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Supplemental information

Cancer-associated fibroblast phenotypes are associated with patient outcome in non-small cell lung cancer

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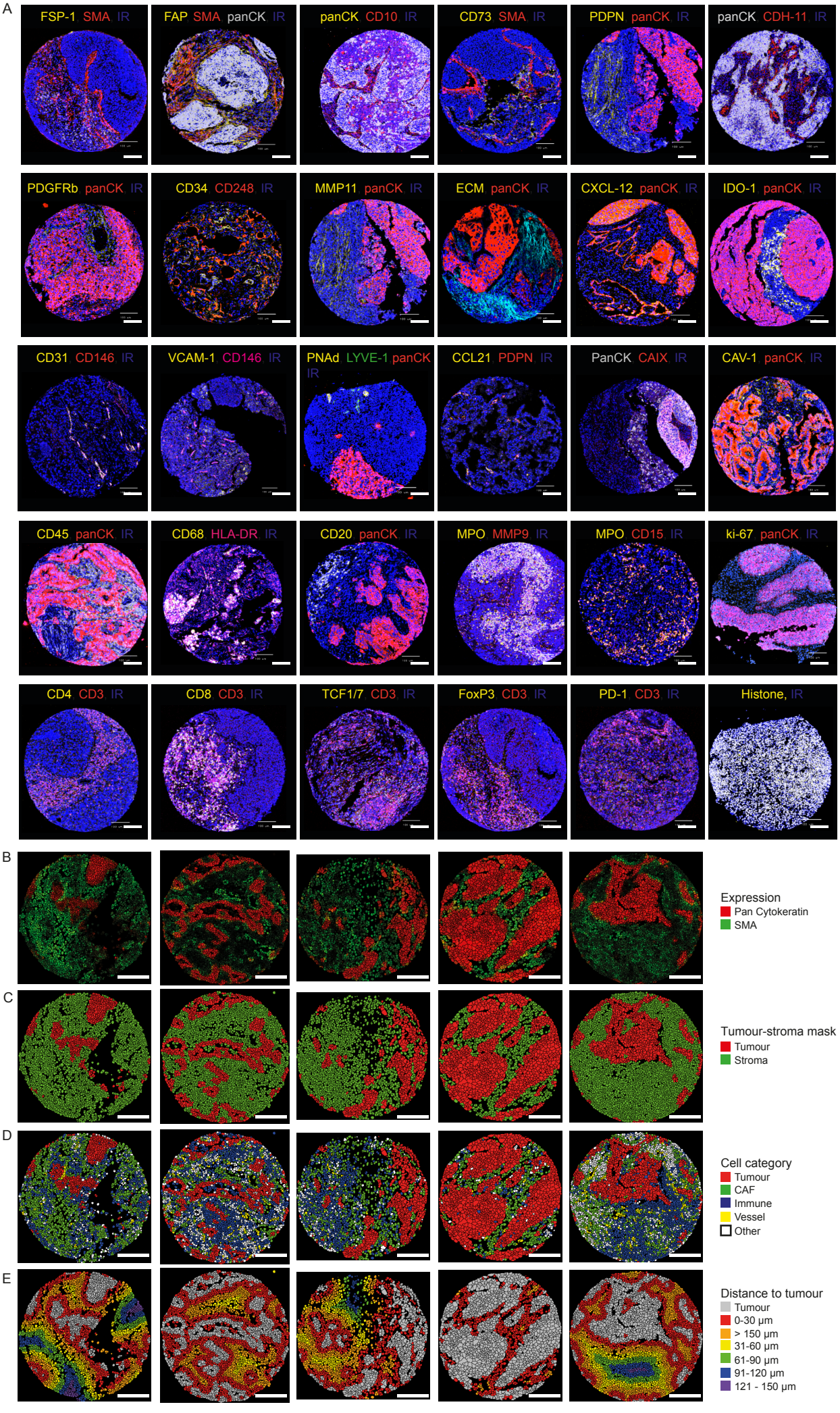

A
FSP-1 SMA IR
FAP SMA panCK IR
panCK CD10 IR
CD73 SMA IR
PDPN panCK IR
panCK CDH-11 IR
PDGFRb panCK IR
CD34 CD248 IR
MMP11 panCK IR
ECM panCK IR
CXCL-12 panCK IR
IDO-1 panCK IR
CD31 CD146 IR
VCAM-1 CD146 IR
PNAd LYVE-1 panCK IR
CCL21 PDPN IR
PanCK CAIX IR
CAV-1 panCK IR
CD45 panCK IR
CD68 HLA-DR IR
CD20 panCK IR
MPO MMP9 IR
MPO CD15 IR
ki-67 panCK IR
CD4 CD3 IR
CD8 CD3 IR
TCF1/7 CD3 IR
FoxP3 CD3 IR
PD-1 CD3 IR
Histone IR
B
Expression
Pan Cytokeratin
SMA
C
Tumour-stroma mask
Tumour
Stroma
D
Cell category
Tumour
CAF
Immune
Vessel
Other
E
Distance to tumour
Tumour
0-30 µm
> 150 µm
31-60 µm
61-90 µm
91-120 µm
121 - 150 µm

Figure S1 – Marker expression in IMC images. Related to figures 1 and 2.

