Supplementary Information

Manuscript title: Visualizing malignant progression: *In situ* CD109-based spatial immunofluorescence assay delineates papillary to anaplastic thyroid carcinoma transformation within the tumor microenvironment

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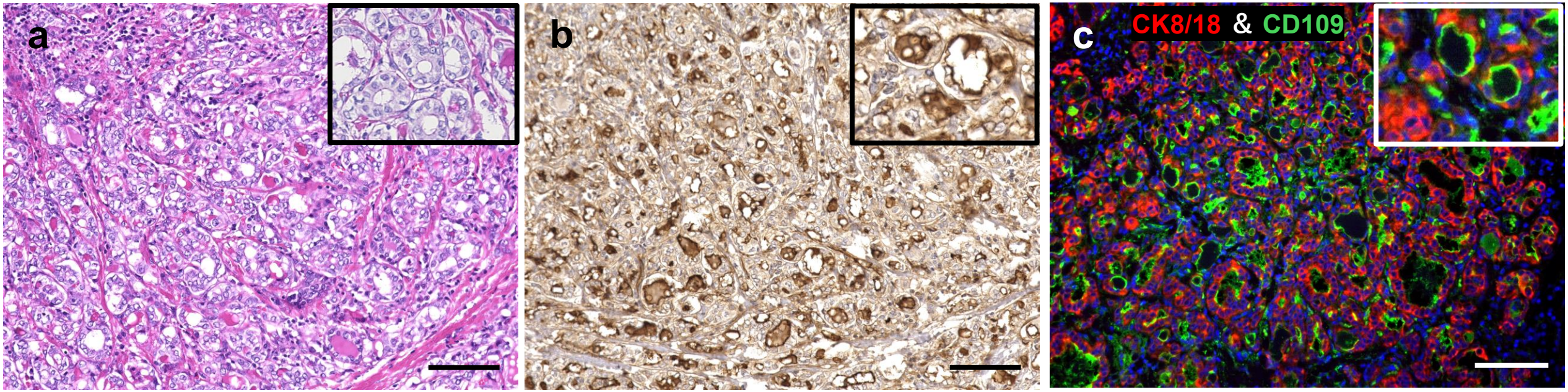
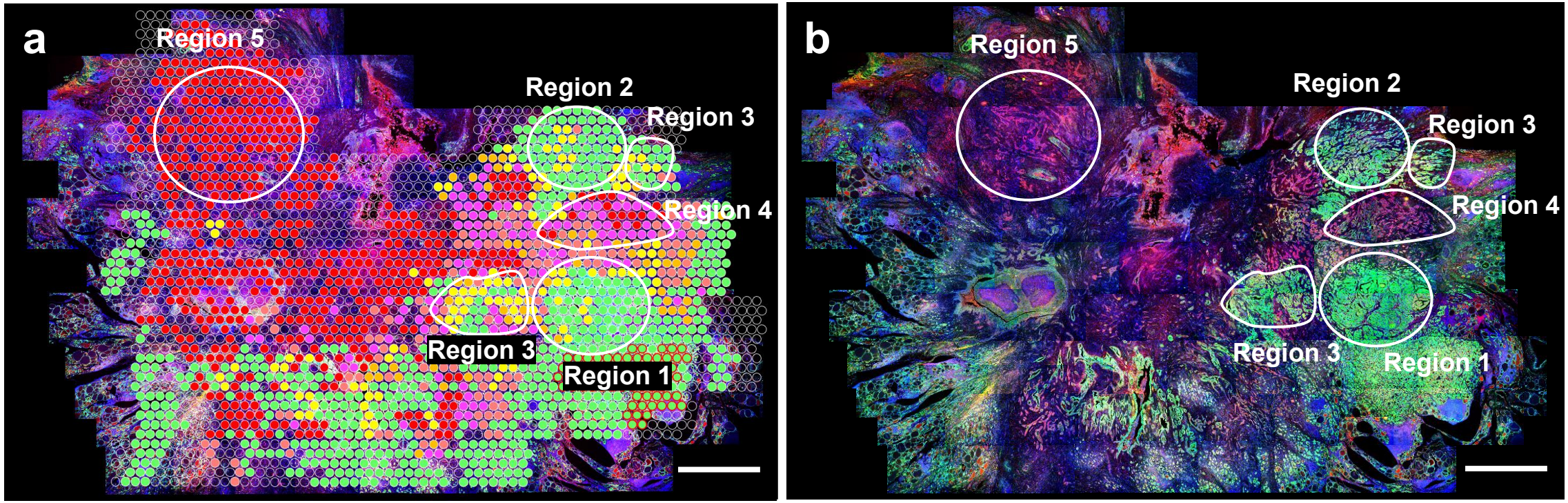


Supplementary Figure 1
Histological findings of a papillary thyroid carcinoma (PTC) component with a follicular structure.
a Hematoxylin and eosin (HE) staining.
b Immunohistochemistry of CD109 using diaminobenzidine (DAB). **c** Multiplex immunofluorescence image stained for CK8/18 (red) and CD109 (green).
The images within the box in the top right are close-up of part of each image.
Scale bars: 100 μm.



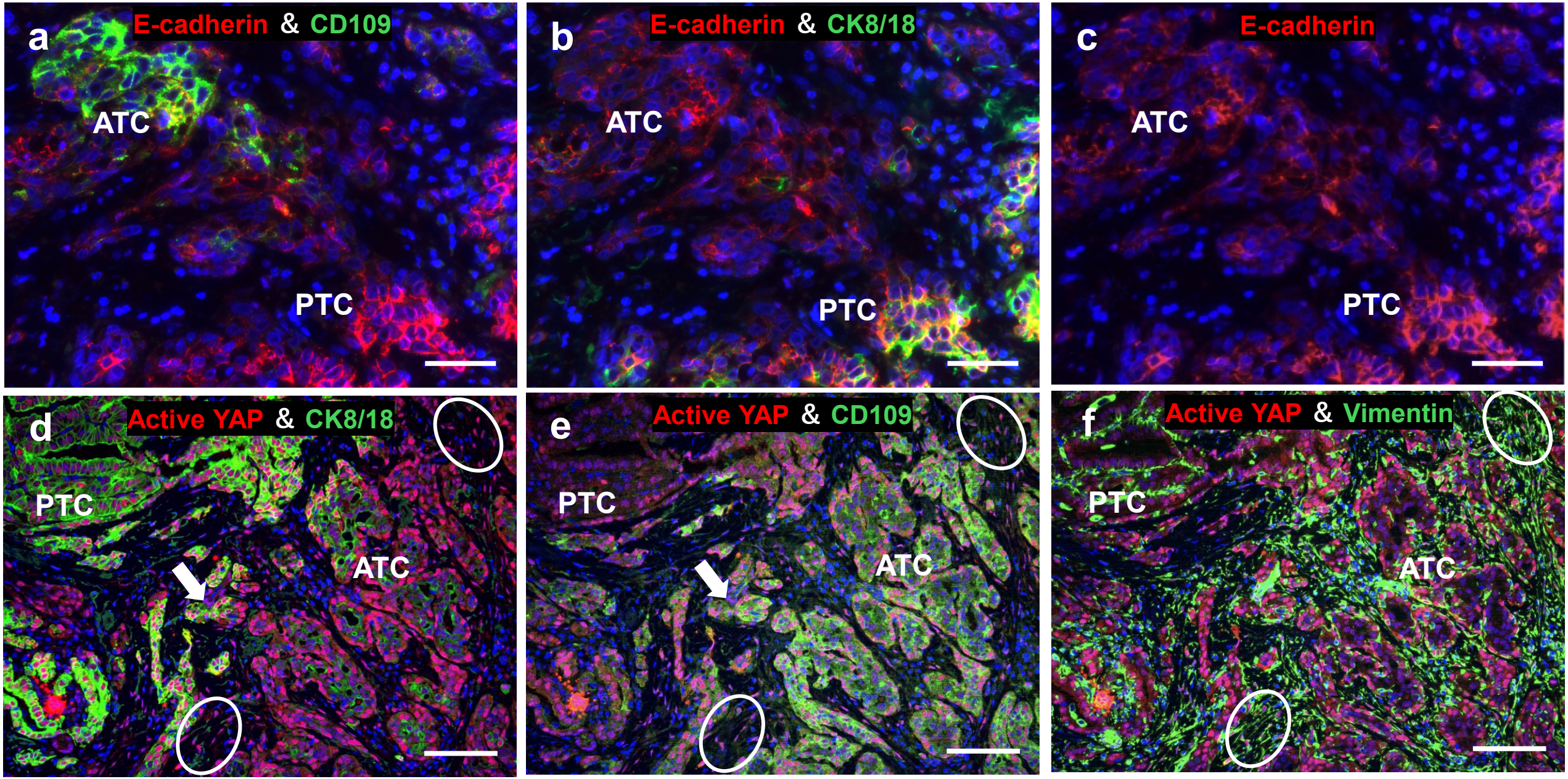
Supplementary Figure 2

Assignment of the five regions.

