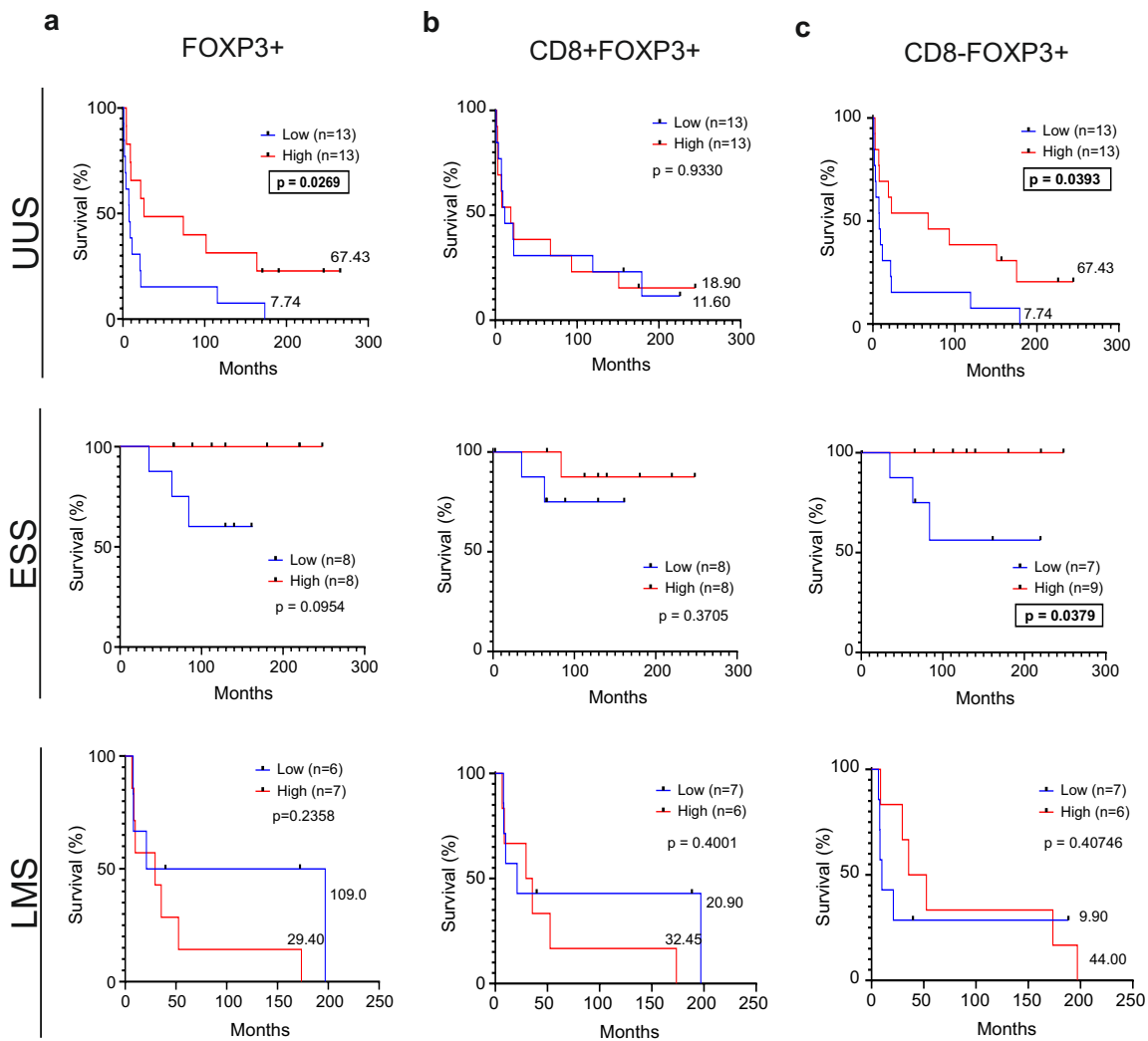
### Supplementary Figure 1

PD-L1 expression and CD8+PD1+ cells were detected in control endometrial carcinoma tissues but not in uterine sarcoma. **a**, Example images of PD-L1 staining and QuPath-based cell type detection (bottom right) showing PD-L1+ cells in endometrial carcinoma tissue. Scale bar indicates 100  $\mu\text{m}$ . **b**, Example images of endometrial carcinoma tissue presenting CD8+PD1+ cells. Arrowhead indicates an example of a CD8+PD1+ cell. Scale bar indicates 40  $\mu\text{m}$ .



## Supplementary Figure 2

Kaplan-Meier curves showing infiltration of CD8+FOXP3- (**a**), M2-like macrophages (CD68+CD163-) (**b**), and M1/M2-like macrophage ratio (**c**), which are not prognostic in uterine sarcomas. Median survival is indicated for each patient group.



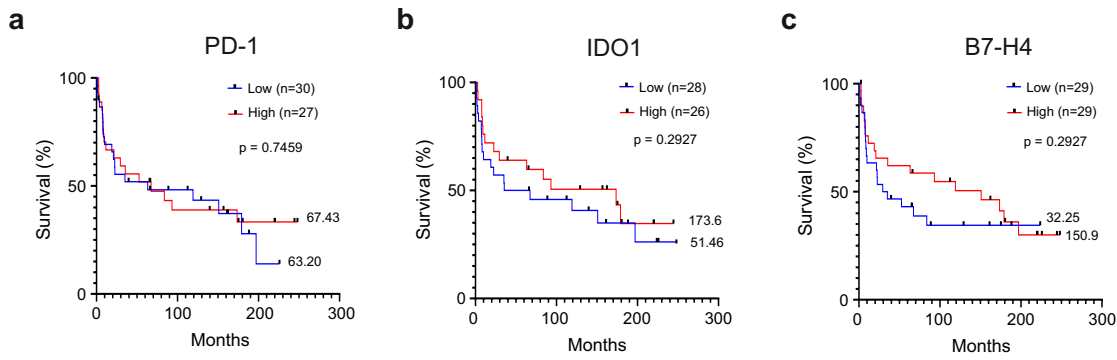
### Supplementary Figure 3

FOXP3+ cell density is associated with better survival in UUS and ESS. **a-c**, Kaplan-Meier curves showing overall survival for the indicated FOXP3+ cell subgroups in UUS, ESS, and LMS patients. Median Survival is indicated for each group.

Tumor type	Marker	Group	n	Median survival (months)	P value (log-rank)
UUS	CD68+CD163-	High	13	67.43	<b>0.0296</b>
		Low	13	7.737	
	CD68+CD163+	High	14	45.06	0.0509
		Low	12	7.582	
ESS	CD68+CD163-	High	8	Undefined	0.9863
		Low	8	Undefined	
	CD68+CD163+	High	8	Undefined	0.7377
		Low	8	Undefined	
LMS	CD68+CD163-	High	6	22.03	0.7196
		Low	7	29.4	
	CD68+CD163+	High	6	18.9833	0.1283
		Low	7	52.5	

### Supplementary Table 2

Summary of median survival and P values for patients grouped according to their M1-like or M2-like macrophage infiltration in ESS, UUS, and LMS tumors.