A) Representative images of all markers. Scale bars indicate 100 µm.
B) Cell masks coloured by the mean SMA (green) and panCK (red) expression per cell. Scale bars indicate 170 µm.
C) Cell masks pseudo-coloured stromal (green) or tumour (red) compartment. Scale bars indicate 170 µm.
D) Cell masks pseudo-coloured by cell category: tumour (red), immune (blue), fibroblast (green), vessel (yellow), other (white). Scale bars indicate 170 µm.
E) Cell masks pseudo-coloured by the distance from each cell to the closest tumour stroma border: tumour (white), 0-30 µm (red), 31-60 µm (orange), 61-90 µm (yellow), 91-120 µm (green), 121-150 µm (blue), > 150 µm (purple). Scale bars indicate 170 µm.

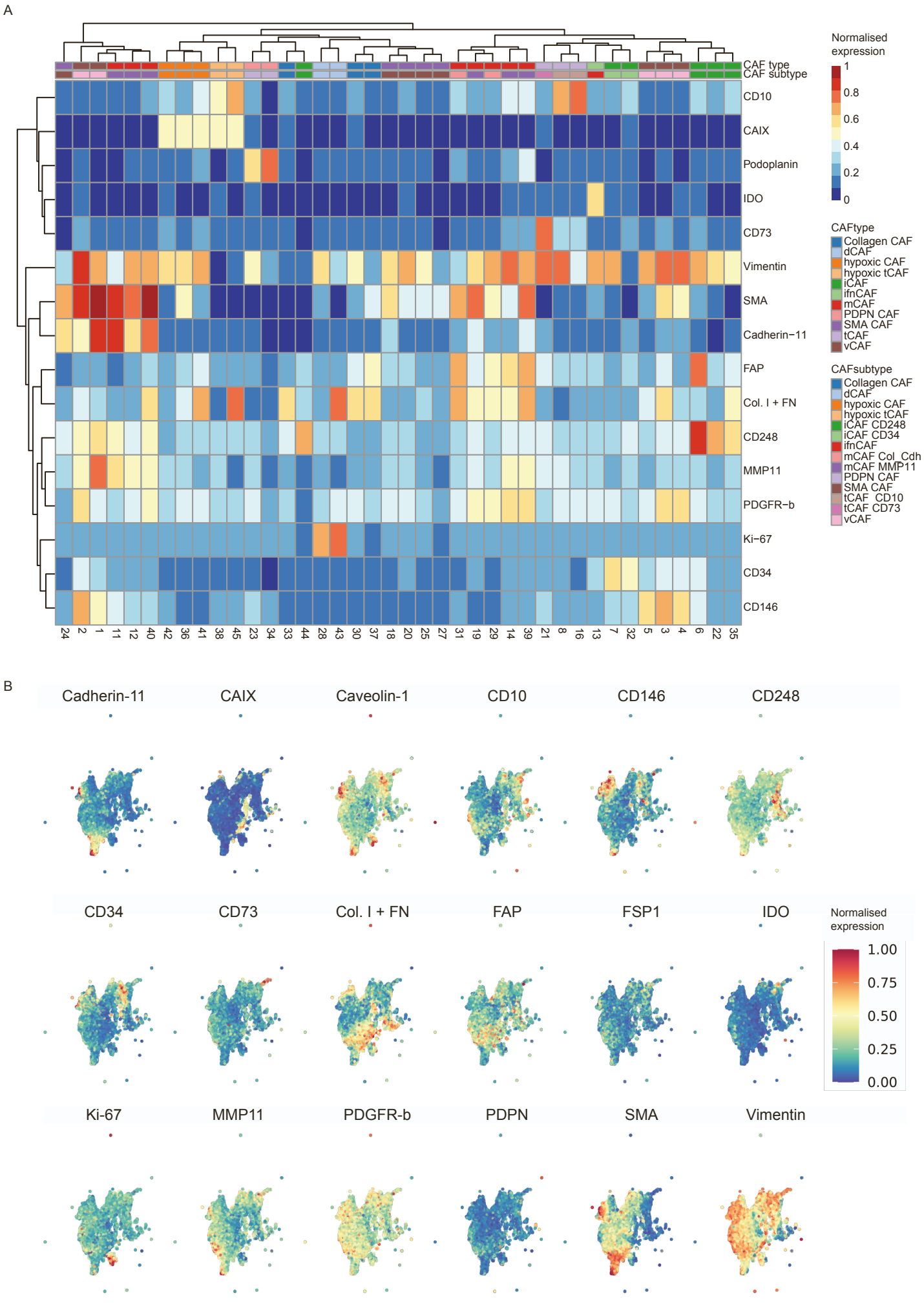

A
CAF type
CAF subtype
CD10
CAIX
Podoplanin
IDO
CD73
Vimentin
SMA
Cadherin−11
FAP
Col. I + FN
CD248
MMP11
PDGFR−b
Ki−67
CD34
CD146
24 2 1 11 12 40 42 36 41 38 45 23 34 33 44 28 43 30 37 18 20 25 27 31 19 29 14 39 21 8 16 13 7 32 5 3 4 6 22 35
Normalised expression
1
0.8
0.6
0.4
0.2
0
CAFtype
Collagen CAF
dCAF
hypoxic CAF
hypoxic tCAF
iCAF
ifnCAF
mCAF
PDPN CAF
SMA CAF
tCAF
vCAF
CAFsubtype
Collagen CAF
dCAF
hypoxic CAF
hypoxic tCAF
iCAF CD248
iCAF CD34
ifnCAF
mCAF Col_Cdh
mCAF MMP11
PDPN CAF
SMA CAF
tCAF CD10
tCAF CD73
vCAF
B
Cadherin-11
CAIX
Caveolin-1
CD10
CD146
CD248
CD34
CD73
Col. I + FN
FAP
FSP1
IDO
Ki-67
MMP11
PDGFR-b
PDPN
SMA
Vimentin
Normalised expression
1.00
0.75
0.50
0.25
0.00

Figure S2 – CAF phenotyping. Relates to Figure 3.

