

SUPPLEMENTARY INFORMATION for

Deciphering the spatial landscape and plasticity of immunosuppressive fibroblasts in breast cancer

Hugo Croizer^{1,2,#}, Rana Mhaidly^{1,2,#}, Yann Kieffer^{1,2,#}, Geraldine Gentric^{1,2}, Lounes Djerroudi^{1,2,3}, Renaud Leclerc³, Floriane Pelon^{1,2}, Catherine Robley^{1,2}, Mylene Bohec^{4,5}, Arnaud Meng^{1,2}, Didier Meseure³, Emanuela Romano⁶, Sylvain Baulande^{4,5}, Agathe Peltier^{1,2}, Anne Vincent-Salomon³ and Fatima Mechta-Grigoriou^{1,2,*}

These authors contributed equally

¹ Institut Curie, Stress and Cancer Laboratory, Equipe labélisée par la Ligue Nationale contre le Cancer, PSL Research University, 26, rue d'Ulm, F-75248 Paris, France

² Inserm, U830, 26, rue d'Ulm, Paris, F-75005, France

³ Department of Diagnostic and Theragnostic Medicine, Institut Curie Hospital Group, 26, rue d'Ulm, F-75248 Paris, France

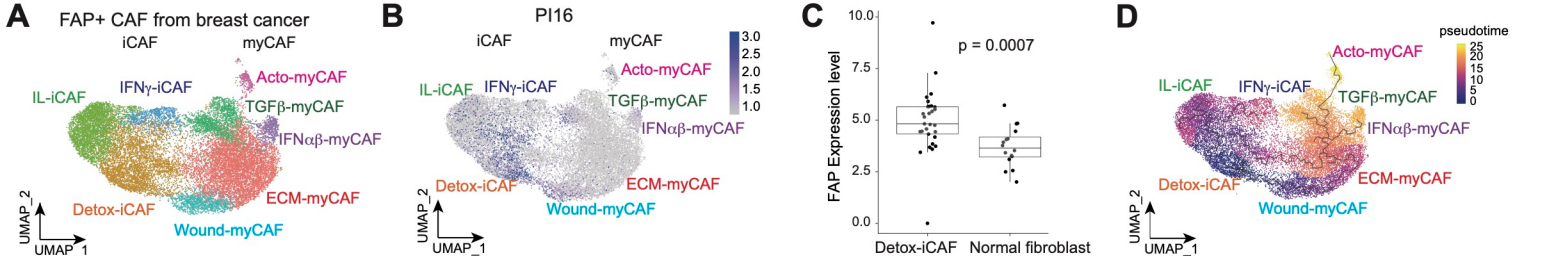
⁴ Institut Curie, PSL Research University, ICGex Next-Generation Sequencing platform, 75005 Paris, France

⁵ Institut Curie, PSL Research University, Single Cell Initiative, 75005 Paris, France

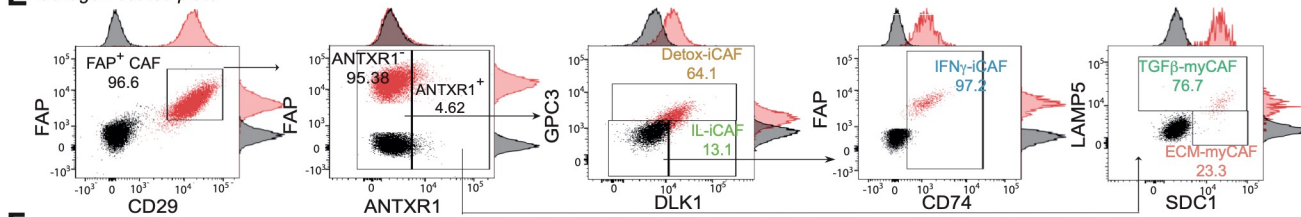
⁶ Department of Medical Oncology, Center for Cancer Immunotherapy, Institut Curie, 26, rue d'Ulm, F-75248 Paris, France

* Corresponding: Fatima Mechta-Grigoriou. Phone: +33 (0)1 56 24 66 53; Fax: +33 (0)1 56 24 66 50; E-mail address: fatima.mechta-grigoriou@curie.fr

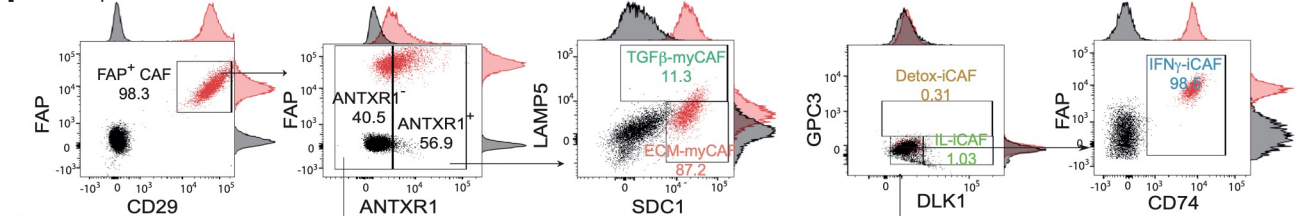
Running title: Topology and plasticity of FAP+ CAF populations



