

## Supplementary information

# A spatially resolved single-cell genomic atlas of the adult human breast

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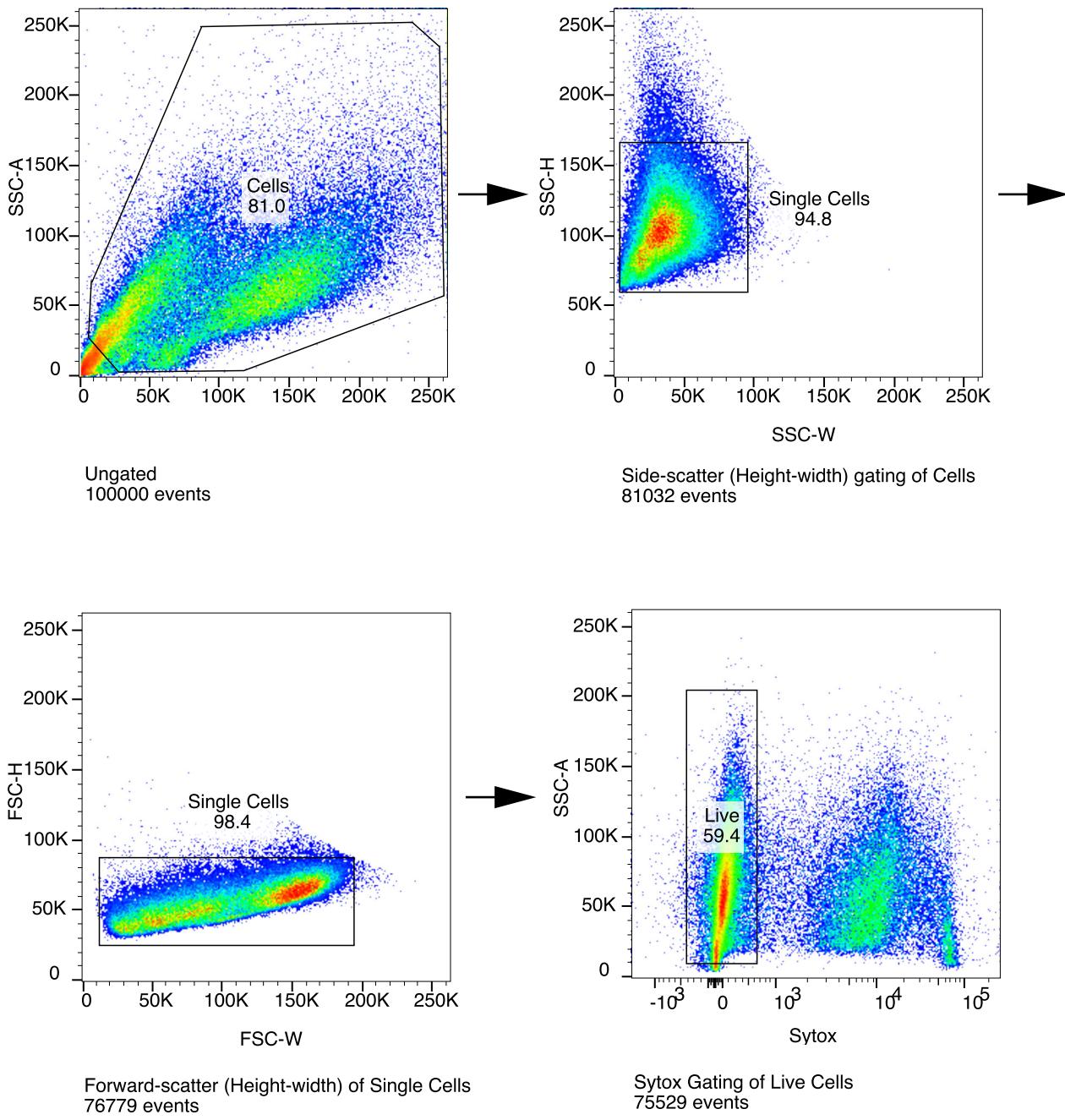
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# **A spatially resolved single cell genomic atlas of the adult human breast**