A) Heatmap of expression levels of CAFs clustered with FLOWSOM (k max = 45, som = 40) together with CAF type and CAF subtype.
B) UMAP of a random subset of 200,000 cells of all fibroblasts showing the mean intensity per cell scaled from 0-1 for all CAF markers.

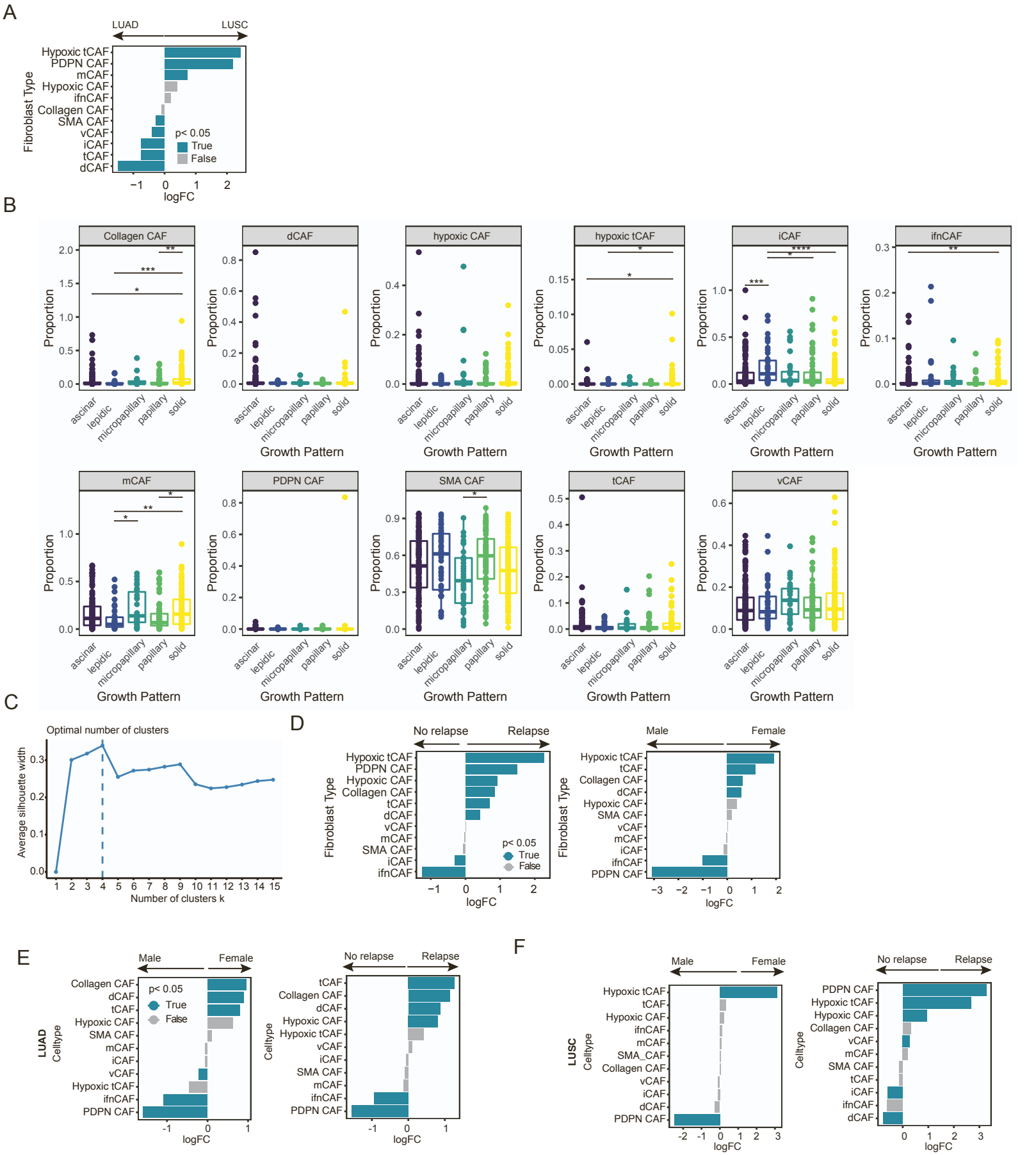

A
LUAD
LUSC
Hypoxic tCAF
PDPN CAF
mCAF
Hypoxic CAF
ifnCAF
Collagen CAF
SMA CAF
vCAF
iCAF
tCAF
dCAF
Fibroblast Type
p< 0.05
True
False
logFC
B
Collagen CAF
dCAF
hypoxic CAF
hypoxic tCAF
iCAF
ifnCAF
mCAF
PDPN CAF
SMA CAF
tCAF
vCAF
Proportion
ascinar
lepidic
micropapillary
papillary
solid
Growth Pattern
C
Optimal number of clusters
Average silhouette width
Number of clusters k
D
No relapse
Relapse
Male
Female
Fibroblast Type
p< 0.05
True
False
logFC
E
Male
Female
LUAD
Celltype
No relapse
Relapse
logFC
F
Male
Female
LUSC
Celltype
No relapse
Relapse
logFC

Figure S3 – Correlation of CAF types with clinical data. Relates to figure 3.