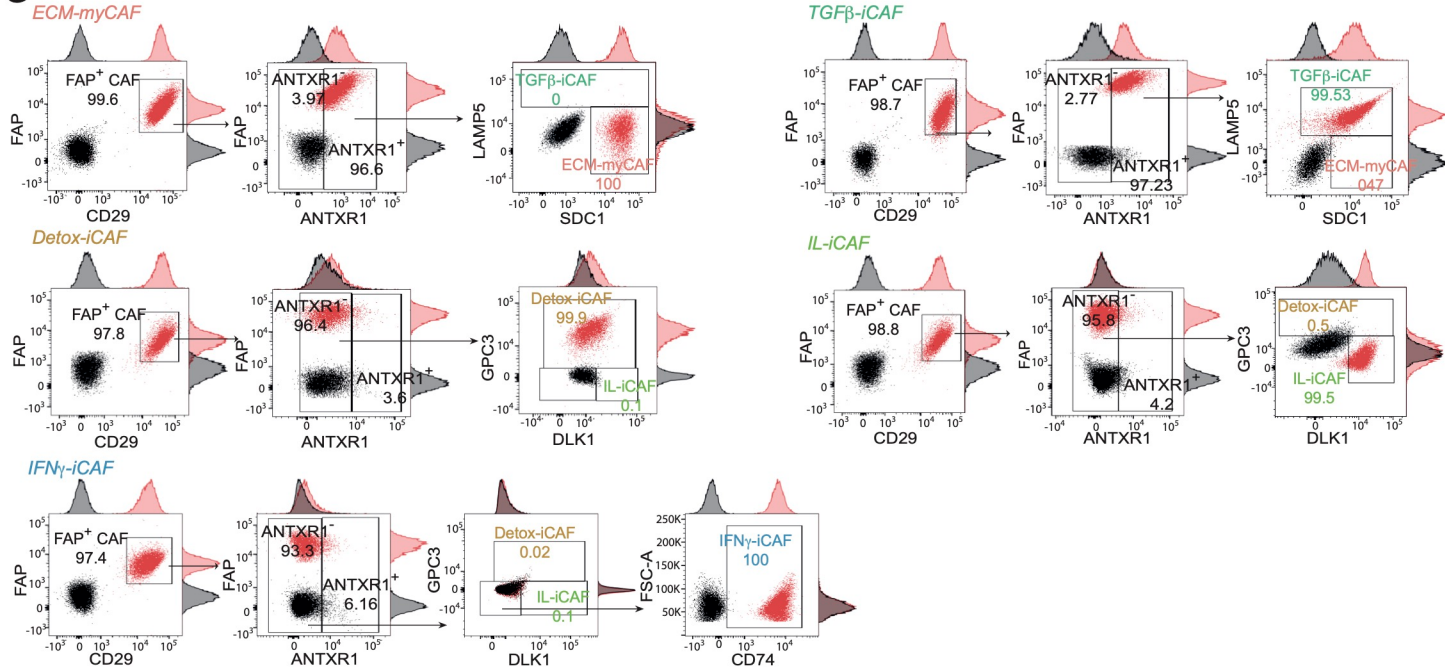
E Collagen-coated plate



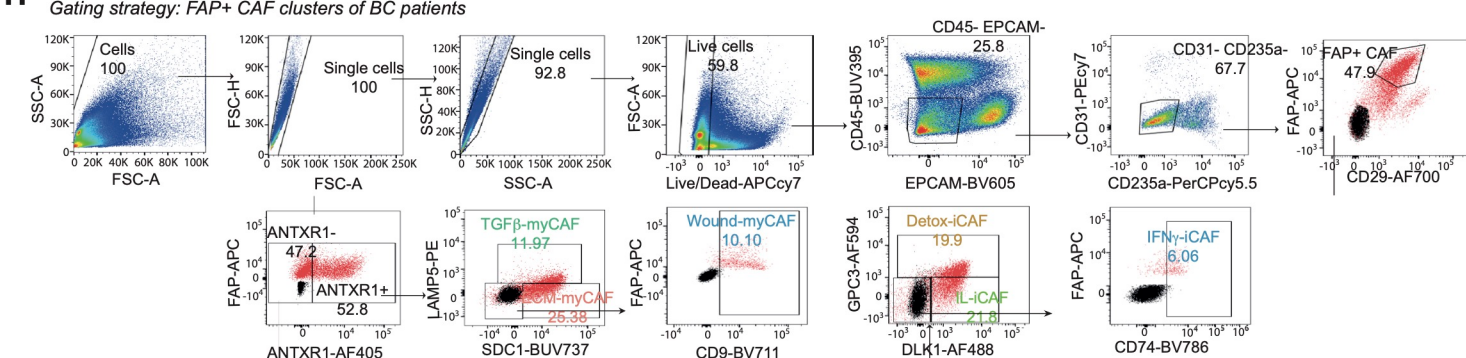
F Plastic plate



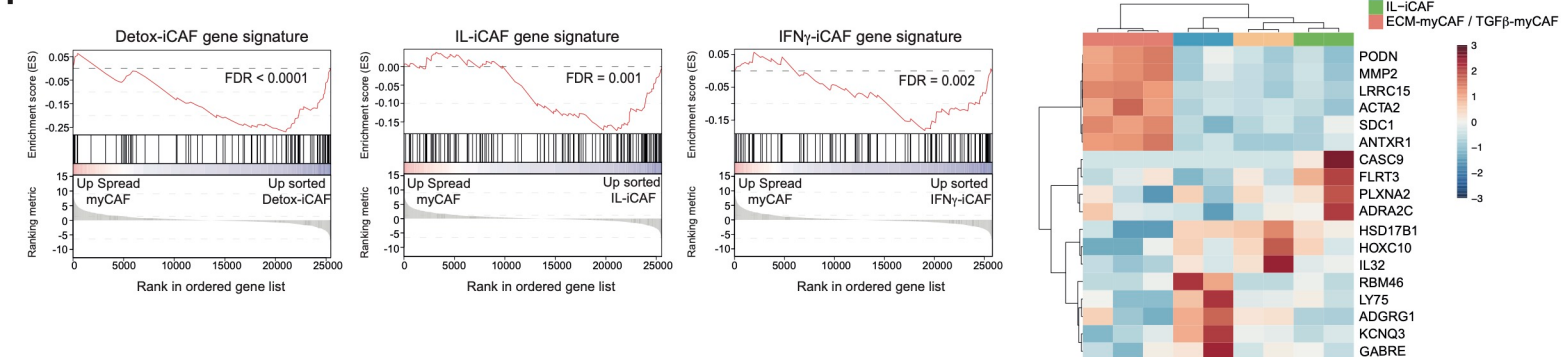
G



H

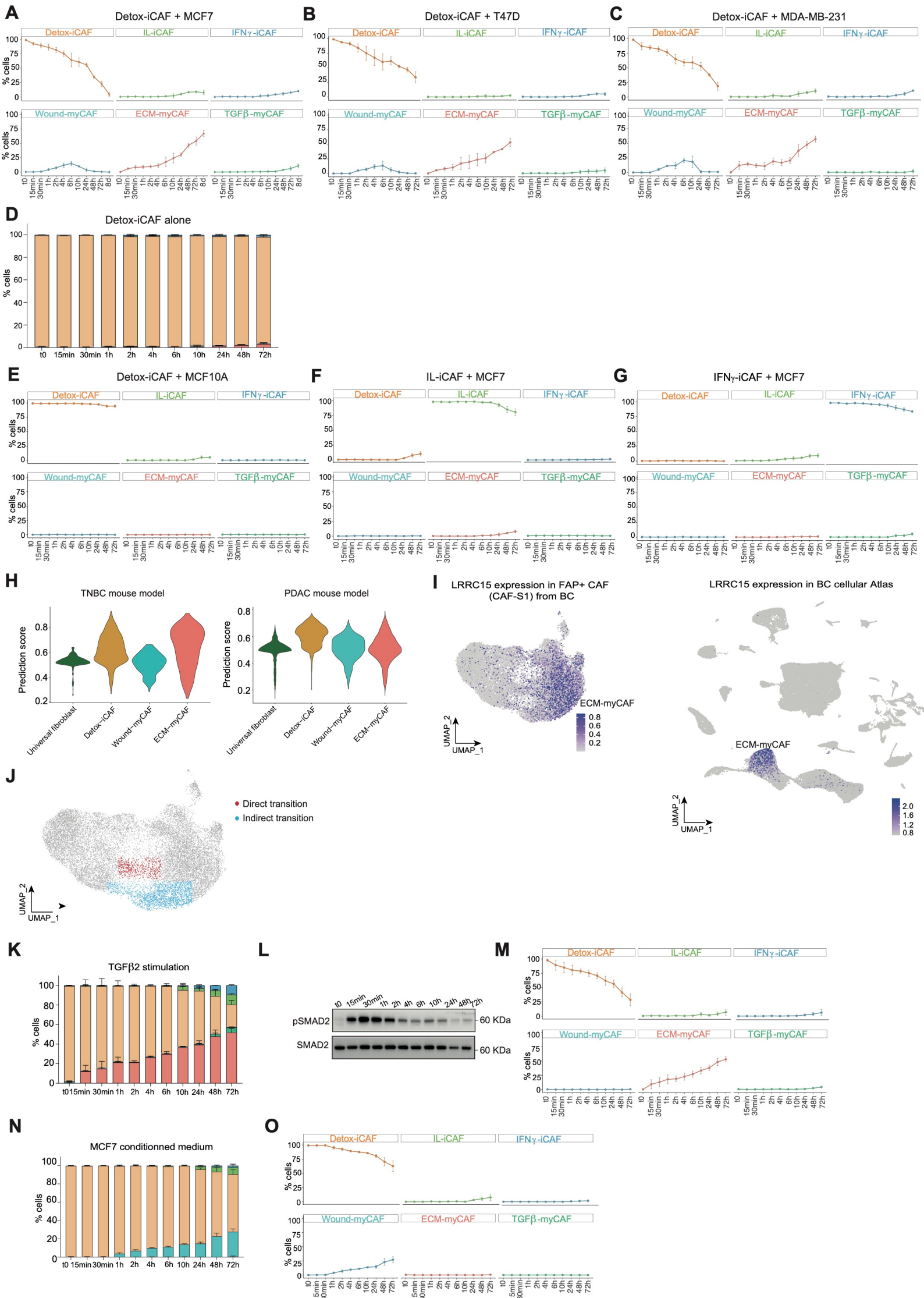


I



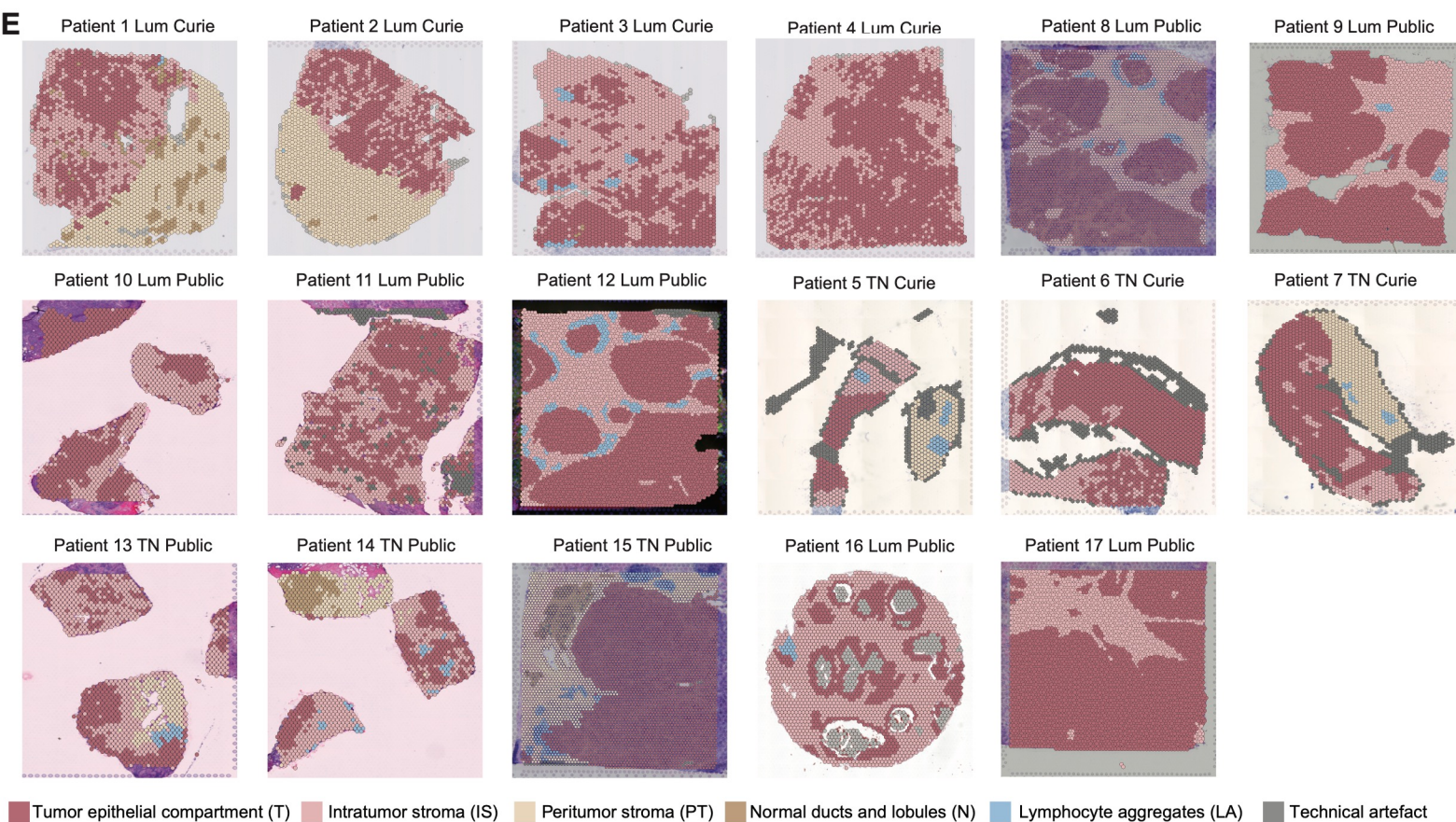
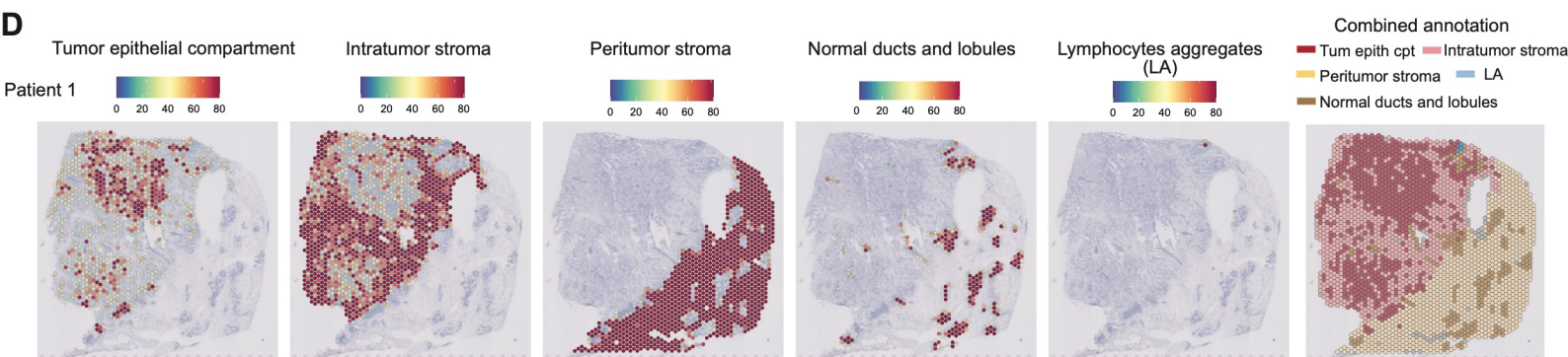
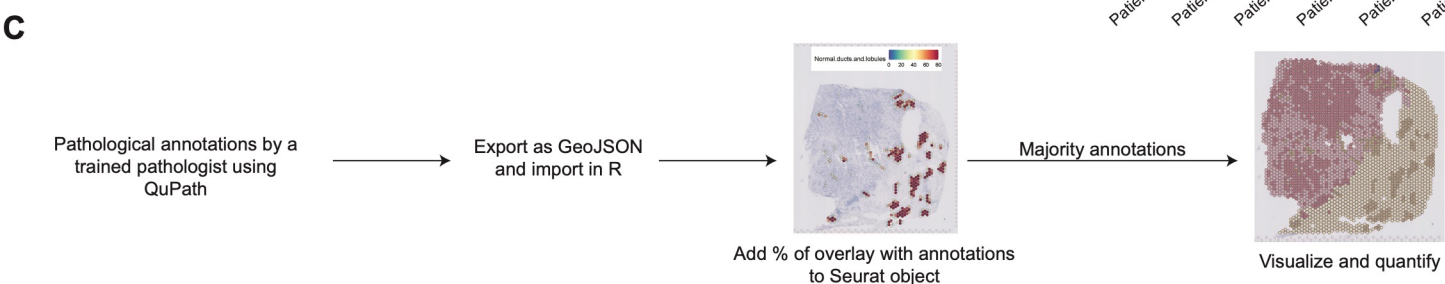
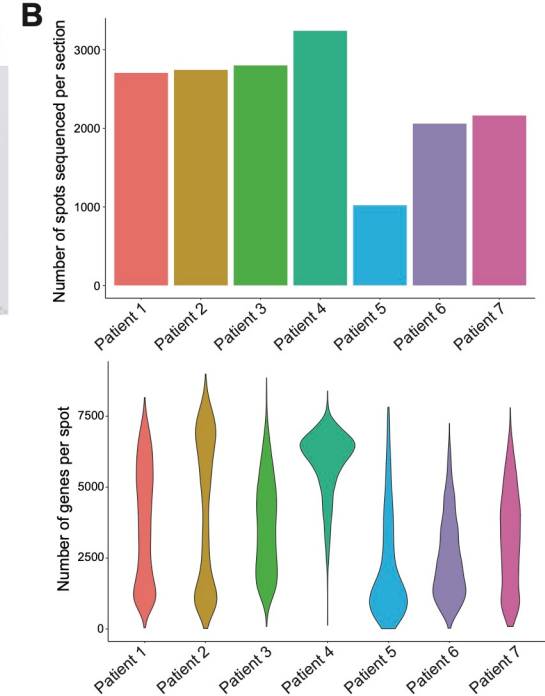
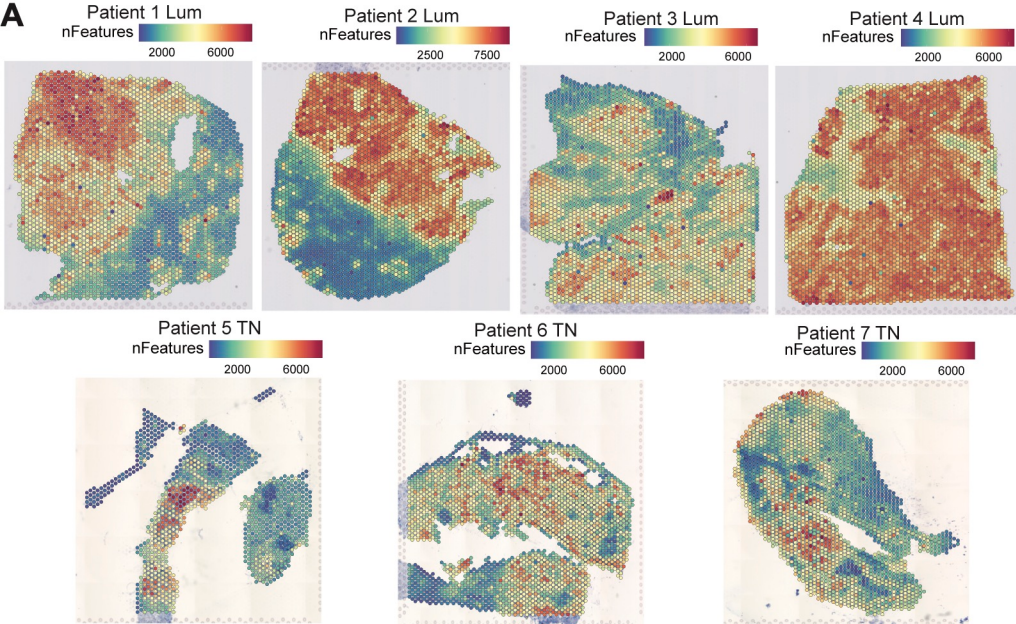
Supplementary Figure 1: FAP+ CAF plasticity origin and characterization of primary clusters by flow cytometry