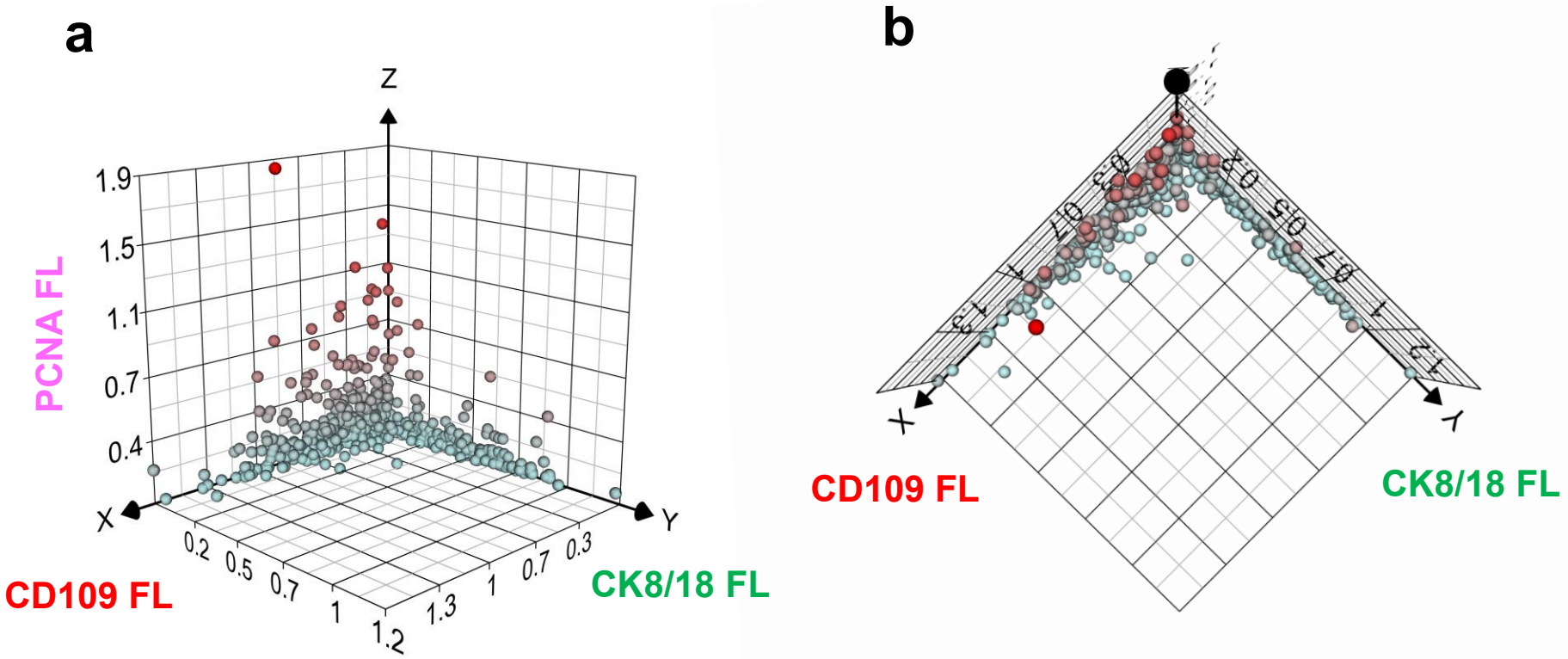
a Five regions are overlaid on the standard SPI assay.

b Five regions are overlaid on the multiplex immunofluorescence image stained for CD109 (red) and CK8/18 (green).

The assigned regions are circled with white lines. Region 1 is a typical PTC area with negative CD109 and positive CK8/18 expression, and shows green markings in the SPI assay. Region 2 is a PTC area with dual expression of CK8/18 and CD109, in which green markings are predominant over yellow markings in the SPI assay. Region 3 corresponds to the transitional zone, which contains areas that are primarily represented by yellow markings in the SPI assay. Region 4 is an ATC area that shows strong CD109 expression and little or no CK8/18 expression, which predominantly appears as pink markings in the SPI assay. Region 5 represents an ATC area that is characterized by strong CD109 expression and complete loss of CK8/18 expression, which is indicated by red markings in the SPI assay. Scale bars: 2000 μm.



Supplementary Figure 3 Multiplex immunofluorescence images stained for **a** E-cadherin (red) and CD109 (green), **b** E-cadherin (red) and CK8/18 (green), **c** E-cadherin (red), **d** Active YAP (red) and CK8/18 (green), **e** Active YAP (red) and CD109 (green), **f** Active YAP (red) and Vimentin (green). The arrows in **d** and **e** indicate cells that co-express CK8/18 and CD109. Circles in **d-f** indicate active YAP-positive cancer-associated fibroblasts (CAFs).
PTC: papillary thyroid carcinoma region. ATC: anaplastic thyroid carcinoma region. Scale bars: 50 μm in **a-c**, 100 μm in **d-f**.



c

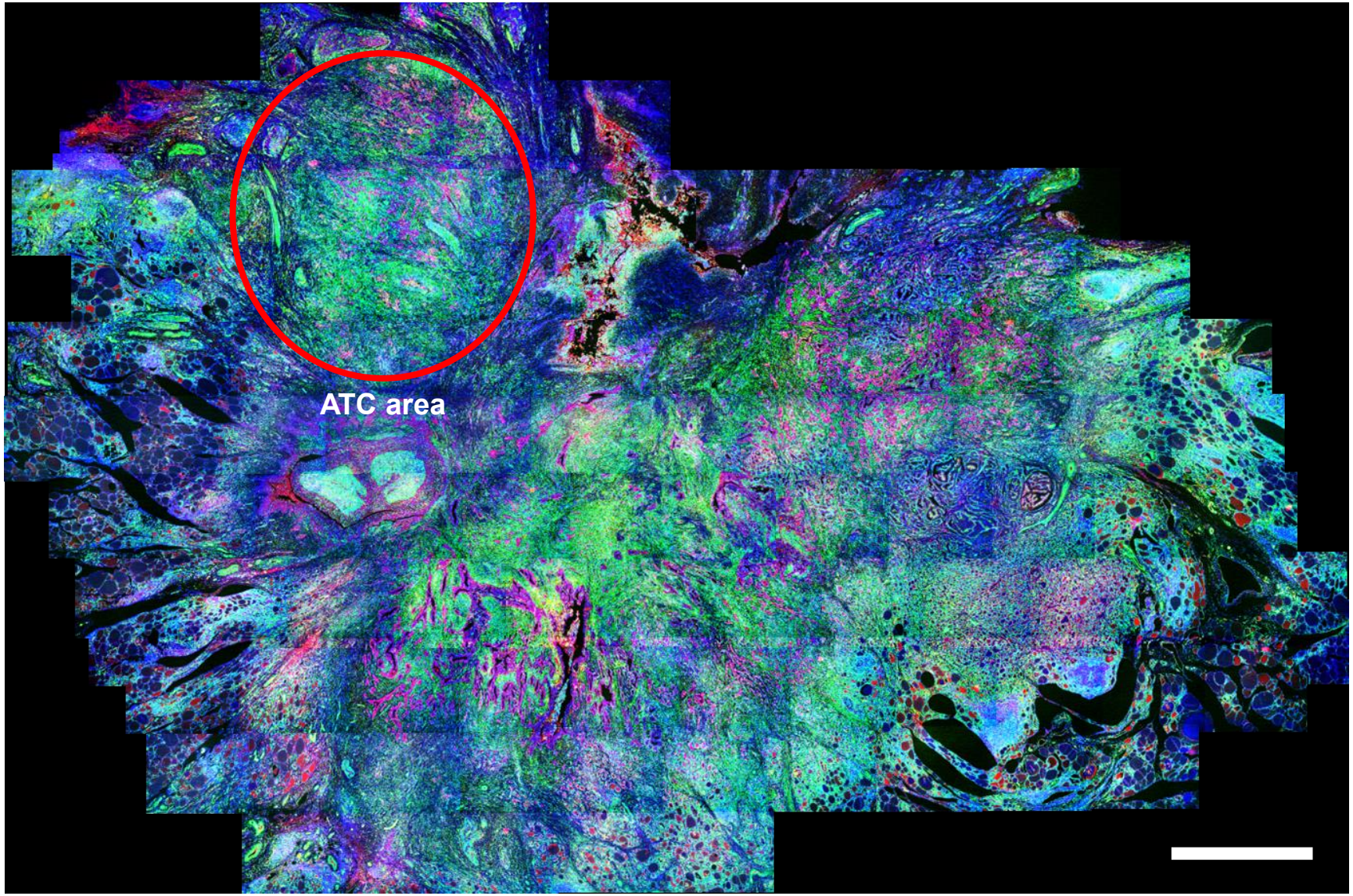
	Total number of cells	PCNA+cells	%PCNA+
CD109-CK8/18+	548	91	16.6
CD109+CK8/18-	550	317	57.6
CD109+CK8/18+	52	30	57.7
CD109-CK8/18-	62	26	41.9