A) Differential abundance analysis for CAF types over tumour types (LUAD/LUSC)
B) Boxplots comparing the proportions of CAF types between LUAD growth patterns (acinar, lepidic, micropapillary, papillary, solid); Kruskal-Wallis: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)
C) Average silhouette width plot showing the optimal calculated cluster number for hierarchical clustering of CAF type proportions calculated per patient.
D) Differential abundance analysis for CAF types over relapse and sex in both tumour types taken together.
E) Differential abundance analysis for CAF type in LUAD for relapse and sex.
F) Differential abundance analysis for CAF type in LUSC for relapse and sex

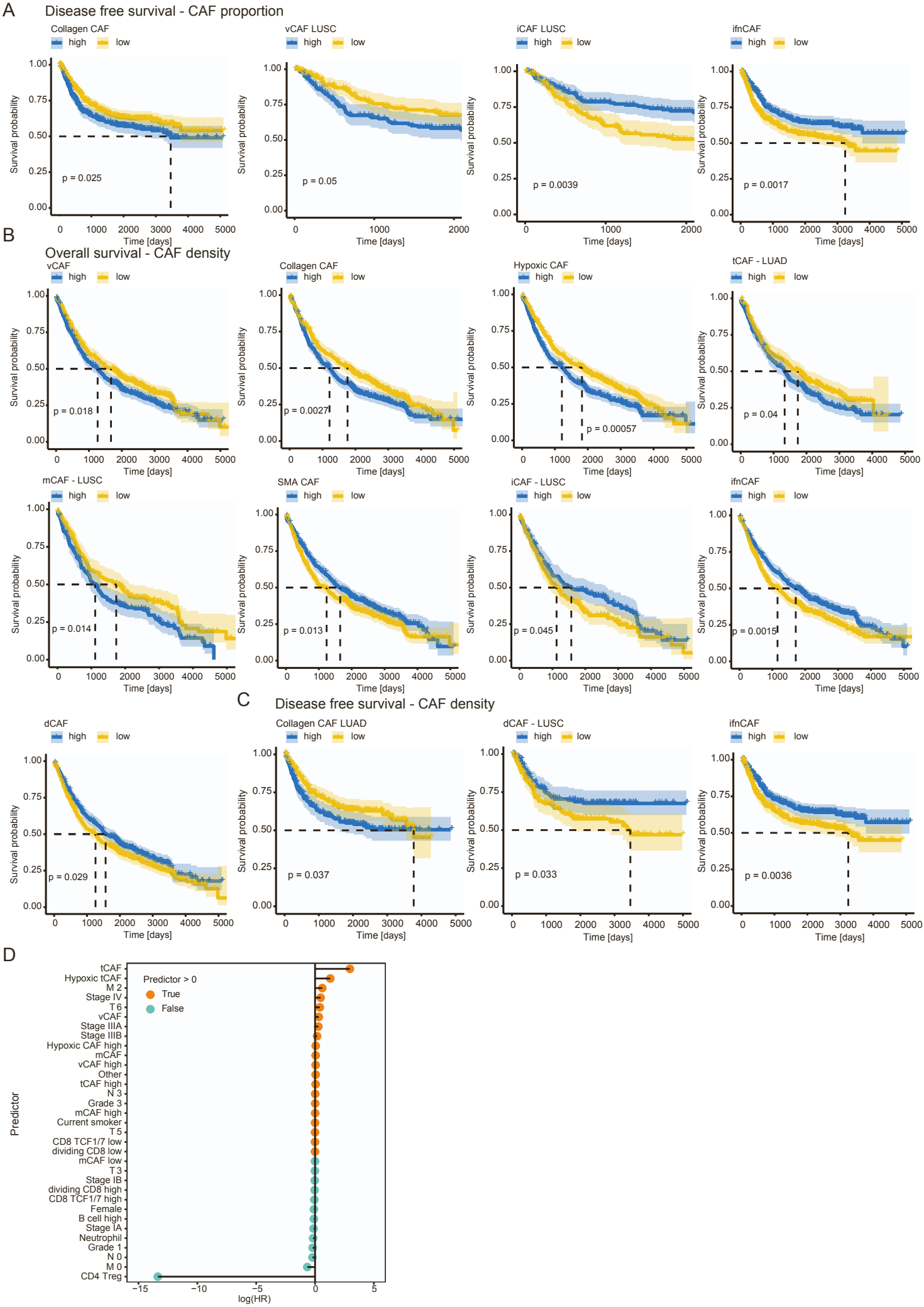

A
Disease free survival - CAF proportion
Collagen CAF
vCAF LUSC
iCAF LUSC
ifnCAF
high
low
Survival probability
Time [days]
p = 0.025
p = 0.05
p = 0.0039
p = 0.0017
B
Overall survival - CAF density
vCAF
Collagen CAF
Hypoxic CAF
tCAF - LUAD
mCAF - LUSC
SMA CAF
iCAF - LUSC
ifnCAF
p = 0.018
p = 0.0027
p = 0.00057
p = 0.04
p = 0.014
p = 0.013
p = 0.045
p = 0.0015
C
Disease free survival - CAF density
dCAF
Collagen CAF LUAD
dCAF - LUSC
ifnCAF
p = 0.029
p = 0.037
p = 0.033
p = 0.0036
D
Predictor > 0
True
False
Predictor
tCAF
Hypoxic tCAF
M 2
Stage IV
T 6
vCAF
Stage IIIA
Stage IIIB
Hypoxic CAF high
mCAF
vCAF high
Other
tCAF high
N 3
Grade 3
mCAF high
Current smoker
T 5
CD8 TCF1/7 low
dividing CD8 low
mCAF low
T 3
Stage IB
dividing CD8 high
CD8 TCF1/7 high
Female
B cell high
Stage IA
Neutrophil
Grade 1
N 0
M 0
CD4 Treg
log(HR)

Figure S4 – Correlation of CAF types with survival data. Relates to figure 4.