#### Supplementary Figure 4

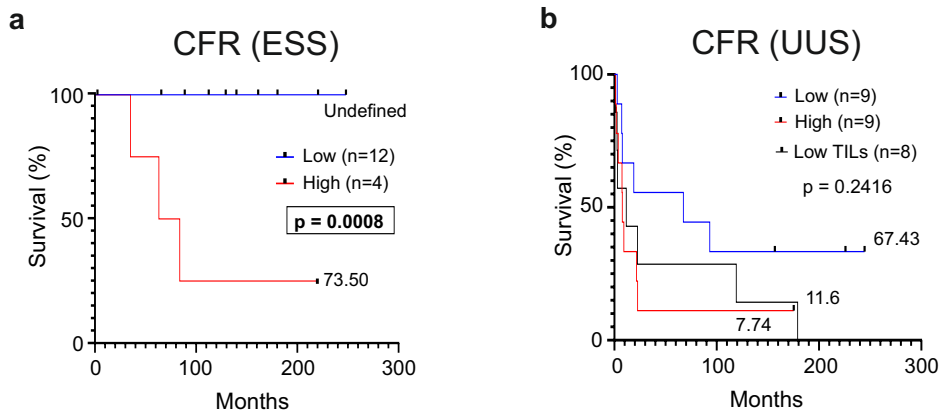
Kaplan-Meier curves of patient groups based on expression of immune regulatory proteins PD-1 (a), IDO1 (b), and B7-H4 (c) showing that the general expression of these proteins does not have prognostic value in uterine sarcomas. Median Survival is indicated for each group.

Tumor type	Marker	Group	n	Median survival (months)	P value (log-rank)
UUS	IDO1	High	12	17.15	0.2063
		Low	13	7.427	
	B7H4	High	13	93.21	0.0606
		Low	13	7.737	
	PD-1	High	13	21.59	0.7571
		Low	13	9.197	
ESS	IDO1	High	9	Undefined	0.47
		Low	7	Undefined	
	B7H4	High	8	Undefined	0.6148
		Low	8	Undefined	
	PD-1	High	7	Undefined	0.4319
		Low	9	Undefined	
LMS	IDO1	High	5	173.567	0.3577
		Low	5	9.9	
	B7H4	High	7	20.9	0.6169
		Low	7	29.4	
	PD-1	High	6	44	0.5934
		Low	7	9.9	

### Supplementary Table 3

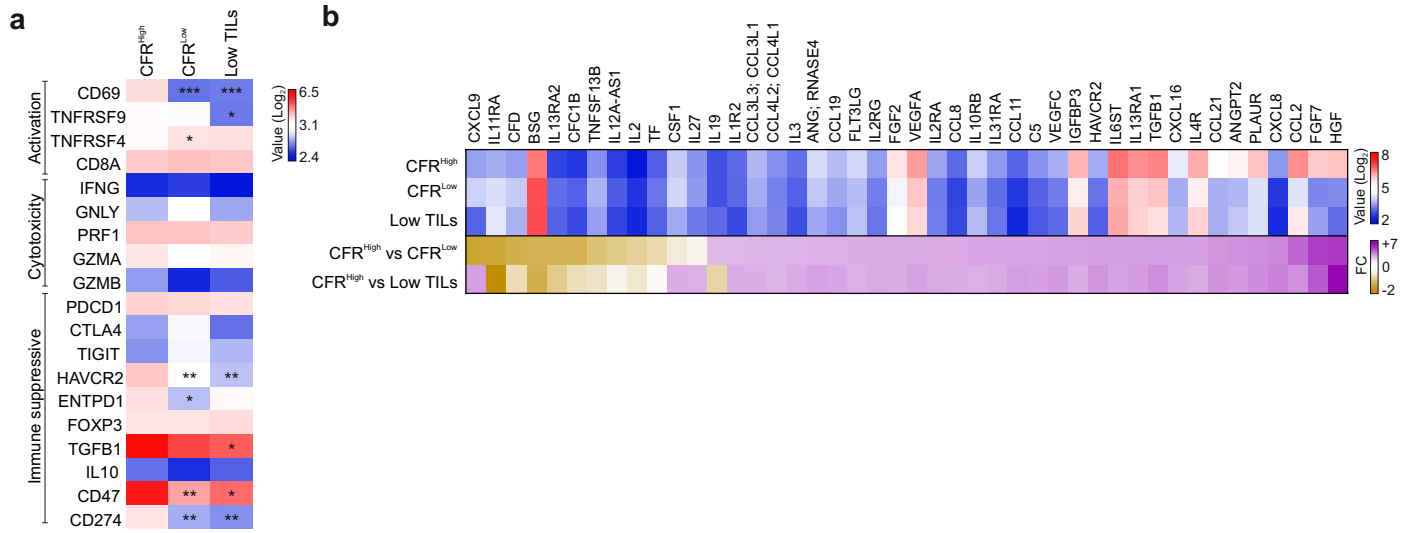
Summary of median survival and P values for patient grouped according to their immune regulatory protein expression in ESS, UUS, and LMS tumors.