## **Supplementary Information**

**Kumar, Nee, Wei, He, Nguyen et al.**

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**Supplementary Information Figure 1 – FACS Gating strategy for isolating live cells.**

A subset of breast tissue samples (HBCA14-24) were FACS sorted to enrich for live cells by staining with Sytox live cell stain and gating positive cells. An example of this gating strategy is shown, in which cells are gated by Side Scatter Area (SSC-A) followed by side-scatter height (SSC-H) and side scatter width (SSC-W) to identify single cell events. The cells were then gated by Forward Scatter Height (FSC-H) and Forward-Scatter Width (FSC-W) to enrich single cell events. Finally live cells were gated from dead cells using Sytox live cell stain.





Sample ID	Patient ID	Institution	Tissue Source	Age	Ethnicity	Parity	Menopause	BMI	BMI Group	Digestion Protocol	Prior Treatment
hbca_c142	P101	bcm	Reduction Mammoplasty	Y	african-american	1	pre	39.72	obese	medium	none
hbca_c143	P102	bcm	Reduction Mammoplasty	Y	african-american	1	pre	32.97	obese	medium	none
hbca_c144	P103	bcm	Reduction Mammoplasty	Y	african-american	1	pre	26.63	overweight	medium	none
hbca_c145	P104	bcm	Reduction Mammoplasty	Y	african-american	1	pre	36.95	obese	medium	none
hbca_c146	P105	bcm	Reduction Mammoplasty	Y	african-american	1	pre	34.19	obese	medium	none
hbca_c147	P106	bcm	Reduction Mammoplasty	Y	african-american	1	pre	27.66	overweight	medium	none
hbca_c148	P107	bcm	Reduction Mammoplasty	Y	african-american	0	pre	27.02	overweight	medium	none
hbca_c149	P108	bcm	Reduction Mammoplasty	Y	caucasian	0	pre	27.13	overweight	medium	none
hbca_c150	P109	bcm	Reduction Mammoplasty	O	caucasian	0	post	32.95	obese	medium	none
hbca_c151	P110	bcm	Reduction Mammoplasty	O	caucasian	0	post	26.73	overweight	medium	none
hbca_c152	P111	bcm	Reduction Mammoplasty	Y	african-american	0	pre	41.15	obese	medium	none
hbca_c153	P112	bcm	Reduction Mammoplasty	Y	caucasian	1	pre	34.00	obese	medium	none
hbca_c154	P113	uci	Cancer Mastectomy	Y	caucasian	1	unknown	23.72	Normal	medium	(Perjeta/Xeloda/Kajinta)
hbca_c155	P114	uci	Cancer Mastectomy	Y	caucasian	1	pre	25.32	overweight	medium	hormonal therapy (aromatase)
hbca_c156	P115	uci	Cancer Mastectomy	O	caucasian	1	post	23.08	Normal	medium	hormonal therapy (aromatase)
hbca_c157	P116	uci	Cancer Mastectomy	O	caucasian	1	post	35.21	obese	medium	chemo
hbca_c158	P117	uci	Cancer Mastectomy	Y	hispanic	1	pre	30.34	obese	medium	none
hbca_c159	P118	uci	Prophylactic Mastectomy	O	caucasian	1	post	23.41	Normal	medium	unknown
hbca_c160	P119	uci	Cancer Mastectomy	Y	caucasian	1	pre	22.31	Normal	medium	hormonal therapy (tamoxifen)
hbca_c161	P120	uci	Cancer Mastectomy	O	hispanic	1	post	30.14	obese	medium	chemotherapy
hbca_c162	P121	uci	Prophylactic Mastectomy	Y	caucasian	unknown	unknown	29.28	overweight	medium	unknown
hbca_c163	P122	uci	Cancer Mastectomy	Y	caucasian	1	pre	21.54	normal	medium	chemotherapy (docetaxel, carboplatin, trastuzumab, and pertuzumab)
hbca_c164	P123	uci	prophylactic Mastectomy	Y	caucasian	1	pre	18.17	normal	medium	hormonal therapy (tamoxifen)
hbca_c165	P124	uci	Cancer Mastectomy	O	caucasian	0	pre	25.40	overweight	medium	none
hbca_c166	P125	uci	prophylactic Mastectomy	Y	caucasian	1	unknown	33.72	obese	medium	none
hbca_c167	P126	uci	prophylactic Mastectomy	O	caucasian	1	pre	23.76	normal	medium	unknown