Supplementary Figure 4

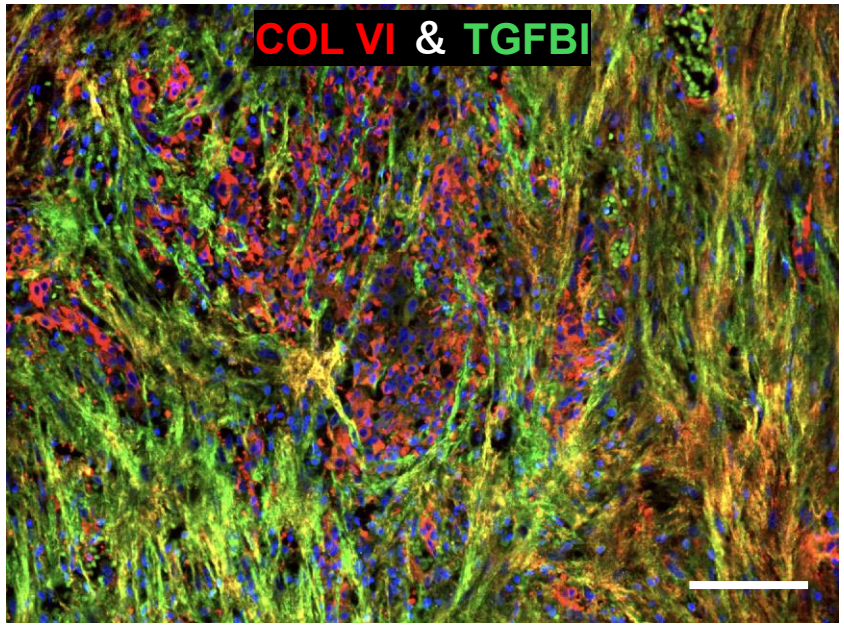
Quantitative analysis of PCNA-positive cells.

a, **b** Three dimensional graphs of CD109 (X-axis), CK8/18 (Y-axis) and PCNA (Z-axis). Each dot represents a region of interest (ROI).

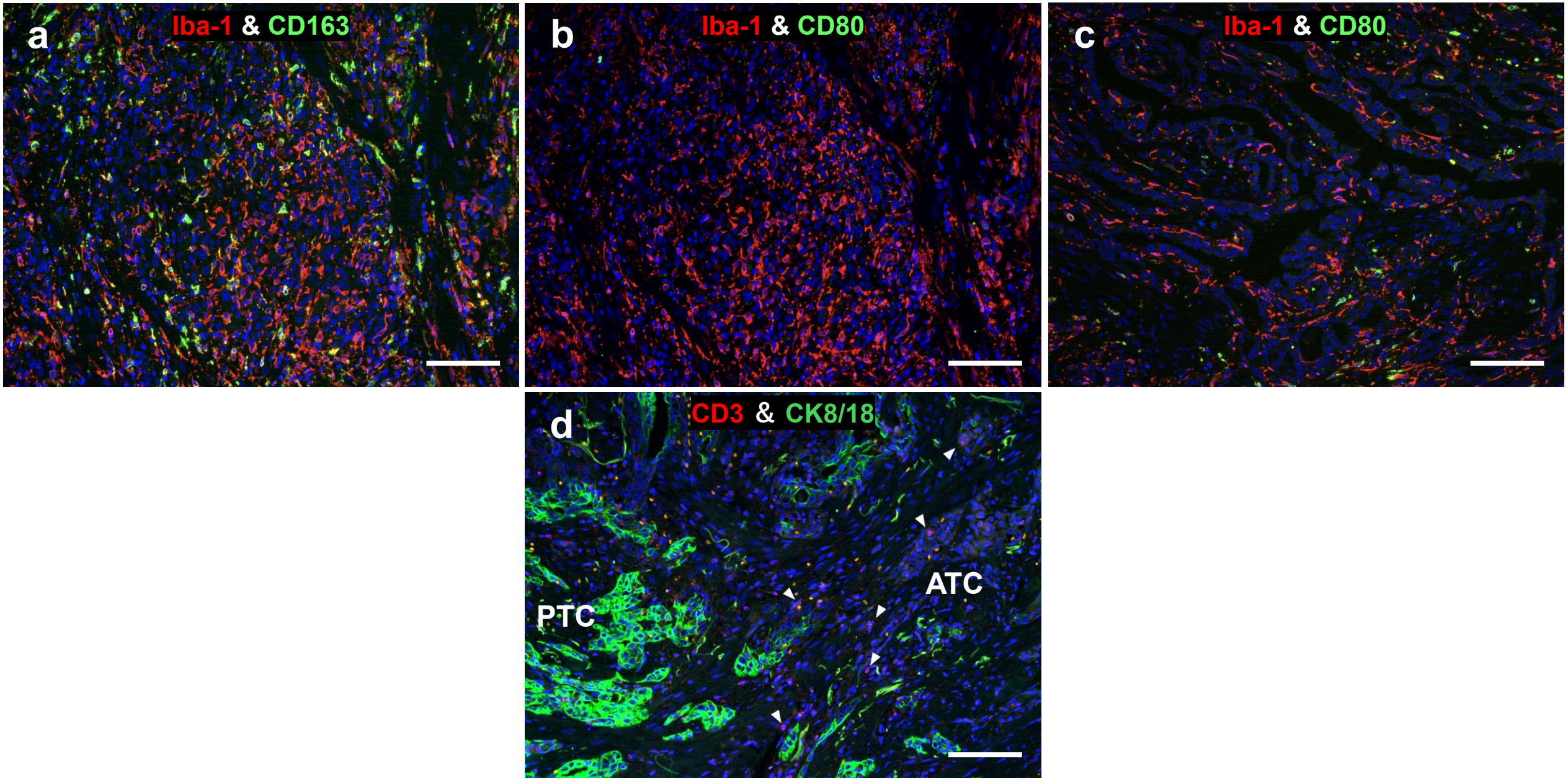
c Summary of percentages of PCNA positive cells in each CD109-CK8/18+, CD109+CK8/18-, CD109+CK8/18+ and CK18-CD109- cells. Cells with fluorescence levels greater than 0.05, 0.07 and 0.047 for CD109, CK18, and PCNA, respectively, were counted. FL: fluorescence.



Supplementary Figure 5
Multiplex immunofluorescence image stained for CD109 (red) and Vimentin (green).
ATC: anaplastic thyroid carcinoma.
Scale bar: 2000 μm.