(A) UMAP of scRNA-seq dataset from 18 297 FAP+ CAF across 7 BC patients. Colors show the different FAP+ CAF clusters defined by graph-based clustering method, as defined in ¹. (B) UMAP showing expression of *PI16* gene. (C) *FAP* expression in Detox-iCAF and in normal fibroblasts after pseudo-bulk reconstruction. P-value from Wald test. (D) UMAP showing pseudotime computed by Monocle 3 with the Detox-iCAF cluster as the root of the trajectory. (E, F) Gating strategy used to characterize primary FAP+ CAF clusters isolated from BC patient samples and cultivated on collagen-coated (E) or plastic (F) dishes. Representative data of 3 independent experiments. (G) Gating strategy used to characterize sorted primary FAP+ CAF from plastic dishes for ECM-myCAF and TGF β -myCAF and from collagen-coated dishes for Detox-iCAF, IL-iCAF and IFN γ -iCAF. This analysis was performed 1 week after cell sorting to allow cell adaptation and proliferation in the aforementioned conditions. All experiments were performed until passage 6 after sorting to avoid cell senescence. FAP+ CAF cells are gated on FAP+ CD29+ fibroblasts. Among FAP+ CAF cells, ANT XR1 staining is used to distinguish inflammatory iCAF (ANT XR1-) from myofibroblastic myCAF (ANT XR1+). Among ANT XR1+ myCAF, SDC1+ LAMP5- were identified as ECM-myCAF, SDC1+LAMP5+ as TGF β -myCAF and SDC1- LAMP5- CD9+ as Wound-myCAF. Among ANT XR1-, DLK1, GPC3 and CD74 protein levels were used to identify Detox-iCAF (DLK1+ GPC3+), IL-iCAF (DLK1+ GPC3-) and IFN γ -iCAF (DLK1- GPC3- CD74+). Representative data of 16 independent experiment using 16 primary FAP+ CAF. (H) FAP+ CAF cluster characterization from fresh BC patient samples. Cells are gated on Live/Dead-, EPCAM-, CD45-, CD31- and CD235a- to exclude dead, epithelial, hematopoietic, endothelial cells and erythrocytes, respectively. FAP+ CAF clusters were then identified using the same gating strategy as described in (E, F). Representative data of 87 patient samples. (I) **Left**, Gene Set Enrichment Analysis (GSEA) plots showing the enrichment score (ES) for Detox-iCAF, IL-iCAF and IFN γ -iCAF gene signatures ¹ in sorted Detox-iCAF (n = 2), sorted IL-iCAF (n = 2) and sorted IFN γ -iCAF (n = 2) clusters, respectively, compared to sorted ECM-myCAF (n = 3) cluster amplified on plastic dishes. **Right**, Heatmap showing differentially expressed genes between sorted CAF-S1 clusters cultured *in vitro*. In all boxplot the center line, box limits and whiskers indicate the median, upper and lower quartiles and 1.5 $\times$ interquartile range.



Supplementary Figure 2: Plasticity of FAP+ CAF clusters upon co-culture with cancer cells and TGF β stimulation

(A) Graphs showing the proportions of the different FAP+ CAF clusters among FAP+ CAF at different timepoints of the co-culture of Detox-iCAF with MCF7 cancer cells. Clusters are identified by flow cytometry analyses using FAP+ CAF cluster-specific markers. $n = 3$ independent experiments. (B) Same as in (A) for the co-culture of Detox-iCAF with T47D cancer cells. (C) Same as in (A) for the co-culture of Detox-iCAF with MDA-MB-231 cancer cells. (D) Bar blots showing the percentages of the distinct FAP+ CAF clusters after culture of primary Detox-iCAF alone, without cancer cells. Timepoints below each bar plot indicate the duration of the culture. Data are mean $\pm$ SEM ($n = 3$). (E) Same as in (A) for the co-culture of Detox-iCAF with MCF10A breast epithelial cells. (F) Same as in (A) for the co-culture of IL-iCAF with MCF7 cancer cells. (G) Same as in (A) for the co-culture of IFN γ -iCAF with MCF7 cancer cells. (H) Violin plots showing prediction scores obtained from label transfer using the BC atlas applied on the BC mouse dataset (Left) ($n = 3363$ cells) and the PDAC mouse dataset (Right) ($n = 44981$ cells). (I) UMAP showing *LRRC15* expression in the ECM-myCAF cluster in FAP+ CAF clusters from BC¹ (Left) and in the BC scRNAseq cellular atlas built in the current study and shown in Fig. 3C (Right). (J) UMAP of the FAP+ CAF dataset from BC highlighting cells implicated in the direct transition from Detox-iCAF to ECM-myCAF (red) and in the indirect transition from Detox-iCAF to Wound-myCAF (blue). (K) Bar plots showing the percentages of each FAP+ CAF cluster after stimulation of the Detox-iCAF cluster (t0) with 10 ng/ml of TGF β 2. Timepoints below each bar plot indicate the duration of the treatment. Data are mean $\pm$ SEM ($n = 3$). (L) Representative western blots showing the phosphorylated isoform (P-SMAD2) and the total SMAD2 protein levels after stimulation of the Detox-iCAF cluster (t0) with 10 ng/ml of TGF β 2. Representative data of 3 independent western blots. (M) Same as in (A) for the stimulation of Detox-iCAF with 10 ng/ml of TGF β 2. (N) Same as in (K) for incubation of Detox-iCAF with MCF-7-derived conditioned medium. (O) Same as in (A) for incubation of Detox-iCAF with MCF-7-derived conditioned medium. Source data are provided as a Source Data file.

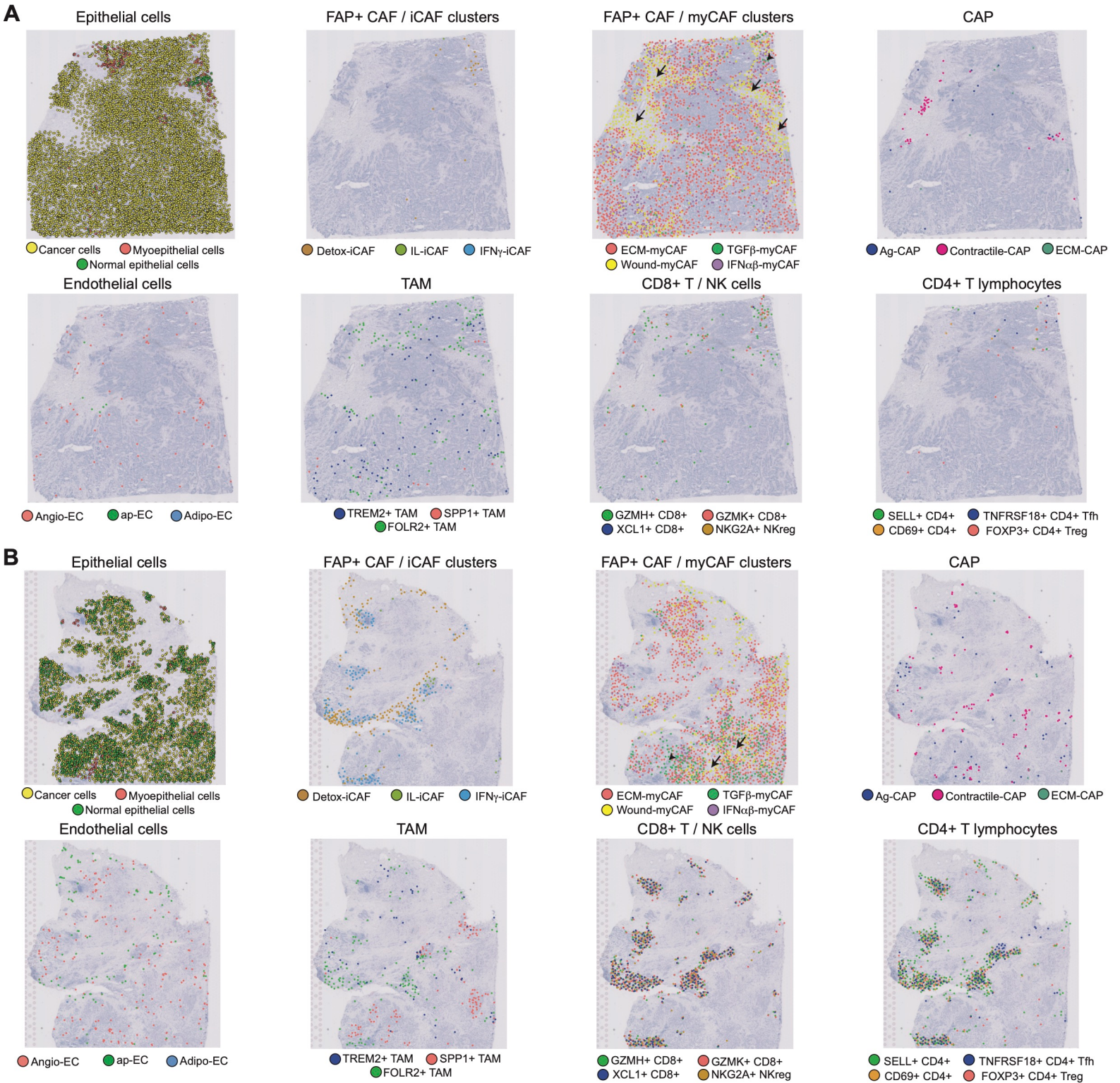


Supplementary Figure 3: Quality control and pathological annotations of spatial breast cancer sections

(A) Number of genes (nFeatures) detected in each spot of the 7 BC sections (4 luminal and 3 triple negative). **(B)** Average number of sequenced spots per section (**Top**) and average number of genes detected (**Bottom**) colored by patient. **(C)** Schematic overview of the workflow allowing the transfer of image-based digital pathologic annotations to the Visium sections for visualization and quantification. Pathological annotation labels were transferred based on a majority area rules, except for tumor epithelial compartment annotation where the label was transferred if more than 30% of the spot covered cancer cells. **(D)** 5 first plots: Visualization of the percentages of overlay between each spot and each pathological annotation in a representative patient (Patient 1). Right plot: Final composite annotations obtained on a majority basis, as detailed in **(C)**. **(E)** Visualization of the 5 pathological annotations on the 17 BC Visium sections.