A) Kaplan-Meier plots of disease-free survival for patients stratified by median proportion into high and low for Collagen CAFs (all patients), vCAFs (LUSC), iCAFs (LUSC), and ifnCAFs (all patients).
B) Kaplan-Meier plots of overall survival for patients stratified by median density into high and low for vCAFs (all patients), Collagen CAFs (all patients), hypoxic CAFs (all patients), tCAFs (LUAD), mCAFs (LUSC), SMA CAFs (all patients), iCAFs (LUSC), ifnCAFs (all patients), and dCAFs (all patients).
C) Kaplan-Meier plots of disease-free survival for patients stratified by median density into high and low for Collagen CAFs (all patients), dCAFs (LUSC), and ifnCAFs (all patients).
D) Lasso-regressed CoxPH model including mean CAF type density calculated per tumour core per patient as well as patient stratification into high and low for each CAF type (by median proportion) together with all available clinical data.

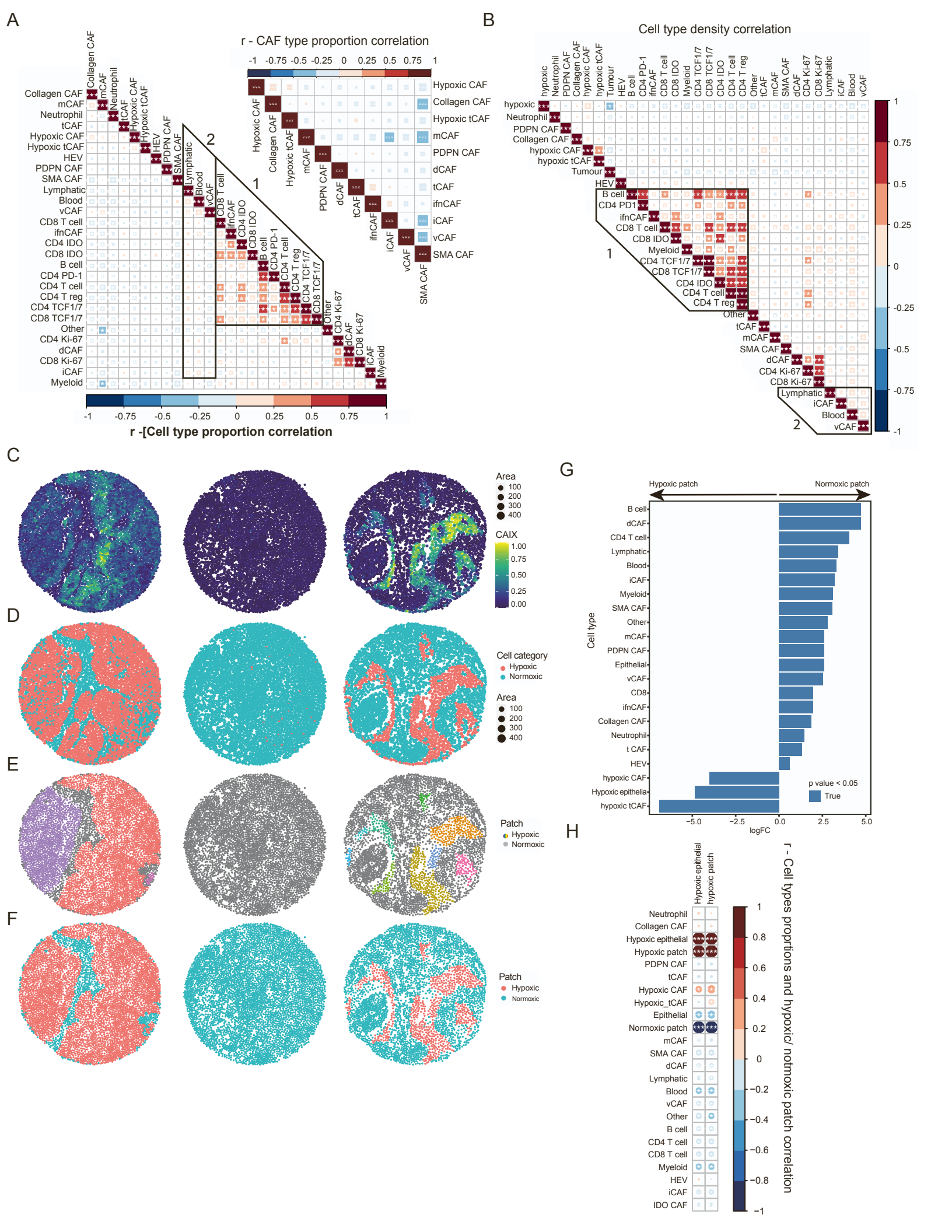

A
r - CAF type proportion correlation
r -[Cell type proportion correlation
B
Cell type density correlation
C
Area
CAIX
D
Cell category
Hypoxic
Normoxic
Area
E
Patch
Hypoxic
Normoxic
F
Patch
Hypoxic
Normoxic
G
Hypoxic patch
Normoxic patch
Cell type
logFC
p value < 0.05
True
H
Hypoxic epithelial
hypoxic patch
r - Cell types proprtions and hypoxic/ notmoxic patch correlation

Figure S5 – Correlation of CAF types with other cells and hypoxia. Relates to figure 6.