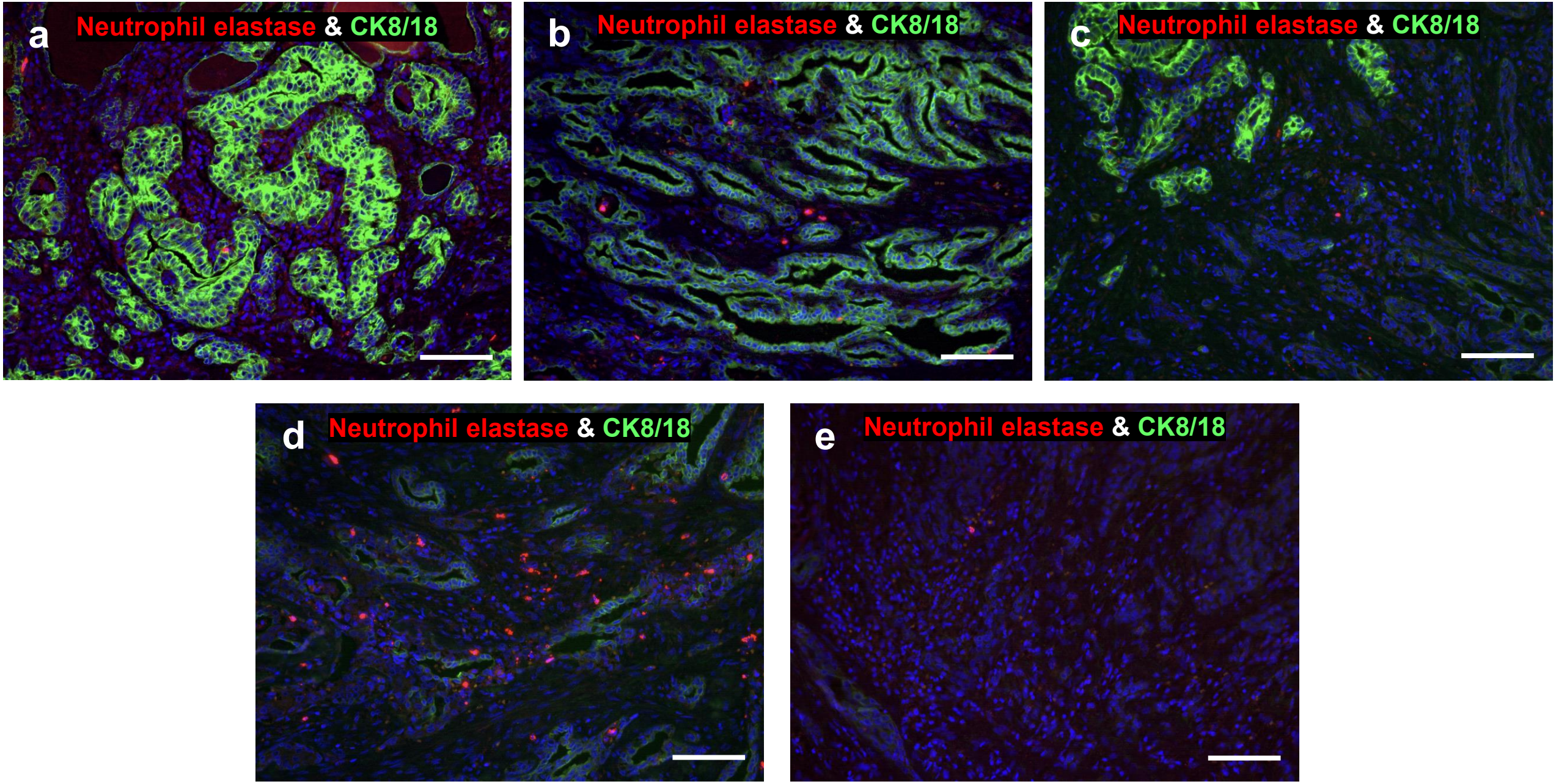
Supplementary Figure 6
Multiplex immunofluorescence image stained for collagen VI (COL VI) (red) and TGFβ1-induced (TGFBI) (green) in Region 5.
Scale bar: 100 μm.



Supplementary Figure 7

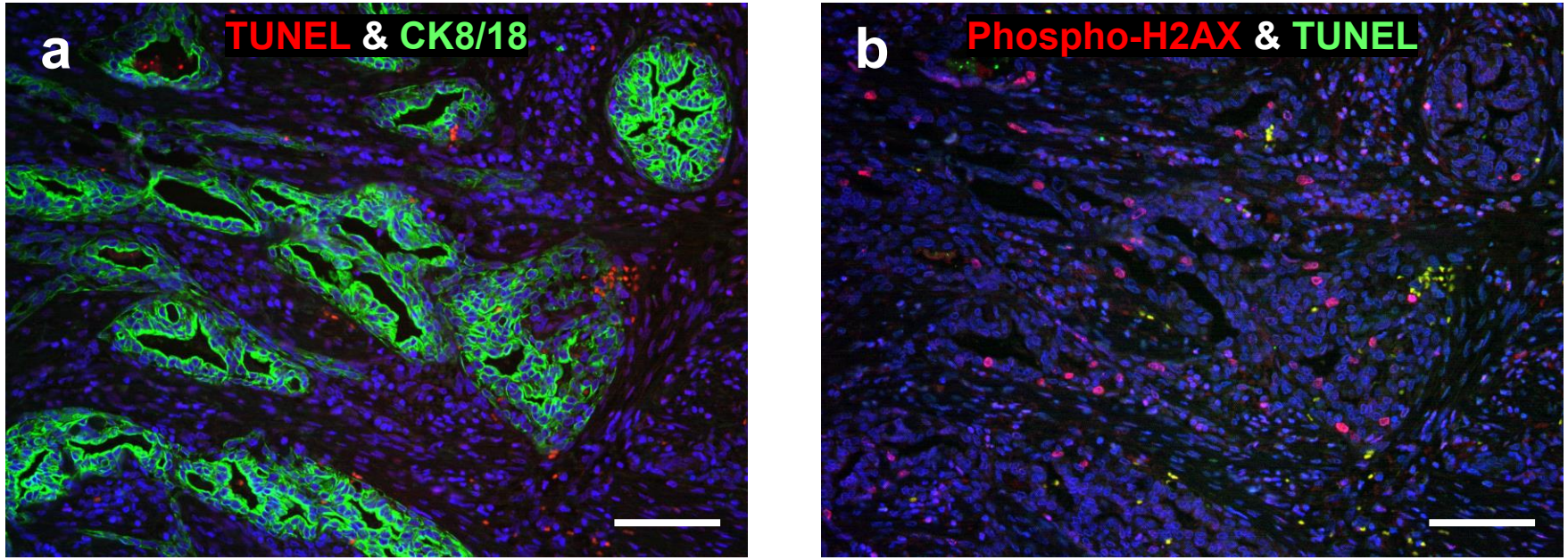
Multiplex immunofluorescence images stained for **a** Iba-1 (red) and CD163 (green) in Region 5, **b** Iba-1 (red) and CD80 (green) in Region 5, **c** Iba-1 (red) and CD80 (green) in Region 2, **d** CD3 (red) and CK8/18 (green) in Region 3. See more detail in Supplementary Table 2.

The arrow heads in **d** indicate CD3-positive cells. PTC: papillary thyroid carcinoma region. ATC: anaplastic thyroid carcinoma region. Scale bars: 100 μm.