### Supplementary Figure 5

High CD8+FOXP3-/FOXP3+ ratio (CFR) is associated with poor survival. Kaplan-Meier curves of ESS (a) and UUS (b) patients grouped based on their CFR. UUS tumours include a low-TIL group defined as tumors with T cell infiltration below the 40th percentile of all tumors analyzed. Median Survival is indicated for each group.

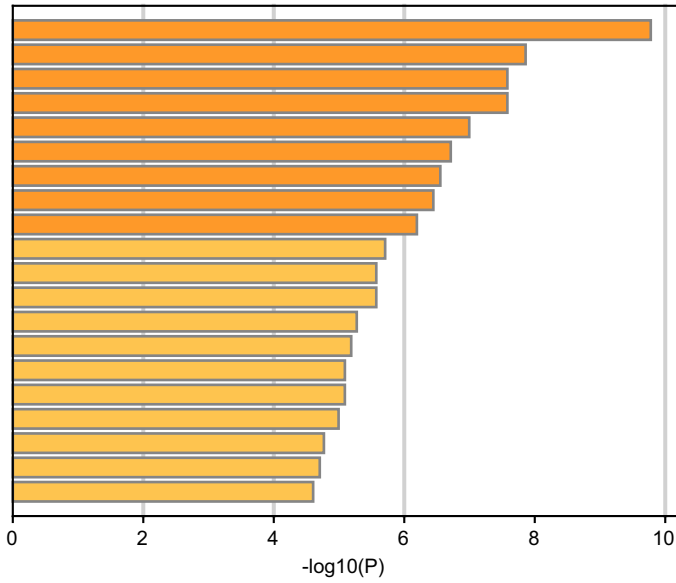


### Supplementary Figure 6

Differential expression of immune marker (a) and cytokine genes (b) between CFR<sup>High</sup> and CFR<sup>Low</sup> or Low TILs (FC, fold change). Significance is indicated as \*(P < 0.05), \*\* (P < 0.01), \*\*\* (P < 0.001)