A) Correlations of the mean proportions per patient of all cell types (bottom left). Cluster 1 shows the correlation of ifnCAFs with various immune cells; cluster 2 shows the correlation of iCAFs with lymphatic and blood vessel cells and vCAFs. The second correlation matrix (top right) displays the correlation only of CAF types. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

B) Correlations of the mean density per patient of all cell types (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Cluster 1 shows the correlation of ifnCAFs with various immune cells; cluster 2 shows the correlation of iCAFs with lymphatic and blood vessel cells and vCAFs.

C) Dot plot of three TMA cores showing the mean cellular expression of CAIX for each cell.

D) Dot plot of three TMA cores (as in E) coloured by their categorisation as hypoxic (hypoxic epithelia, hypoxic tCAFs and hypoxic CAFs) and all normoxic cells.

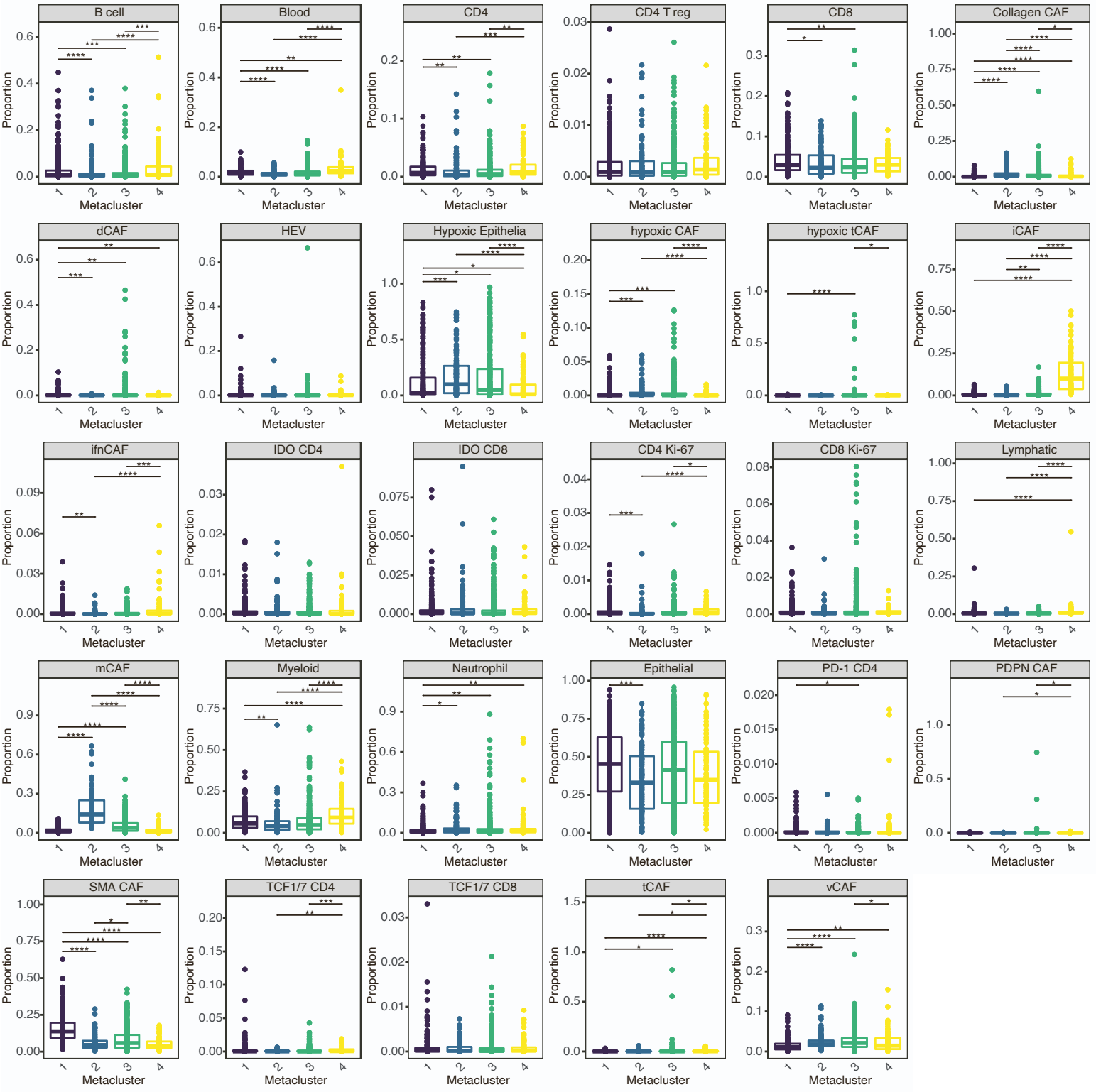
E) Dot plot of three TMA cores (as in E) showing patches calculated based on hypoxic cells.

F) Dot plot of three TMA cores (as in E) showing patches defined as either hypoxic (red) or normoxic patches (blue).

G) Differential abundance analysis comparing hypoxic versus normoxic patches.

H) Correlations of all cells with hypoxic and normoxic patches.

A


B cell
Blood
CD4
CD4 T reg
CD8
Collagen CAF
dCAF
HEV
Hypoxic Epithelia
hypoxic CAF
hypoxic tCAF
iCAF
ifnCAF
IDO CD4
IDO CD8
CD4 Ki-67
CD8 Ki-67
Lymphatic
mCAF
Myeloid
Neutrophil
Epithelial
PD-1 CD4
PDPN CAF
SMA CAF
TCF1/7 CD4
TCF1/7 CD8
tCAF
vCAF
Proportion
Metacluster
1
2
3
4

Supplementary Figure 6 – TME composition of patients stratified by CAF types. Relates to figure 6.