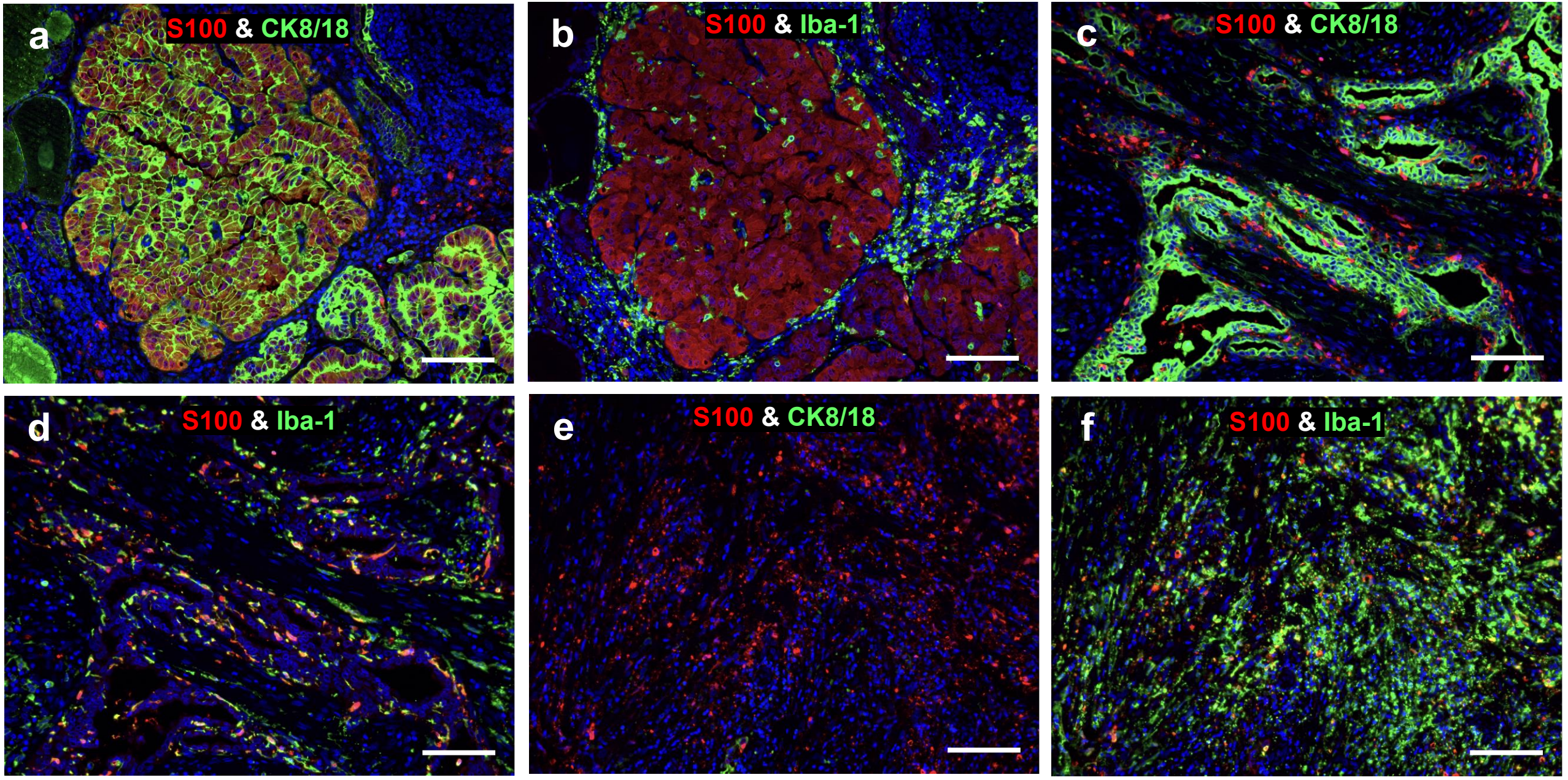


Supplementary Figure 8

Multiplex immunofluorescence images stained for Neutrophil elastase (red) and CK8/18 (green) in Region 1 (**a**), 2 (**b**), 3 (**c**), 4 (**d**), and 5 (**e**). Scale bars: 100 μm.

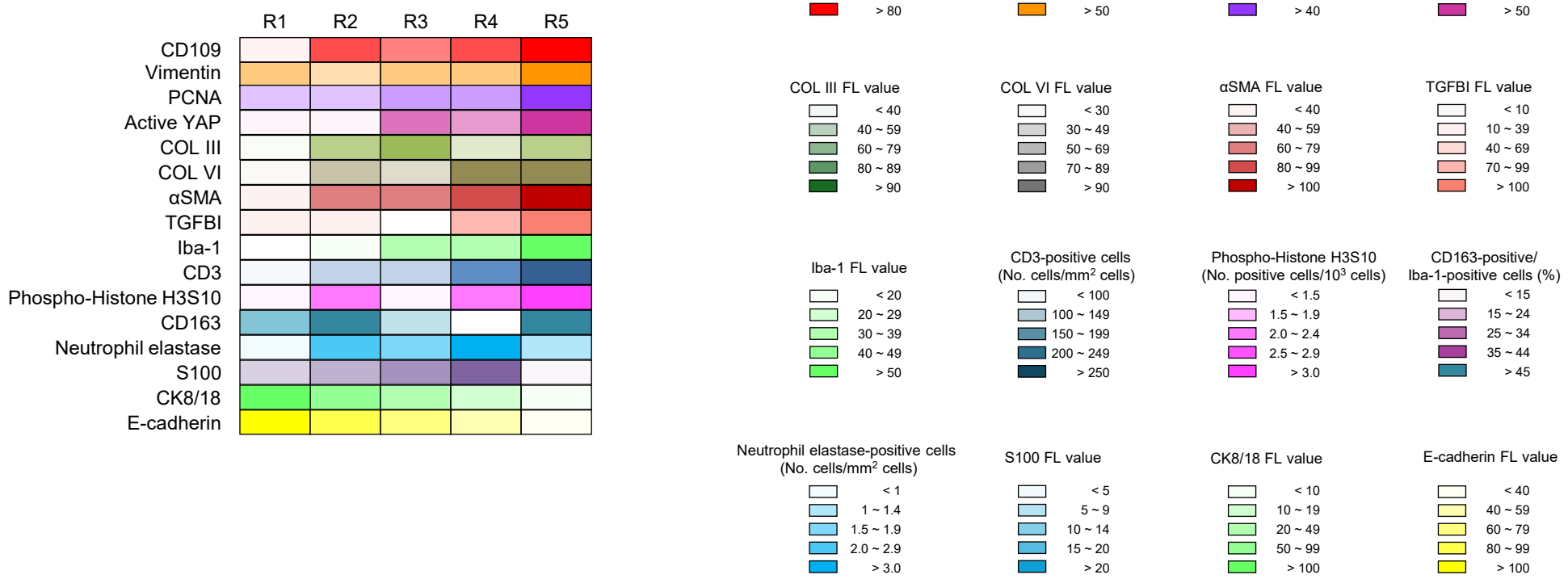


Supplementary Figure 9
Multiplex immunofluorescence images stained for **a** fluorometric TUNEL assay (red) and CK8/18 (green) and **b** Phospho-H2AX (red) and fluorometric TUNEL assay (green) in Region 2.
Scale bars: 100 μm.