**a**

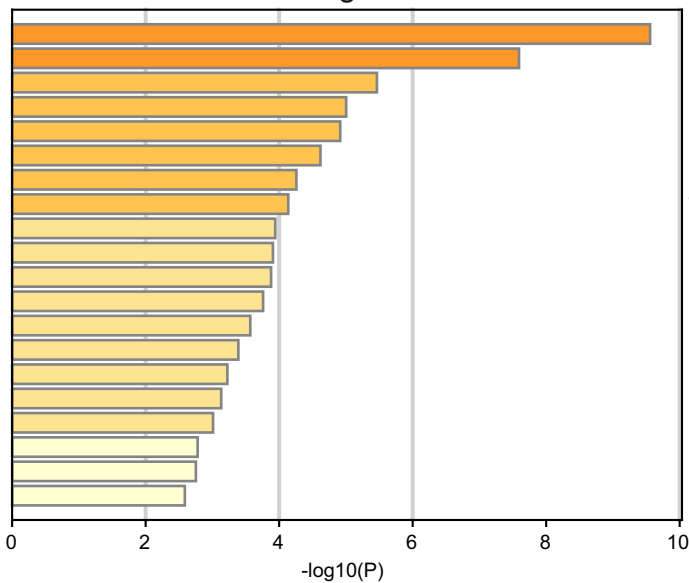
### CD8+FOXP3- High vs Low



GO:0009611: response to wounding  
GO:0030029: actin filament-based process  
ko05205: Proteoglycans in cancer  
GO:0070848: response to growth factor  
GO:0007159: leukocyte cell-cell adhesion  
GO:0006936: muscle contraction  
GO:0007507: heart development  
GO:0051345: positive regulation of hydrolase activity  
GO:0031952: regulation of protein autophosphorylation  
M5884: NABA CORE MATRISOME  
GO:0071634: regulation of transforming growth factor beta production  
GO:0045055: regulated exocytosis  
GO:0030198: extracellular matrix organization  
WP3888: VEGFA-VEGFR2 Signaling Pathway  
GO:0007229: integrin-mediated signaling pathway  
GO:0032870: cellular response to hormone stimulus  
GO:0045862: positive regulation of proteolysis  
ko04530: Tight junction  
R-HSA-109582: Hemostasis  
WP545: Complement Activation

**b**

### FOXP3+ High vs Low



R-HSA-1474244: Extracellular matrix organization  
GO:0031589: cell-substrate adhesion  
GO:0030335: positive regulation of cell migration  
ko04810: Regulation of actin cytoskeleton  
R-HSA-2022090: Assembly of collagen fibrils and other multimeric structures  
GO:0031581: hemidesmosome assembly  
GO:0000904: cell morphogenesis involved in differentiation  
WP3678: Amplification and Expansion of Oncogenic Pathways as Metastatic Traits  
GO:0001667: amoeboid-type cell migration  
GO:0051345: positive regulation of hydrolase activity  
GO:0030029: actin filament-based process  
GO:0060113: inner ear receptor cell differentiation  
GO:1900746: regulation of vascular endothelial growth factor signaling pathway  
GO:0030705: cytoskeleton-dependent intracellular transport  
GO:0050678: regulation of epithelial cell proliferation  
GO:0090066: regulation of anatomical structure size  
GO:0071634: regulation of transforming growth factor beta production  
GO:0060425: lung morphogenesis  
M92: PID ANGIOPOIETIN RECEPTOR PATHWAY  
GO:0045862: positive regulation of proteolysis

## Supplementary Figure 7

Tumors with distinct CD8+ and FOXP3+ cell infiltration show differential expression of extracellular matrix-related genes. **a**, **b**, Metascape pathway analysis reveals the most significantly altered pathways between tumors with high and low CD8+ cell infiltration (**a**), and FOXP3+ cell infiltration (**b**).

Antibody	Company	Product No.	Dilution	Technique
CD8	Dako	M 7103	1 : 100	IF
FOXP3	EuroMAbNET	236A/E7	none	IF
PDCD1 / CD279 (PD1)	HPA	HPA035981	1 : 250	IF
CD68	Dako	M 0876	1 : 100	IF
CD163	HPA	HPA046404	1 : 1600	IF
PDL1	Dako	M 3653	1 : 100	IF
IDO1	HPA	HPA023149	1 : 50	IF
VTCN1 (B7-H4)	HPA	HPA054200	1 : 70	IF
CD4	Ventana	790-4423	none	IHC
Collagen I	Abcam	ab34719	1 : 200	IHC
Collagen VI	Abcam	ab6588	1 : 200	IHC
Fibronectin	Sigma	F3649	1 : 200	IHC
MMP14	Millipore	MAB3328	1 : 100	IHC
YAP1	Abcam	ab56701	1 : 1000	IHC

#### Supplementary Table 4

Summary of the details of the antibodies used for immunofluorescence (IF) and immunohistochemistry (IHC). Human protein atlas is represented as HPA.

### **Supplementary Data 1**

List of differentially expressed genes (> 2-fold) between UUS tumors based on CFR groups and Low TILS

### **Supplementary Data 2**

List of differentially expressed genes between UUS tumors with high and low infiltration of M1-like macrophages.