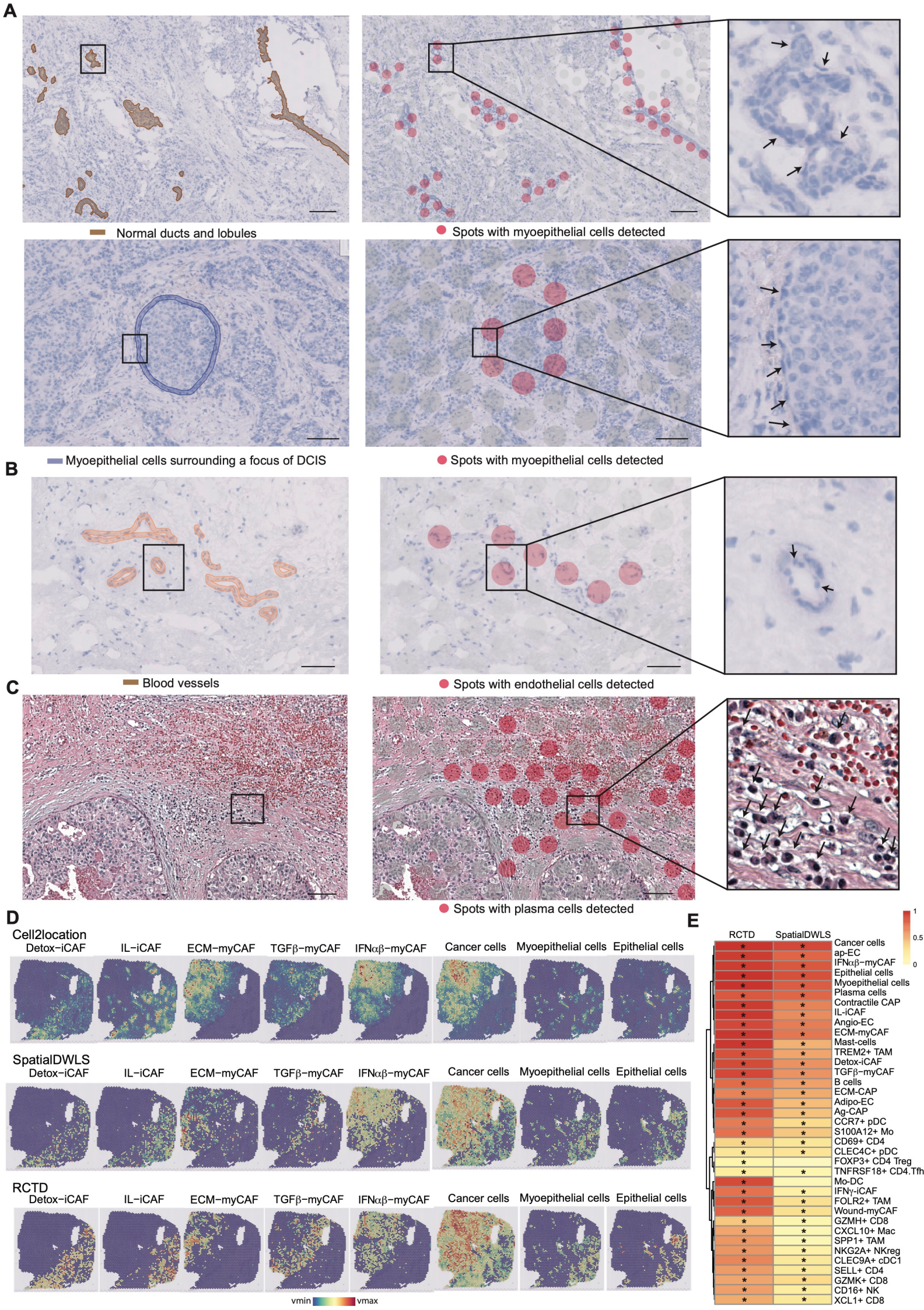
Supplementary Figure 4: Building of a high-resolution breast cancer cellular atlas

(A) Publicly available scRNA-seq datasets ^{2, 3, 4} used for the construction of a human BC cellular atlas that include 73 426 cells from 34 BC patients and 9 healthy donors. (B) UMAP of 5716 CAP across 3 BC patients allowing the visualization of 7 clusters. Colors show the different CAP clusters defined by graph-based clustering. (C) Schematic representation of the 39 different cell types and states defined in the BC cellular atlas. (D) Violin plots showing the prediction score obtained after Label transfer for the annotation of FAP+ CAF and CAP cells from the BC atlas using previously published FAP+ CAF cells ¹ and newly generated CAP from (B). (E) Expression values of specific genes of the 39 cell types and states from the BC atlas. Colors show mean expression level of each gene in each cell type or state. Sizes of circles represent the percentage of cells expressing one given gene. (F) Heatmap showing inferred copy number variation (CNV) for epithelial cells and CAF. scRNA-seq data from normal fibroblasts were used as reference for normalization and are shown in the upper part. Each row corresponds to one cell clustered by cell type, and each column to genes ordered by chromosome position. Amplifications (red) and deletions (blue) are inferred from the gene expression. (G) Heatmap showing the correspondences between original cell type annotations from 2 published BC scRNA-seq datasets ³ (Top) and ⁵ (Bottom) in columns and predicted annotations from Label transfer using BC atlas as reference in rows. Values are centered and scaled in row.



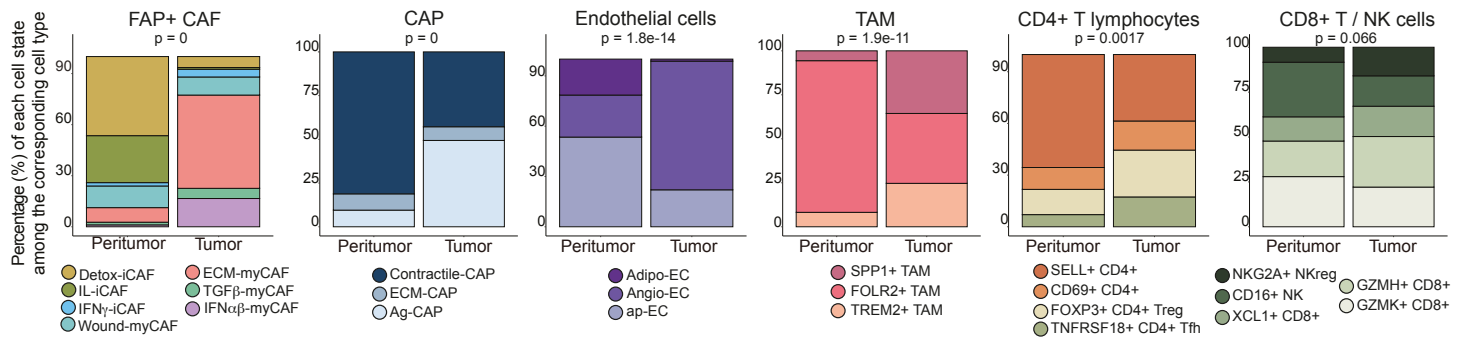
Supplementary Figure 5: Spatial organization of breast cancer micro-environment

(A, B) Deconvolution results based on the scRNA-seq cell atlas showed in **Fig. 3B** displayed on two representative BC sections. Each colored dot corresponds to a single cell and the different colors show distinct cell types and states.