A) Boxplots showing cellular enrichment of all cell types between the four meta-clustered patient groups (1, 2, 3, 4; Kruskal-Wallis: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

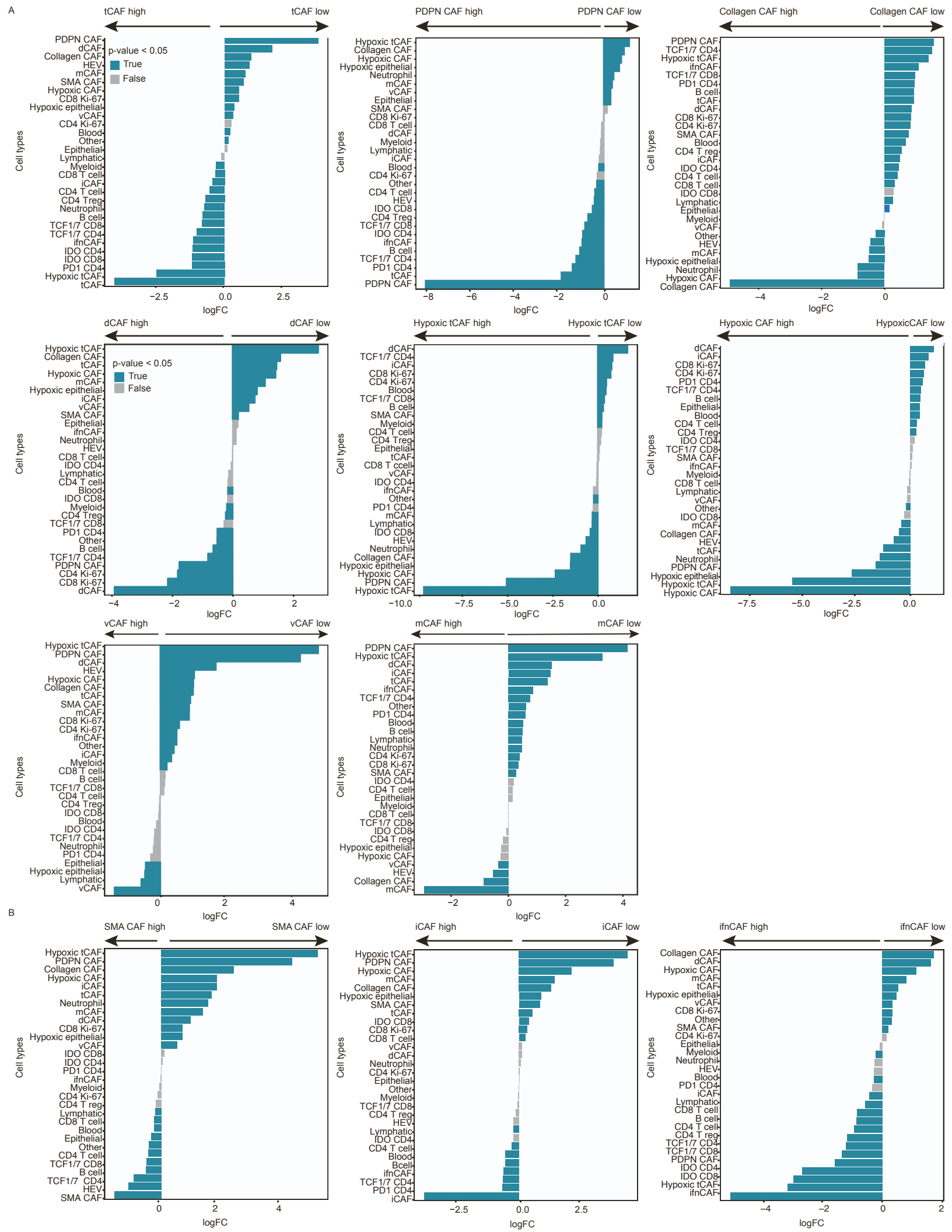

A
tCAF high
tCAF low
p-value < 0.05
True
False
Cell types
logFC
PDPN CAF high
PDPN CAF low
Collagen CAF high
Collagen CAF low
dCAF high
dCAF low
Hypoxic tCAF high
Hypoxic tCAF low
Hypoxic CAF high
HypoxicCAF low
vCAF high
vCAF low
mCAF high
mCAF low
B
SMA CAF high
SMA CAF low
iCAF high
iCAF low
ifnCAF high
ifnCAF low

Figure S7 – TME composition of patients stratified by CAF types.
Relates to figure 6.

A) Differential abundance analysis of all cell types between patients stratified as high or low based on mean proportions of poor-prognosis CAF types (tCAFs, PDPN CAFs, Collagen CAFs, dCAFs, hypoxic tCAFs, hypoxic CAFs, vCAFs and mCAFs).

B) Differential abundance analysis of all cell types between patients stratified as high or low based on mean proportions of good-prognosis CAF types (SMA CAFs, iCAFs and ifnCAFs).