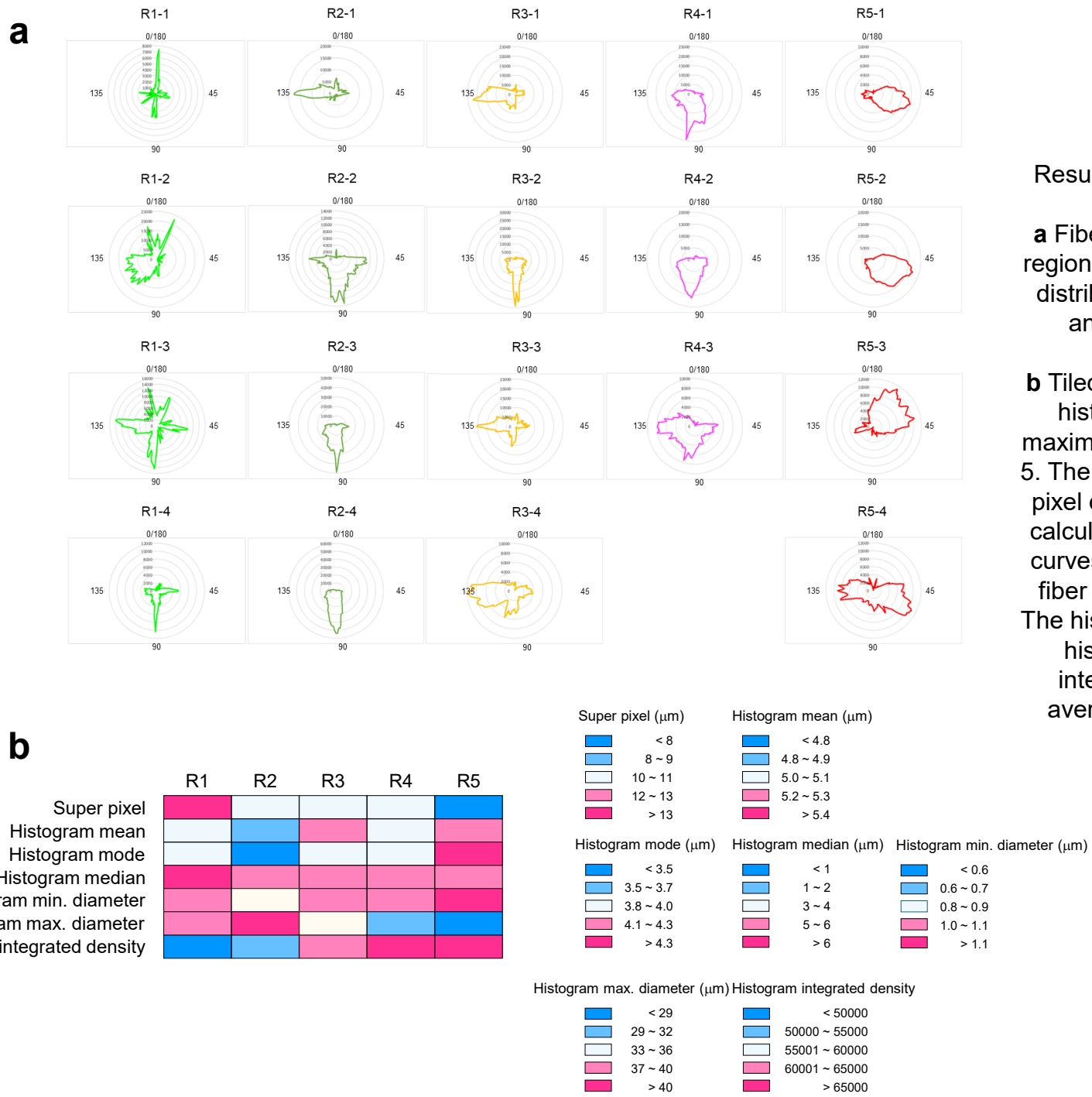
Supplementary Figure 10

Multiplex immunofluorescence images stained for **a** S100 (red) and CK8/18 (green) in Region 1, **b** S100 (red) and Iba-1 (green) in Region 1, **c** S100 (red) and CK8/18 (green) in the Region 4 area where CK8/18 remains expression, **d** S100 (red) and Iba-1 (green) in the Region 4 area where CK8/18 remains expression, **e** S100 (red) and CK8/18 (green) in Region 5, **f** S100 (red) and Iba-1 (green) in Region 5. Scale bars: 100 μm.



Supplementary Figure 11

Tiled immunofluorescence intensity map of Region 1–5. Colors represent fluorescence intensity (FL) values for CD109, Vimentin, PCNA, Active YAP, collagen III (COL III), collagen VI (COL VI), αSMA, TGFβ1-induced (TGFBI), Iba-1, S100, CK8/18, and E-cadherin. For Phospho-Histone H3S10, colors were assigned according to the number of Phospho-Histone H3S10 -positive cells per 10^3 cells. The number of CD3-positive cells per total cells within 1 mm^2, the ratio of CD163-positive cells to Iba-1-positive cells, and the number of Neutrophil elastase-positive cells per total cells within 1 mm^2 were calculated, and the colors were assigned accordingly. Markers related to CD109, extracellular matrix (ECM) remodeling, inflammation, and proliferation exhibited increasing trends, whereas differentiation and cell adhesion markers showed decreasing trends. R1-5: Region1-Region 5.

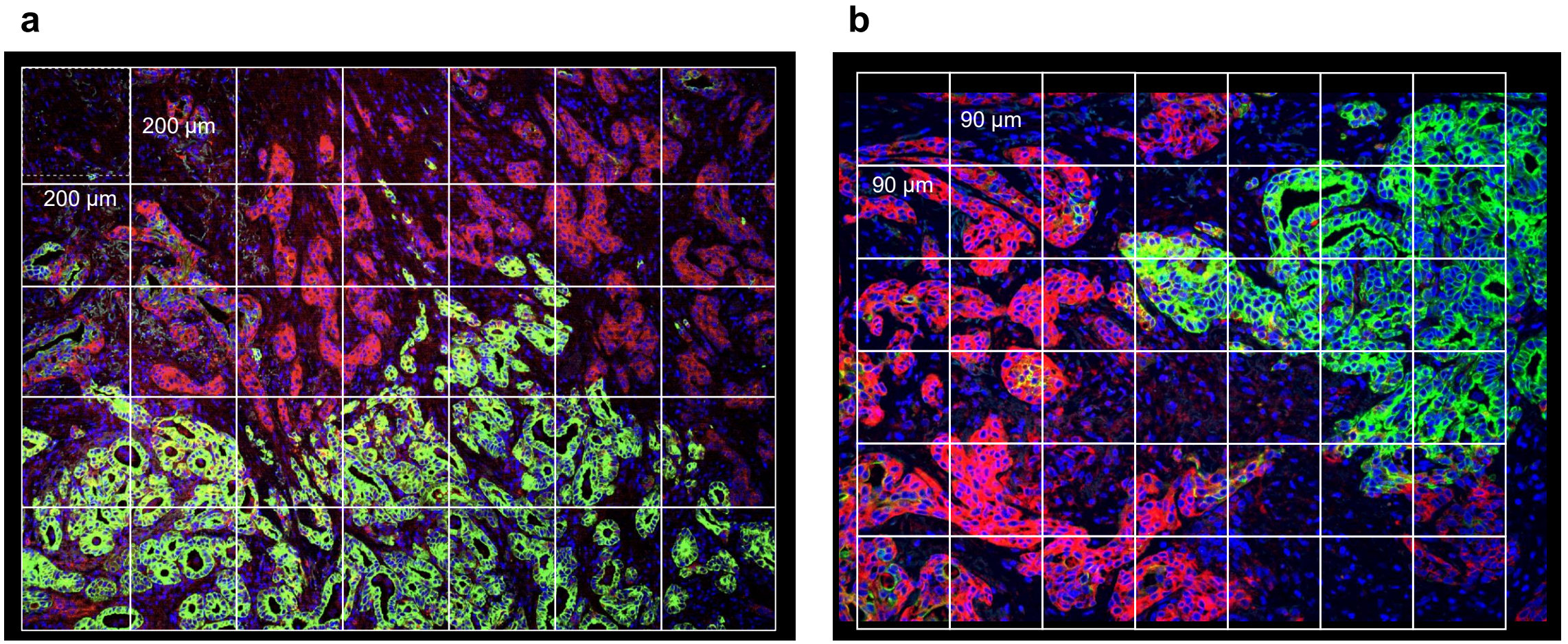


Supplementary Figure 12

Results of qualitative and quantitative analyses of collagen III (COL III) fibers using DiameterJ.

a Fiber orientation distributions of the COL III in Region 1–5, with each region analyzed using 3-4 images. Each polar chart displays the angular distribution of COL III fibers from 0 degree to 180 degree, in which the angle corresponds to fiber orientation and the radius reflects the frequency of fibers detected at each angle.

b Tiled map showing the super pixel, histogram mean, histogram mode, histogram median, histogram minimum (min.) diameter, histogram maximum (max.) diameter and histogram integrated density in Region 1-5. The super pixel is the mean fiber diameter as calculated using a super pixel determination. The histogram mean is the mean fiber diameter as calculated by fitting a Gaussian curve to the radius data and finding the curves mean value. The histogram mode represents the most common fiber diameter, and the histogram median is the middle fiber diameter. The histogram min. diameter is the smallest diameter measured, and the histogram max. is the largest diameter measured. The histogram integrated density is the product of the length of the fibers and the average radius. The assigned colors are defined as indicated. R1-5: Region1-Region 5. See Reference 47 for details.



Supplementary Figure 13

Region of interest (ROI) setting images for the spatial immunofluorescence (SPI) assay.

a A 1400 μm × 1000 μm image stained with CD109 (red) and CK8/18 (green) for the standard SPI assay. Each ROI was 200 × 200 μm in size.

b A 700 μm × 500 μm image stained with CD109 (red) and CK8/18 (green) for the high-density (HD) SPI assay. Each ROI was 90 × 90 μm in size.