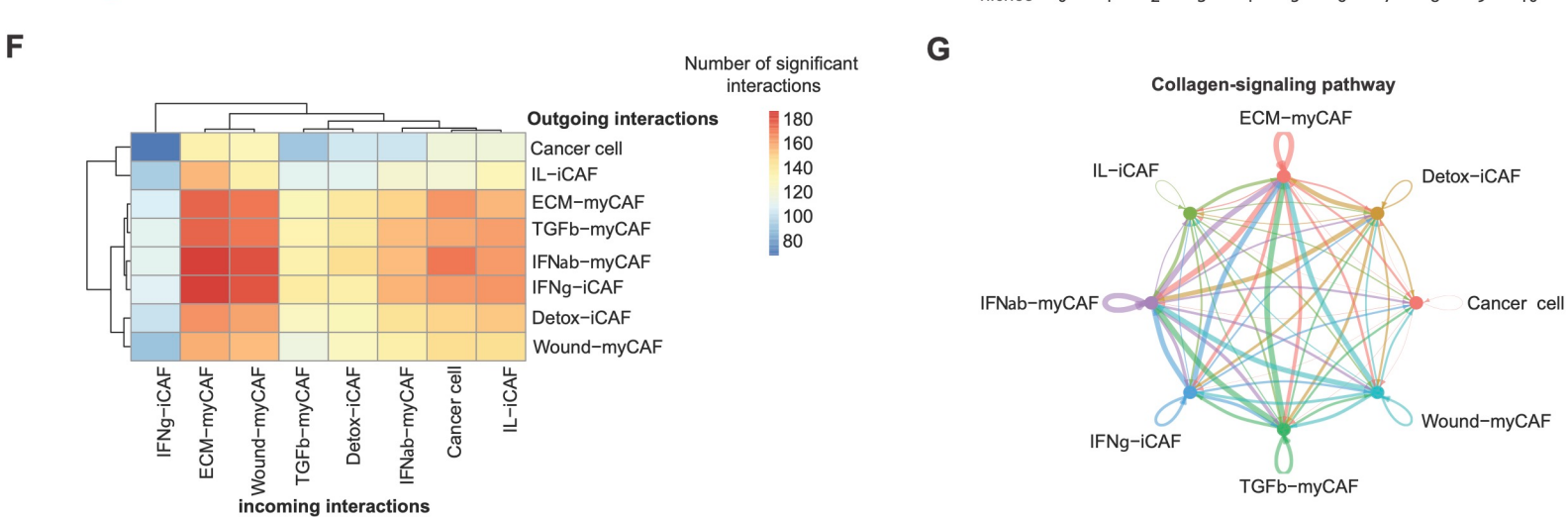
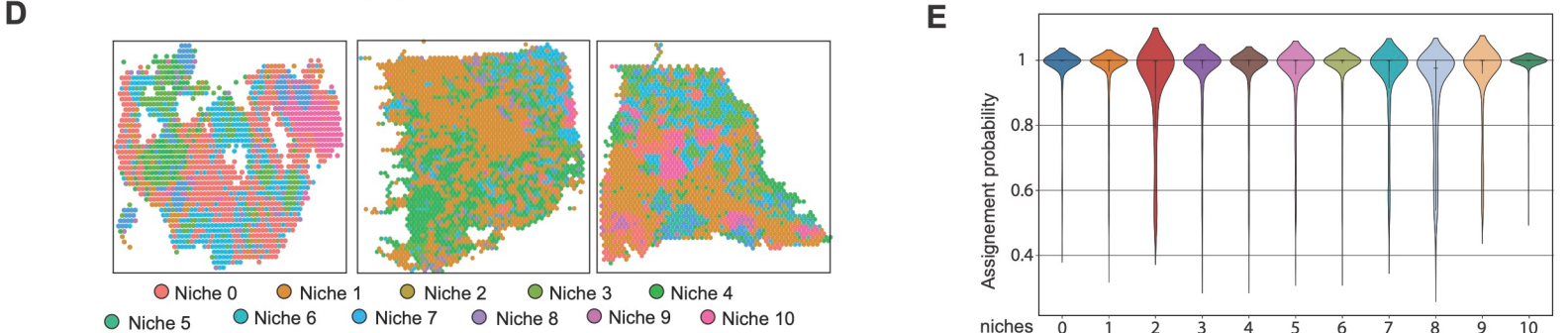
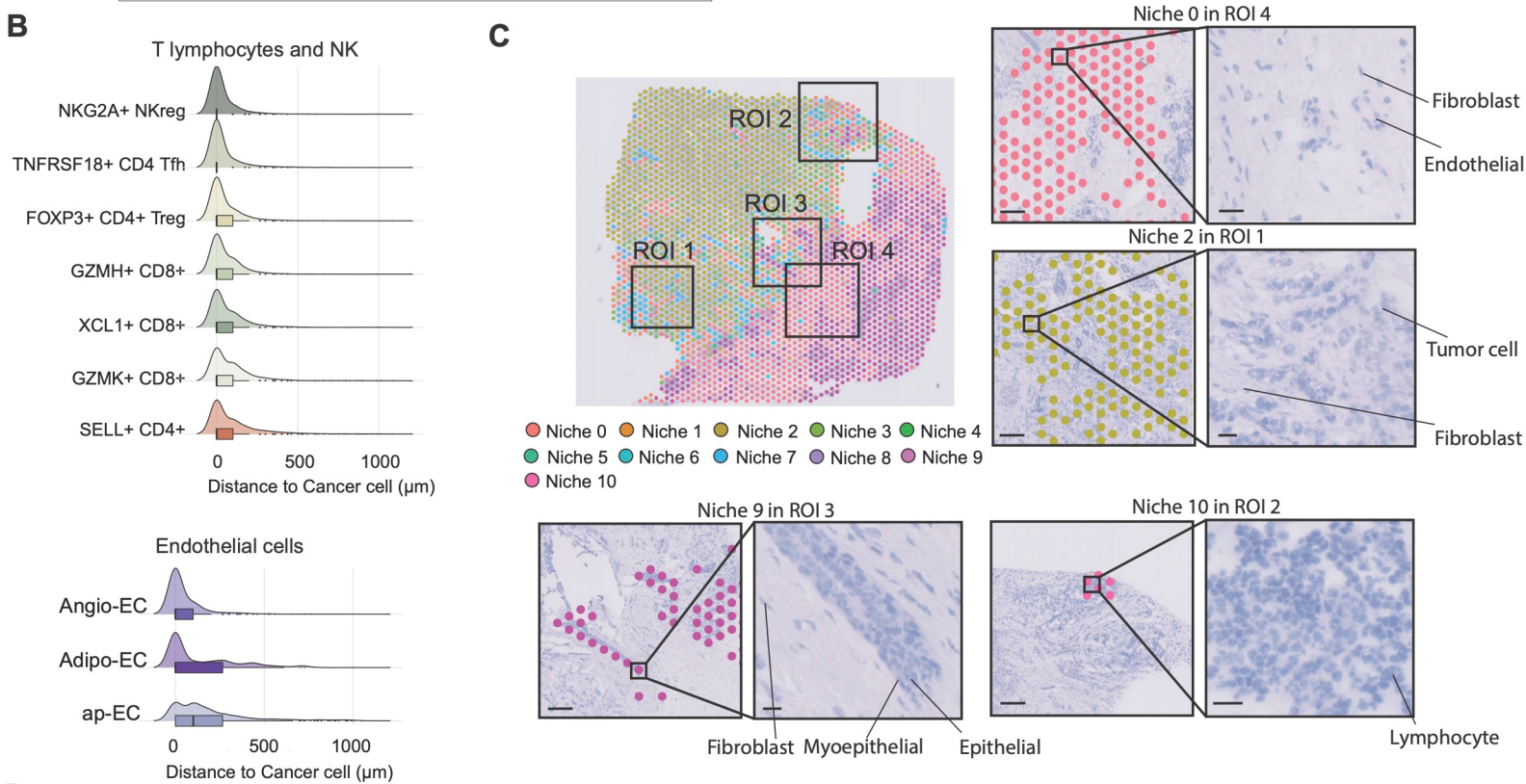
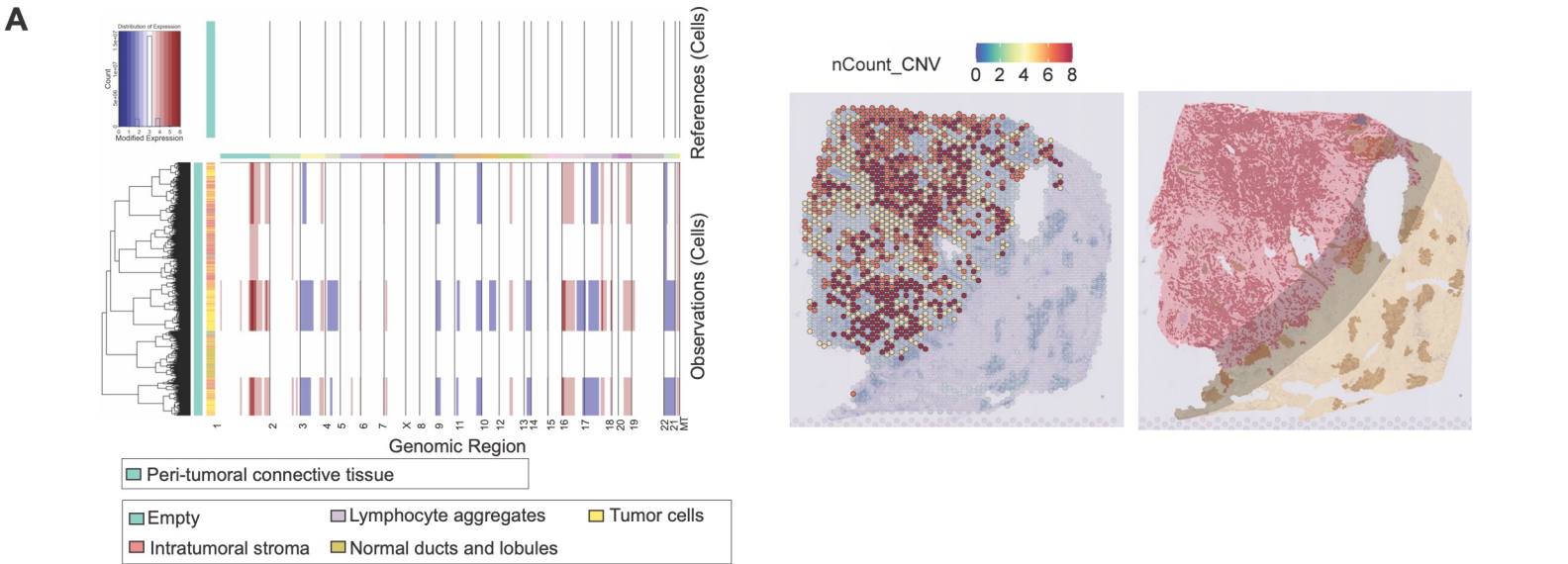
Supplementary Figure 6: Accuracy of cell2location deconvolution compared to pathology morphological annotations

(A) **Left**, Representative H&E images annotated by pathologists based on morphological features showing normal ducts and lobules (**Top**), and myoepithelial cells surrounding a DCIS focus (**Bottom**). **Middle**, Same section analyzed by spatial transcriptomics identifying myoepithelial cells after deconvolution by cell2location. Scale bars: 200 μ m (**Top**) and 100 μ m (**Bottom**). **Right**, Higher magnification of the same area shown in the Middle. Representative image of 17 sections. (B) **Left**, Representative image showing presence of blood vessels identified by pathologists. **Middle** and **Right**, Same section analyzed by spatial transcriptomics identifying endothelial cells after deconvolution. Scale bar = 100 μ m. Representative image of 17 sections. (C) Same as (B) for plasma cells. Scale bar = 100 μ m. Arrows in the magnification panels underline myoepithelial, endothelial and plasma cells under spots where the cell types were detected. Representative image of 17 sections. (D) Comparison of deconvolution results of the section displayed in **Fig. 3C** (representative of the main pathological compartments) for the different cell types and states by using two other deconvolution methods (RCTD and SpatialDWLS). Cell2location values represent the number of deconvoluted cells per spot. RCTD and SpatialDWLS results are expressed as cell type proportions per spot. (E) Data show correlations between Cell2location deconvolution and RCTD or SpatialDWLS methods. The symbol * indicates significant ($P < 0.05$) correlations from Pearson correlation test. Colors show correlation coefficients.