Supplementary tables and legends

Table S1 – Antibody panel. Related to STAR Methods

Metal Tag	Target	Antibody Clone	Lot	Provider	Catalogue Number	RRID
Y89	Myelo-peroxidase	Polyclonal	2003045	Dako	A0398	AB_2335676
In113	FSP1 / S100A4	Polyclonal	3126953	Millipore	136784	AB_10807552
In115	SMA	1A4	2183900	eBioscience	14-9760-82	AB_2572996
Pr141	Histone H3	D1H2	15	Cell Signaling Technology	4499BF	AB_10544537
Nd142	CD11b	SP330	GR3258740-1	Abcam	ab241408	AB_2889379
Nd143	HLA-DR	TAL 1B5	GR3222279-4	Abcam	ab20181	AB_445401
Nd144	CD146	Polyclonal	ECL0319041	R&D Systems	AF932	AB_355721
Nd145	Cadherin-11	283416	VLT0219091	R&D Systems	MAB1790	AB_2076970
Nd146	FAP	Polyclonal	ZKW0315081	R&D Systems	AF3715	AB_2102369
Sm147	CD11b	M1/70	B186484	Biolegend	101202	AB_312785
Nd148	VCAM1	EPR5047	GR3255420-4	Abcam	ab215380	AB_2894839
Sm149	CD20	L26	2059976	eBioscience	14-0202-82	AB_10734340
Nd150	CD68	KP1	2130291	eBioscience	14-0688-82	AB_11151139
Eu151	Indoleamine 2- 3-dioxygenase	SP260	GR3259345-2	Abcam	ab245737	AB_2894840
Sm152	CD3	Polyclonal	GR3229164-6	Dako	A0452	AB_2335677

Eu153	Podoplanin	NC-08	B260834	Biolegend	337002	AB_1595511
Sm154	CD11c	D3V1E	2	Cell Signaling Technology	45581BF	AB_2799286
Gd155	Carbonic Anhydrase IX	Polyclonal	VNQ0319011	R&D Systems	AF2188	AB_416562
Gd156	CD73	D7F9A	2	Cell Signaling Technology	13160BF	AB_2716625
Gd158	MMP9	D6O3H	4	Cell Signaling Technology	13667BF	AB_2798289
Tb159	p75 / CD271	Polyclonal	ANT007AN0702	Alomone labs	ANT-007	AB_2039968
Dy161	CD10	E5P7S	2	Cell Signaling Technology	65534	AB_2894842
Dy162	Vimentin	EPR3776	GR286525-2	Abcam	ab193555	AB_2814713
Dy163	FOXP3	236A/E7	2129676	eBioscience	14-4777-82	AB_467556
Dy164	CXCL13	Polyclonal	BAS0317101	R&D Systems	AF801	AB_355613
Ho165	PNAd	MECA-79	B257139	Biolegend	120802	AB_493555
Er166	CD8a	C8/144B	2132595	eBioscience	14-0085-82	AB_11150240
Er167	Fibronectin	10/Fibro-nectin	6251888	BD Biosciences	610078	AB_397486
Er167	Collagen I	Polyclonal	GR3253239-3	Abcam	ab34710	AB_731684
Er168	LYVE-1	Polyclonal	KPY0119052	R&D Systems	AF2089	AB_355144
Tm169	CD140b	Y92	GR296584-4	Abcam	ab215978	AB_2894841
Er170	CD34	EP373Y	GR3271518-1	Abcam	ab198395	AB_2889381

Yb171	CD4	Polyclonal	YS071 8031	R&D Systems	AF-379-NA	AB_354 469
Yb172	vWF	poly vwf	332299 8	Millipore	AB7356	AB_922 16
Yb172	CD31	EPR3094	GR322 9164-6	Abcam	ab207090	AB_288 9382
Yb173	CXCL12 / SDF-1	79018	JOJ051 9031	R&D Systems	MAB350-100	AB_208 8149
Yb174	CCL21 / 6Ckine	Polyclonal	AYJ218 071	R&D Systems	AF366	AB_355 327
Lu175	Keratin Epithelial	AE3	325545 7	Millipore	MAB1611	AB_213 4409
Lu175	Pan Cytokeratin	AE1	325291 0	Millipore	MAB1612	AB_213 2794
Pt194	Ki-67	B56	811675 1	Becton Dickinson	556003	AB_396 287
Bi209	CD45	2B11	200342 2	eBioscience	14-9457-82	AB_110 63696

Table S1. List of all antibodies used in this study, their respective metal tag, clone number and vendor information, RRID (Research Resource Identifiers) and catalogue number.

Table S2 – Distribution of different cell classes in the stromal and tumour compartment. Related to STAR Methods

	Stroma [n]	Stroma [%]	Tumour [n]	Tumour [%]
Fibroblast	894478	82.3	192200	17.7
Immune	522302	63.5	299903	36.5
Other	387435	65.8	201453	34.2
T cell	265970	74.5	90939	25.5
Tumour	149901	5.0	2829811	95.0
vessel	124863	83.2	25199	16.8

Table S2. Number of cells and percentage within tumour and stroma subsets.

Table S3 – Number of cells in bins measured from the tumour-stroma border. Related to STAR Methods

Bins in µm from tumour-stroma border	Area	Fibroblast [n]	Immune [n]	Other [n]	T cell [n]	Tumour [n]	Vessel [n]
(>0)	Tumour	192100	299765	201349	90902	2829644	25183
-10 to -0	Stroma – tumour interface	299316	179225	127500	76138	126899	45952
-30 to -0	Stroma	598811	359337	257938	164075	144455	89846
-60 to 30	Stroma	155405	90191	71943	52227	3691	19364
-90 to -60	Stroma	63052	34494	27229	22389	995	6887
-120 to -90	Stroma	31572	17255	12560	11408	449	3416
-150 to -120	Stroma	17349	8971	6387	6202	217	1951
(-150)	Stroma	28389	12192	11482	9706	261	3415

Table S3. Number of cells in different bins from the tumour stroma border.