a

Marker	Comparison	*p*-value	Significance
CD109	R1-R2	<0.001	***
	R1-R3	<0.001	***
	R1-R4	<0.001	***
	R1-R5	<0.001	***
	R2-R3	<0.001	***
	R2-R4	1	NS
	R2-R5	<0.001	***
	R3-R4	<0.001	***
	R3-R5	<0.001	***
	R4-R5	<0.001	***

b

Marker	Comparison	*p*-value	Significance
CK8/18	R1-R2	<0.001	***
	R1-R3	<0.001	***
	R1-R4	<0.001	***
	R1-R5	<0.001	***
	R2-R3	<0.001	***
	R2-R4	<0.001	***
	R2-R5	<0.001	***
	R3-R4	<0.001	***
	R3-R5	<0.001	***
	R4-R5	<0.001	***

c

Marker	Comparison	*p*-value	Significance
E-cadherin	R1-R2	<0.001	***
	R1-R3	<0.001	***
	R1-R4	<0.001	***
	R1-R5	<0.001	***
	R2-R3	<0.001	***
	R2-R4	<0.001	***
	R2-R5	<0.001	***
	R3-R4	0.002	**
	R3-R5	<0.001	***
	R4-R5	<0.001	***

d

Marker	Comparison	*p*-value	Significance
Vimentin	R1-R2	0.315	NS
	R1-R3	0.949	NS
	R1-R4	1	NS
	R1-R5	<0.001	***
	R2-R3	<0.001	***
	R2-R4	0.018	*
	R2-R5	<0.001	***
	R3-R4	1	NS
	R3-R5	<0.001	***
	R4-R5	<0.001	***

e

Marker	Comparison	*p*-value	Significance
PCNA	R1-R2	0.003	**
	R1-R3	<0.001	***
	R1-R4	<0.001	***
	R1-R5	<0.001	***
	R2-R3	<0.001	***
	R2-R4	<0.001	***
	R2-R5	<0.001	***
	R3-R4	1	NS
	R3-R5	<0.001	***
	R4-R5	<0.001	***

f

Marker	Comparison	*p*-value	Significance
αSMA	R1-R2	<0.001	***
	R1-R3	<0.001	***
	R1-R4	<0.001	***
	R1-R5	<0.001	***
	R2-R3	1	NS
	R2-R4	<0.001	***
	R2-R5	<0.001	***
	R3-R4	<0.001	***
	R3-R5	<0.001	***
	R4-R5	<0.001	***

g

Marker	Comparison	*p*-value	Significance
Active YAP	R1-R2	1	NS
	R1-R3	<0.001	***
	R1-R4	<0.001	***
	R1-R5	<0.001	***
	R2-R3	<0.001	***
	R2-R4	<0.001	***
	R2-R5	<0.001	***
	R3-R4	0.062	NS
	R3-R5	<0.001	***
	R4-R5	<0.001	***

h

Marker	Comparison	*p*-value	Significance
COL Ⅲ	R1-R2	<0.001	***
	R1-R3	<0.001	***
	R1-R4	<0.001	***
	R1-R5	<0.001	***
	R2-R3	<0.001	***
	R2-R4	<0.001	***
	R2-R5	1	NS
	R3-R4	<0.001	***
	R3-R5	<0.001	***
	R4-R5	<0.001	***

i

Marker	Comparison	*p*-value	Significance
COL VI	R1-R2	<0.001	***
	R1-R3	<0.001	***
	R1-R4	<0.001	***
	R1-R5	<0.001	***
	R2-R3	<0.001	***
	R2-R4	<0.001	***
	R2-R5	<0.001	***
	R3-R4	<0.001	***
	R3-R5	<0.001	***
	R4-R5	1	NS

j

Marker	Comparison	*p*-value	Significance
TGFBI	R1-R2	<0.001	***
	R1-R3	<0.001	***
	R1-R4	<0.001	***
	R1-R5	<0.001	***
	R2-R3		NS
	R2-R4	<0.001	***
	R2-R5	<0.001	***
	R3-R4	<0.001	***
	R3-R5	<0.001	***
	R4-R5	<0.001	***

k

Marker	Comparison	*p*-value	Significance
Iba-1	R1-R2	0.003	**
	R1-R3	<0.001	***
	R1-R4	<0.001	***
	R1-R5	<0.001	***
	R2-R3	<0.001	***
	R2-R4	<0.001	***
	R2-R5	<0.001	***
	R3-R4	0.184	NS
	R3-R5	<0.001	***
	R4-R5	<0.001	***

l

Marker	Comparison	*p*-value	Significance
S100	R1-R2	0.108	NS
	R1-R3	<0.001	***
	R1-R4	<0.001	***
	R1-R5	<0.001	***
	R2-R3	<0.001	***
	R2-R4	<0.001	***
	R2-R5	<0.001	***
	R3-R4	<0.001	***
	R3-R5	<0.001	***
	R4-R5	<0.001	***

m

Marker	Comparison	*p*-value	Significance
POSTN	R1-R2	<0.001	***
	R1-R3	<0.001	***
	R1-R4	<0.001	***
	R1-R5	<0.001	***
	R2-R3	0.051	NS
	R2-R4	<0.001	***
	R2-R5	<0.001	***
	R3-R4	<0.001	***
	R3-R5	<0.001	***
	R4-R5	1	NS

Supplementary Table 1

Results of Bonferroni's post-hoc tests for pairwise comparisons among the regions (R1-5).
The markers are **a** CD109, **b** CK8/18, **c** E-cadherin, **d** Vimentin, **e** PCNA, **f** αSMA, **g** Active YAP, **h** Collagen III (COL III), **i** Collagen VI (COL VI), **j** TGFβ1-induced (TGFBI), **k** Iba-1, **l** S100, and **m** Periostin (POSTN). *p<0.05, **p<0.01, ***p<0.001. NS: Not significant.

a

	Frequency/mm^2		
	Phospho-Histone H3S10	Neutrophil elastase	CD3
R1	1.27 ± 0.35	0.90 ± 0.57	90.8 ± 20.7
R2	2.19 ± 0.64	2.73 ± 0.29	130.6 ± 34.4
R3	1.16 ± 1.14	1.88 ± 1.68	130.6 ± 43.2
R4	2.10 ± 0.61	37.2 ± 16.5	207.7 ± 16.3
R5	6.26 ± 0.65	1.43 ± 0.60	337.2 ± 62.2

Significance brackets: R1–R2: *; R1–R5: ***; R2–R4: **; R3–R4: **; R3–R5: ***; R4–R5: ***

b

	% of positive cells/Iba-1-positive cells	
	CD163	CD80
R1	27.5 ± 8.04	0.042 ± 0.025
R2	54.9 ± 11.5	0.133 ± 0.049
R3	19.6 ± 13.3	0.028 ± 0.0056
R4	14.1 ± 5.16	0.019 ± 0.0038
R5	40.85 ± 3.86	0.0015 ± 0.0059

Significance brackets: R1–R2: **; R1–R5: *; R2–R4: *; R3–R5: *; R1–R2: *

c

	% of Active YAP-positive cells						
	CK18+ and/or CD109+		CK18+	CD109+	CK18+CD109+	CAF	
R1	33.4 ± 23.0	*	33.4 ± 23.0	-	-	3.1 ± 1.4	***
R2	6.8 ± 3.1		6.8 ± 3.1	-	-	3.1 ± 1.1	
R3	71.3 ± 5.8	**	34.7 ± 21.9	81.4 ± 7.1	83.1 ± 21.9	25.8 ± 19.0	***
R4	55.4 ± 10.9		-	55.4 ± 10.9	-	60.3 ± 7.4	
R5	87.9 ± 5.4		-	87.9 ± 5.4	-	81.6 ± 4.3	

Supplementary Table 2

Quantitative analysis of Phospho-Histone H3S10-, Neutrophil elastase-, CD3-, CD163-, CD80-, and active YAP-positive cells across Region 1-5. The proportions of marker-positive cells were compared among regions to evaluate changes in cell proliferation, immune cell infiltration, and YAP activation during anaplastic transformation.

Data are presented as mean ± standard deviation (SD). R: Region. * $p<0.05$, ** $p<0.01$, ***$p<0.001$ by Welch's *t*-test.