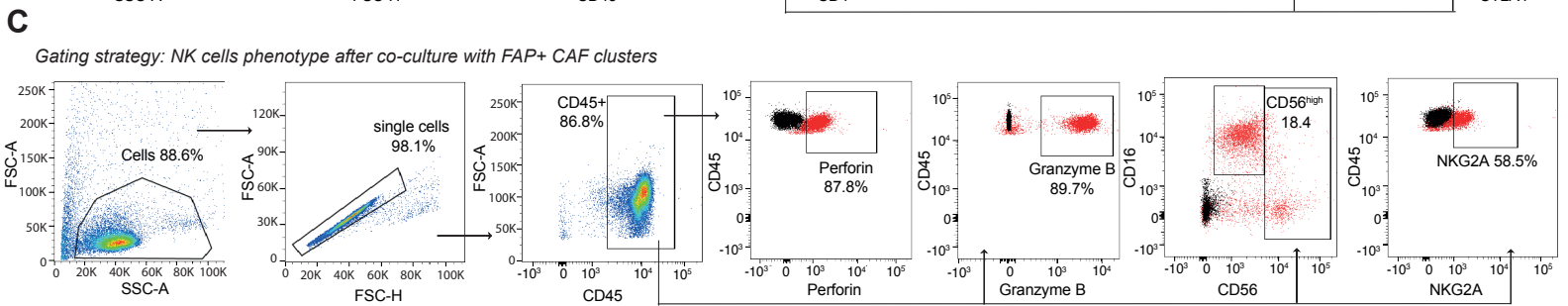
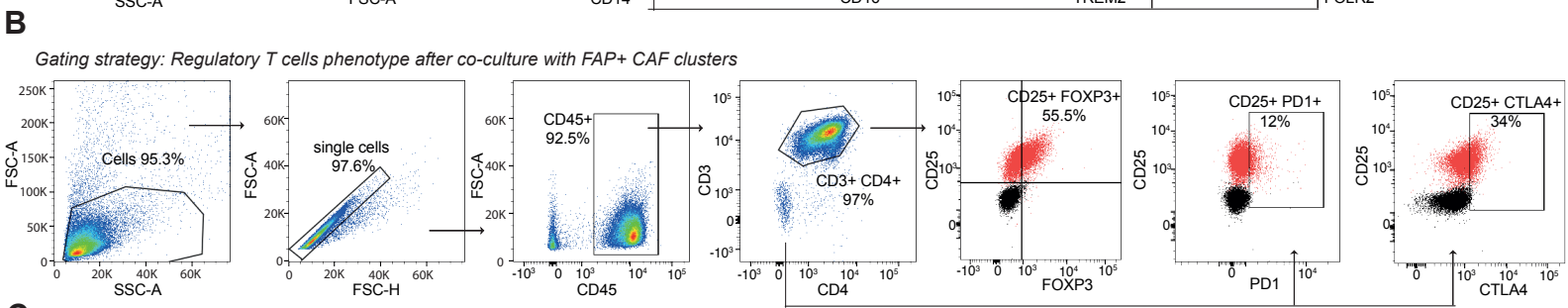
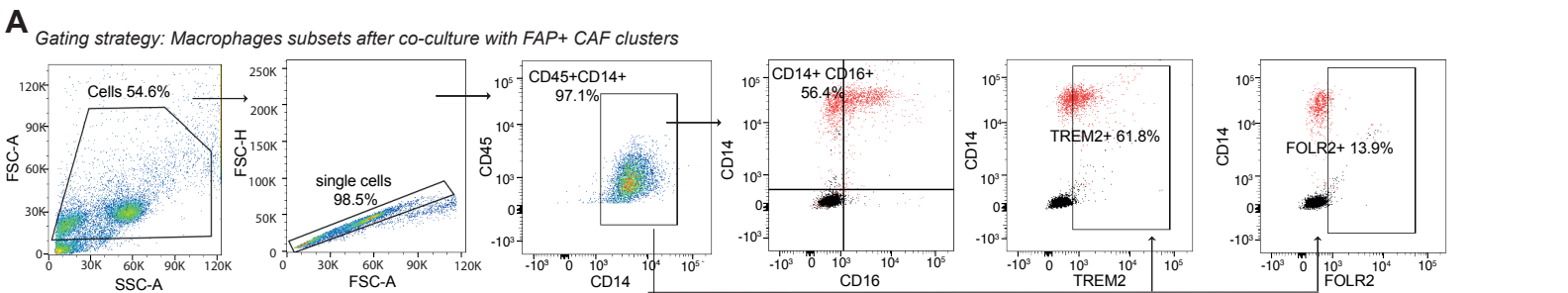
Supplementary Figure 7: Proportion of each cell state between peritumor and tumor compartments

Mean proportion of each cell state between the peritumor and the tumor bed (Tumor epithelial compartment and intratumor stroma) among total FAP+ CAF, CAP, endothelial cells, TAM, CD4+ T lymphocytes and CD8+ T cells and NK cells (N = 17 BC sections for Tumor and N = 7 for Peritumor). P-values from Fisher's exact test.



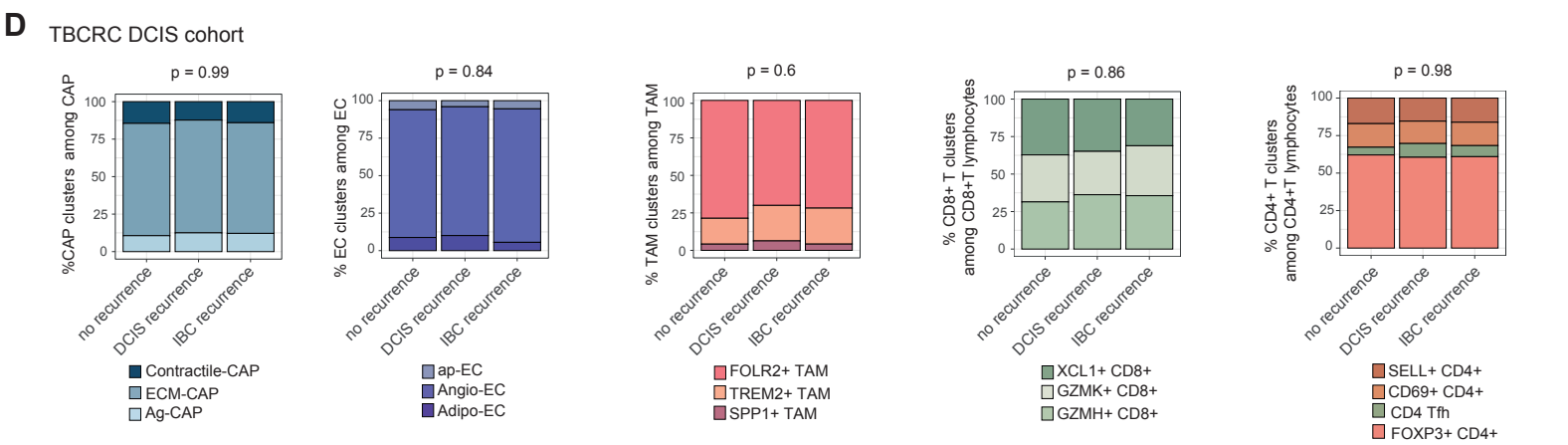
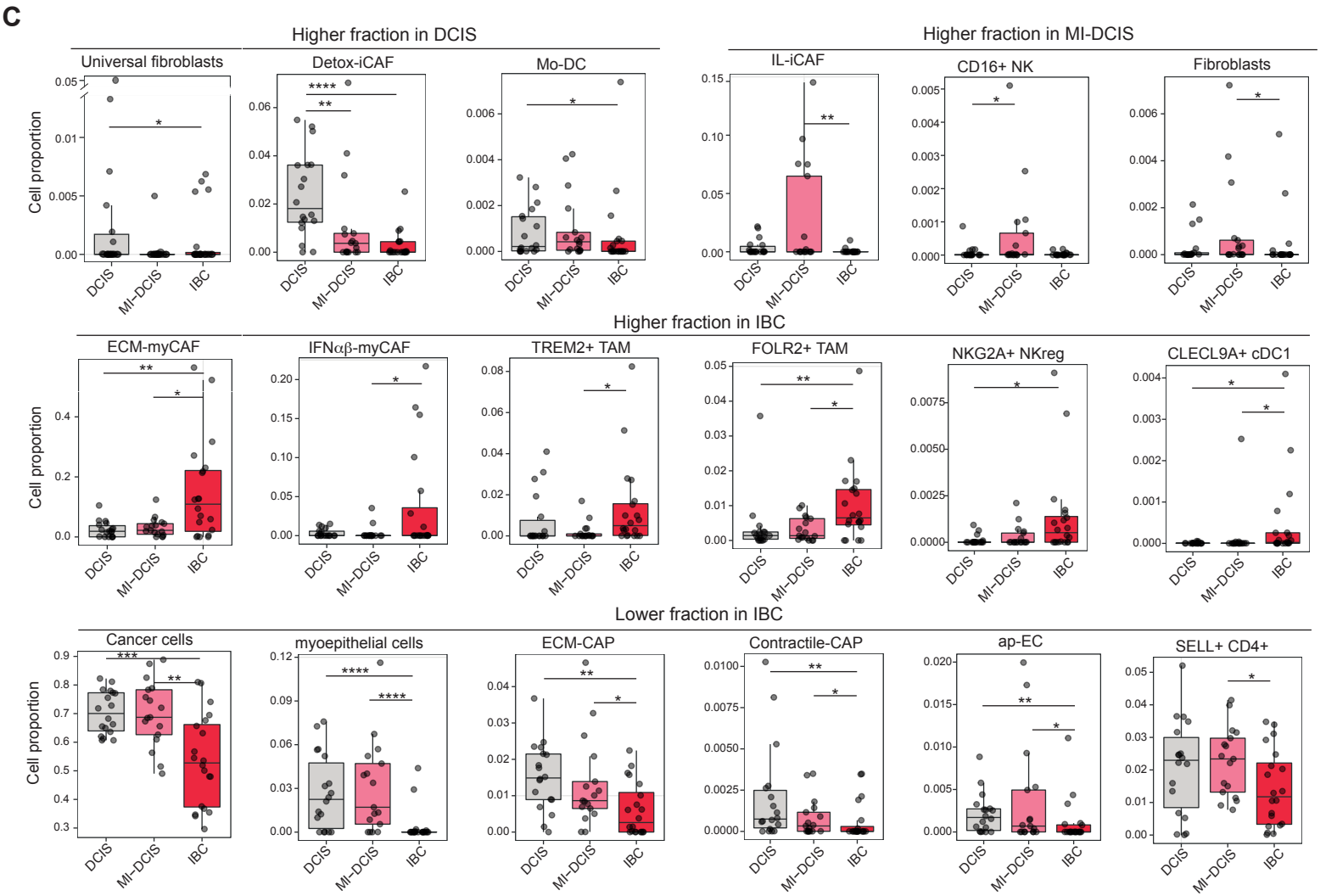
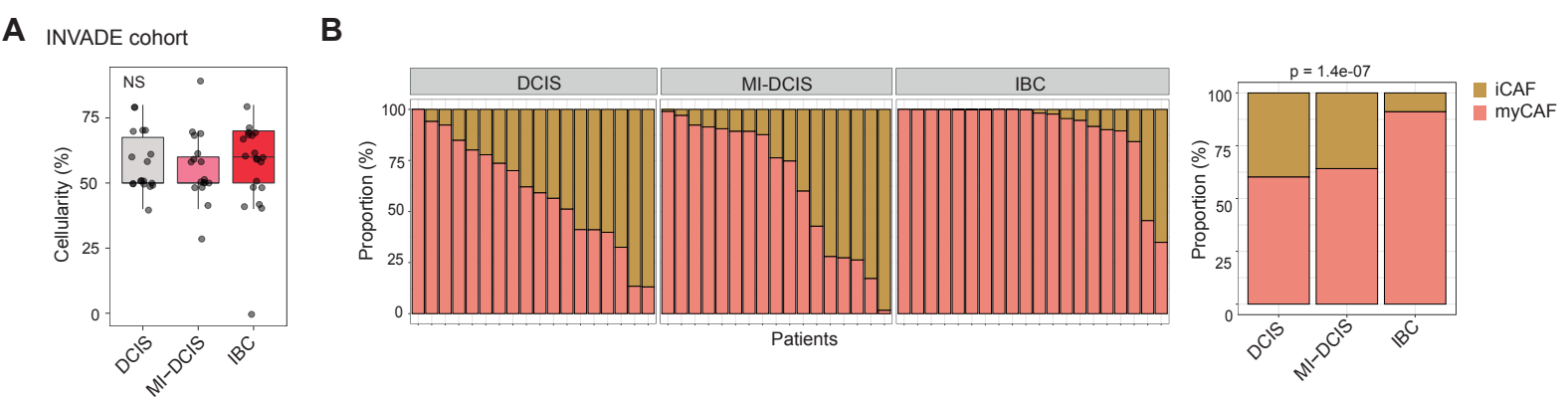
Supplementary Figure 8: Cell-cell distances and communications in breast cancer micro-environment

(A) Left, scRNA-seq derived CNV analysis for each spatial transcriptomic barcoded spot from patient 1, which contained a mixture of stroma and cancer cells. Spot annotated as peritumoral connective tissue were used as reference (red, gain; blue, loss). **Right**, number of CNV detected per spot on the section and matching pathological annotation on the same section. **(B)** Distribution of T lymphocytes, NK and endothelial cells (EC) populations according to the distance to cancer cells (in μm). Distances are computed between the closest cancer cell obtained by deconvolution and T lymphocytes, NK and EC. Cell types are ranked based on their median distance to cancer cells. **(C)** Section displayed in **Fig.3C**. Colors indicate the niches. Black boxes correspond to representative areas (Regions of Interest, ROI) for 4 selected niches shown at 2 different magnifications on the Bottom and on the Right. For each ROI: **Left**: Spots indicating the niche; Scale bar = 150 μm .; **Right**: Corresponding histological section of the niche; Scale bar = 25 μm . **(D)** Predicted BC cellular niches on an external cohort of BC sections⁶ analyzed by spatial transcriptomics. **(E)** Assignment probability of the niches for the spots from the external cohort. **(F)** Number of significant interactions between FAP+ CAF clusters and cancer cells inferred by CellChat. The direction of interactions is represented from the cell type indicated in rows (outgoing interactions) to the cell type indicated in columns (incoming interactions). **(G)** Chord diagram showing inferred Collagen-signaling pathway between FAP+ CAF clusters and cancer cells. Edges width represents the communication probability computed by CellChat, colored by cell type. In all boxplot the center line, box limits and whiskers indicate the median, upper and lower quartiles and $1.5 \times$ interquartile range.



Supplementary Figure 9: Gating strategies used to characterize isolated immune cells after co-culture with FAP+ CAF clusters *in vitro*

(A) Gating Strategy used to characterize TAM subsets after 24h of co-culture with FAP+ CAF clusters. Cells were first gated based on their size (FSC-A) and granularity (SSC-A). Myeloid cells were identified as CD45⁺ CD14⁺ and their phenotype was assessed by flow cytometry for CD16, TREM2 and FOLR2 protein levels. Representative data of 9 independent experiments. (B) Gating strategy used to characterize regulatory T cells among total CD4⁺ CD3⁺ T lymphocytes after 16h of co-culture with FAP+ CAF clusters. Cells were first gated based on their size (FSC-A) and granularity (SSC-A). Among CD45⁺ CD3⁺ CD4⁺ cells, regulatory T lymphocytes were identified based on CD25 and FOXP3 protein levels. The percentage of CTLA4 as well as PD1 were then evaluated. Representative data of 8 independent experiments. (C) Gating strategy used to characterize NK cells after 24h of co-culture with FAP+ CAF clusters. Cells were first gated based on their size (FSC-A) and granularity (SSC-A). Among CD45⁺ hematopoietic cells, the percentage of NKG2A as well as granzyme B and perforin levels were evaluated. NK cells were also separated in two subsets according to CD56 and CD16 protein levels and defined as cytotoxic NK (CD16^{high} CD56^{Med}) and noncytotoxic NK (CD16⁻ CD56^{high}). Representative data of 7 independent experiments.



Supplementary Figure 10: Deconvolution of bulk RNA-seq from retrospective BC cohorts

(A-C) Data from the Invasive BC patient cohort (N = 55). **(A)** Boxplot showing percentage of tumor cellularity across DCIS (N = 18), MI-DCIS (N = 17) and IBC (N = 20) samples. P-value from Welch's t-test. **(B)** Bar plots representing the relative composition in iCAF and myCAF cells among total FAP+ CAF cells per patient **(Left)** or according to BC invasive status (DCIS, MI-DCIS and IBC) **(Right)**. P-value from Fisher's exact test. **(C)** Boxplots of the relative cell type composition across DCIS (N = 18), MI-DCIS (N = 17) and IBC (N = 20) samples. Cell types with significant differences between BC invasive status are represented. P-values from Mann-Whitney test. Symbols for significance: *, $p < 0.05$, **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$. **(D)** Data from the TBCRC cohort of DCIS patients (N = 216 patients, N = 66 with DCIS recurrence, N = 55 with IBC recurrence and N = 95 without recurrence). Bar plots showing the relative proportions of clusters among CAP, endothelial, myeloid and lymphoid cells according to recurrence. P-values from Fisher's exact test. In all boxplot the center line, box limits and whiskers indicate the median, upper and lower quartiles and $1.5 \times$ interquartile range.

Supplementary Table 1

		Prospective Cohort 1 (FACS analysis)	Prospective Cohort 2 (primary cell lines)
Number of patients		84	16
Age at diagnosis			
	< 50 years	28 (33.3%)	8 (50%)
	> or = 50 years	56 (66.7%)	8 (50%)
Multifocality			
	no	51 (60.7%)	7 (43.7%)
	yes	31 (37%)	9 (56.3%)
	NA	2 (2.3%)	0
Histological type			
	ductal	54 (64.3%)	12 (75%)
	lobular	16 (19%)	2 (12.5%)
	other	14 (16.7%)	2 (12.5%)
Grade			
	G1	12 (14.3%)	2 (12.5%)
	G2	45 (53.6%)	10 (62.5%)
	G3	27 (32.1%)	4 (25%)
	NA	0	0
Tumor size			
	pT1	35 (41.7%)	5 (31.1%)
	pT2	41 (48.8%)	11 (68.9%)
	pT3	8 (9.5%)	0
	NA	0	0
Lymph node metastases			
	pN0	56 (66.7%)	7 (43.7%)
	pN1	20 (23.8%)	6 (37.5%)
	pN2	4 (4.7%)	2 (12.5%)
	pN3	2 (2.4%)	1 (6.3%)
	NA	2 (2.4%)	0
ER status			
	ER-	5 (6%)	0
	ER+	79 (94%)	16 (100%)
PR status			
	PR-	15 (17.8%)	3 (18.75%)
	PR+	69 (82.2%)	13 (81.25%)
HER2 status			
	Negative	81 (96.4%)	15 (93.75%)
	Positive	3 (3.6%)	1 (6.25%)
HistoMolecular class			
	LumA	45 (53.6%)	9 (56.25%)
	LumB	33 (39.4%)	6 (37.5%)
	LumB /HER2	3 (3.5%)	1 (6.25%)
	TN	3 (3.5%)	0
Surgery			
	Mastectomy	46 (54.7%)	6 (37.5%)
	Lumpectomy	38 (45.3%)	10 (62.5%)
Radiotherapy			
	Yes	60 (71.5%)	14 (87.5%)
	No	16 (19%)	2 (12.5%)
	NA	8 (9.5%)	0
Hormonotherapy			
	Yes	67 (79.8%)	15 (93.75%)
	No	10 (12%)	1 (6.25%)
	NA	7 (8.2%)	0
Adjuvant chemotherapy			
	Yes	40 (47.5%)	11 (68.75%)
	No	36 (43%)	5 (31.25%)
	NA	8 (9.5%)	0

Supplementary Table 1: Description of the prospective cohorts used in this study

Two prospective cohorts have been studied here. Tumor samples from cohort 1 have been used for multicolor flow cytometry and spatial transcriptomics. Tumor samples from cohort 2 have been used for culture of primary FAP+ CAF clusters. Abbreviations: pT: Pathological tumor size; pN: Pathological lymph node status; ER: Estrogen receptor; PR: Progesterone receptor; LumA: Luminal A; LumB: Luminal B; TN: Triple Negative; HER2: Human epidermal growth factor receptor 2; NA: Not available.

Supplementary Table 2

		ALL	DCIS	MI-DCIS	IBC
Number of patients		55	18	17	20
Sex					
	Female	55	18	17	20
Age at diagnosis					
	min-max (median)	23 - 84 (50)	24 - 56 (44.5)	23 - 70 (56)	33 - 84 (53)
TILs					
	< 10%	11 (20%)	9 (50%)	2 (11.8%)	
	10-30%	18 (32.7%)	7 (38.9%)	11 (64.7%)	
	> 30%	6 (10.9%)	2 (11.1%)	4 (23.5%)	
	NA	20 (36.4%)			20 (100%)
Grade (DCIS and MI-DCIS)					
	low	2 (5.7%)	2 (11.1%)		
	Intermediate	10 (28.6%)	6 (33.3%)	4 (23.5%)	
	High	23 (65.7%)	10 (55.6%)	13 (76.5%)	
Grade (IBC)					
	1	9 (45%)			9 (45%)
	2	3 (15%)			3 (15%)
	3	8 (40%)			8 (40%)
pT (UICC)					
	pT1	9 (16.4%)			9 (45%)
	pT2	11 (20%)			11 (55%)
	pTis	18 (32.7%)	18 (100%)		
	pT1Mi	17 (30.9%)		17 (100%)	
pN (UICC)					
	pN0	34 (61.8%)	11 (61.1%)	12 (70.6%)	11 (55%)
	pN1	9 (16.4%)	1 (5.6%)	1 (5.9%)	7 (35%)
	pN2	2 (3.6%)			2 (10%)
	NA	10 (18.2%)	6 (33.3%)	4 (23.5%)	
ER status					
	ER-	18 (32.7%)	4 (22.2%)	9 (52.9%)	5 (25%)
	ER+	37 (67.3%)	14 (77.8%)	8 (47.1%)	15 (75%)
PR status					
	PR-	26 (47.3%)	7 (38.9%)	12 (70.6%)	7 (35%)
	PR+	29 (52.7%)	11 (61.1%)	5 (29.4%)	13 (65%)
HER2 status					
	Negative	41 (74.5%)	16 (88.9%)	9 (52.9%)	16 (80%)
	Positive	14 (25.5%)	2 (11.1%)	8 (47.1%)	4 (20%)
HistoMolecular class					
	LumA	27 (49.1%)	11 (61.1%)	6 (35.3%)	10 (50%)
	LumB	9 (16.4%)	3 (16.7%)	2 (11.8%)	4 (20%)
	HER2	11 (20%)	1 (5.6%)	7 (41.2%)	3 (15%)
	LumB /HER2	3 (5.5%)	1 (5.6%)	1 (5.9%)	1 (5%)
	TN	5 (9.1%)	2 (11.1%)	1 (5.9%)	2 (10%)
Surgery					
	Mastectomy	31 (56.4%)	12 (66.7%)	7 (41.2%)	12 (60%)
	Lumpectomy	24 (43.6%)	6 (33.3%)	10 (58.8%)	8 (40%)
Radiotherapy					
	Yes	33 (60%)	7 (38.9%)	11 (64.7%)	15 (75%)
	No	22 (40%)	11 (61.1%)	6 (35.3%)	5 (25%)
Hormonotherapy					
	Yes	16 (29.1%)	1 (5.6%)	1 (5.9%)	14 (70%)
	No	39 (70.9%)	17 (94.4%)	16 (94.1%)	6 (30%)
Adjuvant chemotherapy					
	Yes	11 (20%)	1 (5.6%)		10 (50%)
	No	44 (80%)	17 (94.4%)	17 (100%)	10 (50%)

Supplementary Table 2: Description of the retrospective INVADE cohort

Retrospective BC cohort of 55 patients suffering from pre-invasive Ductal Carcinoma in Situ (DCIS) lesions (N = 18), micro-invasive DCIS (MI-DCIS) corresponding to DCIS lesions with invasive foci not exceeding 1 mm (N = 17) and Invasive Breast Cancer (IBC) (N = 20). Abbreviations: TILs: Tumor infiltrating lymphocytes; pT: Pathological tumor size; pN: Pathological lymph node status; ER: Estrogen receptor; PR: Progesterone receptor; LumA: Luminal A; LumB: Luminal B; TN: Triple Negative; HER2: Human epidermal growth factor receptor 2; NA: Not available.