Metric	R1		R2		R3		R4		R5	
	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
Super Pixel (um)	15.5743917	10.5644468	10.906455	1.18756497	10.70296	1.90867125	11.4124778	1.11290462	7.75627	1.32767257
Histogram Mean (um)	5.18497	0.75306221	4.97638	0.50268315	5.32423	0.3079861	5.17744722	0.35877681	5.23671833	0.92402603
Histogram SD (um)	2.883885	0.83409419	2.91295667	0.58247891	2.71544333	0.26335828	2.55392778	0.31379792	2.31671167	0.39171701
Histogram Mode (um)	3.88034	0.28532	3.3097	4.44E-16	4.023	0	4.023	0	4.4138	0.7816
Histogram Median (um)	6.07193	1.07266688	5.81955333	0.52076057	5.86686667	0.21204126	5.61541667	0.42738282	5.51722	0.97704
Histogram Min. Diameter (um)	0.97004	0.07132	0.8274	1.11E-16	1.0057	0	1.0057	0	1.10342	0.19544
Histogram Max. Diameter (um)	37.102085	5.14426564	40.0196983	4.15330208	35.6202133	5.6267951	29.2784111	4.42505965	28.1992267	7.07715886
Histogram Integrated Density	35325.5969	10859.0388	51146.1219	5071.61455	61376.9085	4610.82081	67036.6499	3075.51637	68909.1552	10709.6436
Histogram Raw Integrated Density	35325.5969	10859.0388	51146.1219	5071.61455	61376.9085	4610.82081	67036.6499	3075.51637	68909.1552	10709.6436
Diameter Skewness	1.78946833	0.40872951	1.848005	0.29312923	1.65601167	0.39156051	1.45294444	0.19548143	1.44733333	0.1473094
Diameter Kurtosis	4.217125	2.05528802	5.01332	1.76808042	4.91986333	3.12774852	3.48343056	1.3173735	3.370515	0.94331252
Fiber Length (um)	4622.22133	1590.71817	5854.93598	509.282772	9010.63936	705.24868	10674.3854	1120.5335	12015.0532	1938.80085
Mean Pore Area (um^2)	1874.71318	3030.7378	337.144972	243.479532	182.995933	26.6034354	126.166356	24.7006424	146.41501	42.4661394
Pore Area SD (um^2)	5704.04475	6755.77085	1629.33371	1033.42512	932.24636	222.886775	495.312922	130.56432	540.86377	192.147987
Min. Pore Area (um^2)	8.99409333	6.81271868	2.105245	0.10269	3.0346	3.44E-16	3.0346	3.63E-16	3.76736	1.46552
Max. Pore Area (um^2)	42145.9272	48035.7929	15646.5933	8231.62511	13175.5348	3761.07404	5916.06483	2223.30174	7302.27952	3472.79983
Percent Porosity	0.783315	0.09153397	0.53078167	0.00820924	0.55841167	0.02630392	0.54454444	0.01100717	0.57154	0.01313367
Number of Pores	76.45	55.6239257	148.366667	42.8771047	271.666667	59.3815581	288.583333	14.44001	380.816667	37.2303789
Number of Intersections	304.566667	202.366269	602.183333	106.376068	923.366667	117.216059	1091.69444	98.7326247	1302.93333	78.8034545
Intersection Density (Intersections/um^2)	0.00088667	0.00051698	0.00242833	0.00043229	0.00251	0.00033526	0.00298056	0.00027473	0.00315167	0.00076693
Characteristics Length (um)	16.6301133	4.08074949	12.4590333	1.30177694	12.04078	0.35445507	11.1776528	0.67047576	11.2640567	2.03118485
SD Characteristics Length (um)	17.8061767	4.9962699	11.2316633	1.91718333	10.322155	0.6900543	9.31208056	0.69170127	8.6985	1.58087796
Max. Span Length (um)	140.796923	30.9827622	100.592803	23.9119099	109.023162	41.1904287	88.5988361	9.25579844	74.679085	21.2120847
Old Characteristics Length (Total Fiber Length/Number of intersections) (um)	25.335895	2.79450546	19.749965	2.47501087	18.2960467	2.98996182	15.0787611	1.18811288	18.72731	3.28308713

Supplementary Table 3

Results of the quantitative analysis of collagen III (COL III) fibers using DiameterJ, including averages and standard deviations (SD) in Region 1-5. The highlighted metrics are emphasized. The super pixel is the mean fiber diameter as calculated using the super pixel determination. The histogram mean is the mean fiber diameter as calculated by fitting a Gaussian curve to the radius data and finding the curves mean value. Histogram mode is most occurring fiber diameter, and the histogram median represents the middle fiber diameter. Histogram minimum (min.) diameter is the smallest diameter measured, and histogram maximum (max.) diameter is the largest diameter measured. The histogram integrated density is the product of the length of the fibers and the average radius. R1-5: Region1-Region 5. See Reference 47 for details.

a

Antibodies	Dilution	Cat. number	Source
Anti-CD109 (E4I2V) rabbit monoclonal	1:250	24765	Cell Signaling Technology, Danvers, MA, USA
Anti-keratin K8/K18 (GP11) guinea pig polyclonal	1:500	GP11	Progen Biotechnik, Heidelberg, Germany
Anti-CK8 (TROMA-1) rat monoclonal	1:100	MABT329	EMD Millipore Corp., Temecula, CA, USA
Anti-E-cadherin (36/E-cadherin) mouse monoclonal	1:50	610182	BD Biosciences, Tokyo, Japan
Anti-vimentin chicken polyclonal	1:500	POLY29191	BioLegend, San Diego, CA, USA
Anti-PCNA (PC-10) mouse monoclonal	1:100	307902	BioLegend, San Diego, CA, USA
Anti-phospho-Histone H3 Serine10 rabbit polyclonal	1:100	06-570	EMD Millipore Corp., Temecula, CA, USA
Anti-α-Smooth Muscle Actin antibody (4A4) mouse monoclonal	1:200	ab19952	Abcam, Cambridge, UK
Anti-active YAP1 rabbit monoclonal	1:100	ab205270	Abcam, Cambridge, UK
Anti-collagen III rabbit polyclonal	1:100	ab7778	Abcam, Cambridge, UK
Anti-collagen VI rabbit polyclonal	1:50	ab182744	Abcam, Cambridge, UK
Anti-IBA 1 Guinea Pig (Gp311H9) monoclonal	1:100	HS-234	SYSY, Gottingen, Germany
Anti-S100 rabbit polyclonal	1:100	Z0311	DAKO, Glostrup, Denmark
Anti-Periostin (1A11A3) mouse monoclonal	1:100	66491-1-Ig	Proteintech, Rosemont, IL, USA
Anti-TGFBI (3E11D11) mouse monoclonal	1:100	60007-1-Ig	Proteintech, Rosemont, IL, USA
TUNEL assay kit		64936	Cell Signaling Technology, Danvers, MA, USA
Anti-phospho-Histone H2AX (Ser139) rabbit monoclonal	1:100	9718	Cell Signaling Technology, Danvers, MA, USA
Anti-CD80/B7-1 mouse monoclonal	1:100	66406-1-Ig	Proteintech, Rosemont, IL, USA
Anti-CD163 rabbit polyclonal	1:100	ab182422	Abcam, Cambridge, UK
Anti-CD3 (SP7) rabbit monoclonal	1:50	ab16669	Abcam, Cambridge, UK
Anti-Neutrophil elastase rabbit polyclonal	1:100	27642-1-Ig	Proteintech, Rosemont, IL, USA

b

Antibodies	Dilution	Cat. number	Source
Alexa Fluor 488 goat anti-rabbit IgG (H+L) cross-adsorbed secondary antibody	1:200	A-11008	Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA
Alexa Fluor 555 Plus goat anti-rabbit IgG (H+L) cross-adsorbed secondary antibody	1:200	A-32732	Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA
Alexa Fluor 647 Plus goat anti-rabbit IgG (H+L) highly cross-adsorbed secondary antibody	1:200	A-32733	Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA
Alexa Fluor Plus 555 goat anti-mouse IgG (H+L) highly cross-adsorbed secondary antibody	1:200	A32727	Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA
Alexa Fluor 647 Plus goat anti-mouse IgG (H+L) highly cross-adsorbed secondary antibody	1:200	A32728	Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA
Alexa Fluor 488 goat anti-guinea pig IgG (H+L) highly cross-adsorbed secondary antibody	1:200	A-11073	Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA
Alexa Fluor 647 goat anti-guinea pig IgG (H+L) highly cross-adsorbed secondary antibody	1:200	A-21450	Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA
Alexa Fluor Plus 800 goat anti-guinea chicken IgY (H+L) cross-adsorbed secondary antibody	1:200	A32935	Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA
Alexa Fluor 488 Plus goat anti-rat IgG (H+L) cross-adsorbed secondary antibody	1:200	A-48262	Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA
DyLight 800 goat anti-rat IgG (H+L) cross-adsorbed secondary antibody	1:200	SA5-10024	Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA

Supplementary Table 4

Lists of antibodies used in this study.

a List of primary antibodies, including dilution, catalog (cat.) number, and source.

b List of secondary antibodies, including dilution, catalog (cat.) number, and source.

Supplementary animation (Provided as a separate file)

An animation of the high-density (HD) SPI assay showing the dynamics of CD109 (red) and CK8/18 (green) expression levels in the region of interest (ROI) column. The x-axis represents consecutive 90 μm sections, and the y-axis represents the mean fluorescence intensity (FL) values.