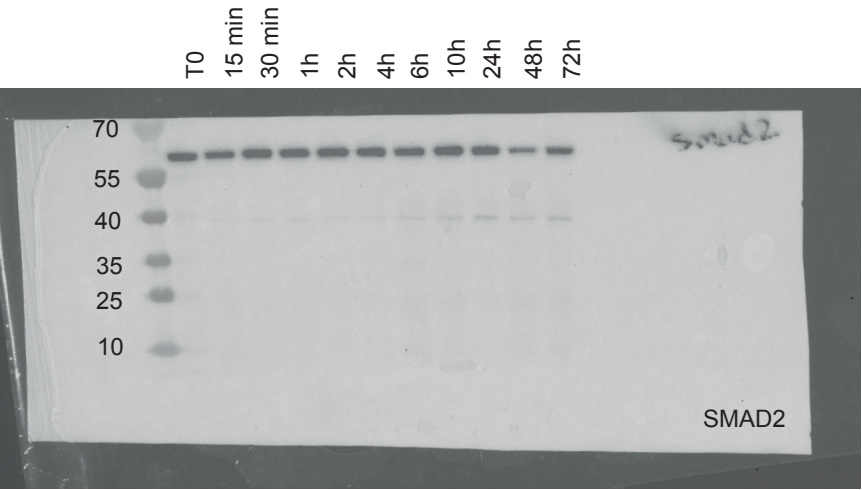
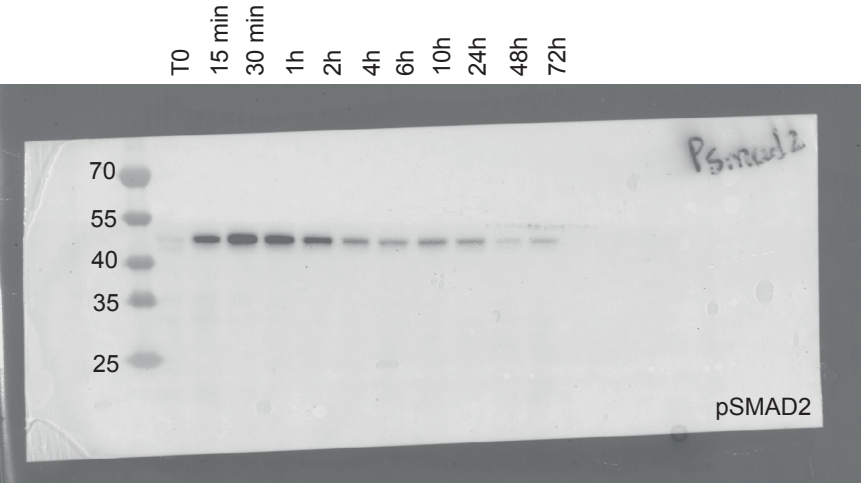
Supplementary Table 3

Antibodies for CAF-S1 clusters characterization	Reference	Dilution	Clone
Brilliant Violet 605 anti-human CD326 (EpCAM)	BioLegend, #324224	1/50	9C4
PE/Cyanine7 anti-human CD31 antibody	BioLegend, #303118	1/100	WM59
BUV395 Anti-human CD45	BD Biosciences, #563792	1/50	HI30
PerCP/Cyanine5.5 anti-human CD235a	Biolegend, #349110	1/50	HI264
Human Fibroblast Activation Protein Alpha/FAP Antibody	R&D Systems, #MAB3715	1/100	427819
Alexa Fluor700 anti-human CD29	BioLegend, #303020	1/100	TS2/16
Alexa Fluor 405 TEM8/ANTXR1 antibody	Novus Biologicals #NB100-56585AF405	1/25	200C1339(SB20)
BUV737 anti human CD138 (SDC1)	BD Biosciences #612834	1/25	MI15
Human Glypican 3 Alexa Fluor 594 Antibody	R&D systems #FAB2119T	1/20	307801
Human DLK1 Alex Fluor 488 Antibody	R&D systems #FAB1144G	1/20	211309
BV711 Anti-human CD9	BD Biosciences #743050	1/100	M-L13
BV786 Anti-human CD74	BD Biosciences #743736	1/100	LN2
PE anti-human LAMP5 antibody	Miltenyi Biotec #130-109-156	1/10	REA590
Fluorescent dye Zenon APC Mouse IgG1 labeling kit	Thermo Fisher Scientific, #Z25051	1/100	
IgG controls	Reference	Dilution	Clone
Mouse IgG1 isotype control FAP	R&D Systems, #MAB002	1/200	11711
Alexa Fluor® 700 Mouse IgG1, κ- Isotype control CD29	BioLegend, #400144	1/25	400144
Alexa Fluor 405 Mouse IgG1 Isotype Control ANTXR1	Novus Biologicals #IC002V	1/25	IC002V
BUV737 Mouse IgG1 Isotype control SDC1	BD Biosciences #612758	1/25	X40
Alexa Fluor 594 Mouse IgG2A Isotype Control GPC3	R&D systems #IC003T	1/20	IC003T
Alexa Fluor 488 Mouse IgG2B Isotype Control DLK1	R&D systems #IC0041G	1/20	IC0041G
BV711 Mouse IgG1 Isotype Control CD9	BD Biosciences #563044	1/100	563044
BV786 Mouse IgG1 Isotype Control CD74	BD Biosciences #563330	1/100	563330
REA control Antibody, human IgG1, PE isotype control LAMP5	Miltenyi Biotec #130-104-613	1/10	REA293
Antibodies for NK cells characterization	Reference	Dilution	Clone
APCcy7 Mouse Anti-human CD45	BD Biosciences #557833	1/50	2D1
Alexa Fluor 700 Mouse anti-human CD3	BD Biosciences #557943	1/50	UCHT1
BV510 Mouse anti-human CD14	BD Biosciences #563079	1/50	MΦP9
PerCP-Cyanine5.5 Anti-human CD19	BD Biosciences #561295	1/50	H1B19
BV650 Mouse Anti-human CD16	BD Biosciences #563692	1/50	3G8
BUV395 Mouse Anti-human CD56	BD Biosciences #563554	1/50	NCAM16.2
BV786 Mouse Anti-human NKG2A	BD Biosciences #747917	1/50	131411
PE anti-human Granzyme B	BD Biosciences #561142	1/50	GB11
Alexa Fluor 488 Anti human Perforin	BD Biosciences #563764	1/50	δG9
IgG controls	Reference	Dilution	Clone
BV650 Mouse IgG1 Isotype Control (CD16)	BD Biosciences #563231	1/50	X40
BUV395 Mouse IgG2b Isotype Control (CD56)	BD Biosciences #563558	1/50	27-35
BV786 Mouse IgG1 Isotype Control (NKG2A)	BD Biosciences #563330	1/50	X40
PE Mouse IgG1 Isotype Control (Granzyme B)	BD Biosciences #555749	1/50	MOPC-21
Alexa Fluor 488 Mouse IgG2b Isotype Control (Perforin)	BD Biosciences #558716	1/50	27-35
Antibodies for macrophages subsets characterization	Reference	Dilution	Clone
APCcy7 Mouse Anti-human CD45	BD Biosciences #557833	1/50	2D1
Alexa Fluor 700 Mouse anti-human CD3	BD Biosciences #557943	1/50	UCHT1
PEcy7 Mouse anti-human CD14	BD Biosciences #557742	1/50	M5E2
PerCP-Cyanine5.5 Anti-human CD19	BD Biosciences #561295	1/50	H1B19
BV650 Mouse Anti-human CD16	BD Biosciences #563692	1/50	3G8
BUV395 Mouse Anti-human CD56	BD Biosciences #563554	1/50	563554
PE anti-human FOLR2	Biolegend #391704	1/50	94b
Unconjugated anti-human TREM2	Novus Biologicals #MAB17291-100	1/50	237920
APC Goat anti-rat IgG secondary antibody	Novus Biologicals #F0105B	1/50	
IgG controls	Reference	Dilution	Clone
BV650 Mouse IgG1 Isotype Control (CD16)	BD Biosciences #563231	1/50	X40
BUV395 Mouse IgG2b Isotype Control (CD56)	BD Biosciences #563558	1/50	27-35
PEcy7 Mouse IgG2a Isotype Control	BD Biosciences #557907	1/50	G155-178
PE Mouse IgG1 Isotype Control	Biolegend #400112	1/100	400112
Unconjugated Rat IgG2b Isotype Control	Novus Biologicals #MAB0061	1/50	
Antibodies for Regulatory T cells characterization	Reference	Dilution	Clone
APCcy7 Mouse Anti-human CD45	BD Biosciences #557833	1/50	2D1
Alexa Fluor 700 Mouse anti-human CD3	BD Biosciences #557943	1/50	UCHT1
APC Anti human CD4	Miltenyi #130-113-210	1/10	VIT4
PE anti-human CD25	Miltenyi #130-113-282	1/20	4.00E+03
BUV737 Anti human PD-1	BD Biosciences #612791	1/50	EH12.1
PEcy5 Anti human CTLA4	BD Biosciences #555854	1/50	BN13
AF488 Anti human FOXP3	ThermoFischer Scientific #53-4776-42	1/40	PCH101
IgG controls	Reference	Dilution	Clone
PE Isotype Control Antibody, mouse IgG2b	Miltenyi #130-092-215	1/20	IS6-11E5.11
BUV737 Mouse IgG1, κ Isotype Control	BD Biosciences #564299	1/50	X40
PE-Cy5 Mouse IgG2a, κ Isotype Control	BD Biosciences, #555575	1/50	G155-178
Rat IgG2a kappa Isotype Control (eBR2a), Alexa Fluor 488	eBiosciences, #53-4321-80	1/200	eBR2a
Antibodies for CAF-S1 clusters isolation	Reference	Dilution	Clone
Alexa Fluor 405 TEM8/ANTXR1 antibody	Novus Biologicals #NB100-56585AF405	1/25	200C1339(SB20)
APC anti-human LAMP-5 antibody	Miltenyi Biotec #130-109-204	1/10	REA590
Alexa Fluor 700 anti-human GPC3 antibody	R&D systems #FAB2119N	1/25	307801
FITC Mouse anti-human CD74	BD Biosciences #555540	1/50	MB 741

Supplementary Table 3: List of primary antibodies used in this study

List of primary antibodies used for multicolor flow cytometry and cell sorting in order to characterize FAP⁺ CAF clusters and immune cell populations. Each antibody was titrated separately to find the best concentration to use. Appropriate isotype controls corresponding to the matched antibody were also used.

Uncropped images for western blot shown in Supplementary Fig. 2L



REFERENCES

1. Kieffer Y, *et al.* Single-Cell Analysis Reveals Fibroblast Clusters Linked to Immunotherapy Resistance in Cancer. *Cancer Discov* **10**, 1330-1351 (2020).
2. Bassez A, *et al.* A single-cell map of intratumoral changes during anti-PD1 treatment of patients with breast cancer. *Nat Med* **27**, 820-832 (2021).
3. Wu SZ, *et al.* Stromal cell diversity associated with immune evasion in human triple-negative breast cancer. *EMBO J* **39**, e104063 (2020).
4. Pal B, *et al.* A single-cell RNA expression atlas of normal, preneoplastic and tumorigenic states in the human breast. *EMBO J* **40**, e107333 (2021).
5. Wu SZ, *et al.* A single-cell and spatially resolved atlas of human breast cancers. *Nat Genet* **53**, 1334-1347 (2021).
6. Coutant A, *et al.* Spatial Transcriptomics Reveal Pitfalls and Opportunities for the Detection of Rare High-Plasticity Breast Cancer Subtypes. *Lab Invest* **103**, 100258 (2023).