### Supplementary Table 1 – Breast Atlas Tissue Samples and Clinical Metadata

This table lists the n =167 tissue samples and clinical metadata for the 126 women included in the breast atlas project. The columns list from left to right the sample identifier (Sample ID), the patient identifier (Patient ID), the institution where the tissue sample was collected from MD Anderson (MDA), UC Irvine (UCI) or Baylor College of Medicine (BCM), the procedure from which the tissue source was collected reduction mammoplasties (RM=111), prophylactic mastectomies (PM=18), and contralateral mastectomies (CM=38), the age of the women as < 50 (Y), >= 50 (O), the ethnicity the women self-identifies as, the parity status as positive (1), negative (0) or unknown, the menopause status (pre/post), the Body Mass Index (BMI), the BMI Group classification and digestion protocol time use during sample dissociation (short, medium, or long) and whether the woman received any prior treatment before the tissue was collected.





<b>Sample ID</b>	<b>Number of Cells</b>	<b>Mean Reads per Cell</b>	<b>Median Genes per Cell</b>	<b>Number of Reads</b>	<b>Fraction Reads in Cells</b>	<b>Genes Detected</b>	<b>Median UMI Counts per Cell</b>
hbca_c125	11,846	29,921	1,630	354,452,553	80.0%	25,290	4,845
hbca_c126	13,775	28,931	1,529	398,533,174	84.5%	25,967	4,386
hbca_c127	15,262	23,761	1,479	362,643,192	89.3%	25,860	4,269
hbca_c128	11,606	29,845	1,236	346,390,571	84.4%	24,230	2,776
hbca_c129	13,665	25,641	1,398	350,385,998	83.7%	25,781	3,754
hbca_c130	12,946	27,950	1,616	361,852,867	84.2%	26,059	4,459
hbca_c131	10,004	39,896	1,461	399,123,293	77.7%	25,154	4,185
hbca_c132	11,895	18,060	1,726	214,835,483	84.9%	24,574	4,741
hbca_c133	11,820	23,403	1,565	276,623,881	81.1%	25,851	4,179
hbca_c134	11,735	36,751	1,690	431,277,850	75.7%	26,152	4,780
hbca_c135	3,025	135,406	1,986	409,604,334	86.7%	24,779	6,867
hbca_c136	15,721	25,999	1,832	408,737,174	86.3%	26,111	5,724
hbca_c137	15,523	26,608	1,544	413,049,215	87.3%	26,070	4,342
hbca_c138	13,432	33,324	2,074	447,616,500	85.2%	26,380	5,930
hbca_c139	13,461	28,347	1,406	381,590,819	82.9%	26,185	3,909
hbca_c140	13,719	28,310	1,440	388,386,621	79.4%	25,702	4,386
hbca_c141	8,643	50,086	1,804	432,898,337	76.3%	25,749	5,456
hbca_c142	14,040	32,229	1,819	452,499,084	84.0%	26,384	5,369
hbca_c143	11,377	36,111	1,886	410,842,800	79.9%	25,902	6,072
hbca_c144	14,623	28,942	1,368	423,224,604	80.8%	26,247	3,911
hbca_c145	7,779	51,558	1,924	401,069,920	83.7%	26,191	5,740
hbca_c146	10,420	41,819	2,246	435,762,558	87.6%	26,590	7,428
hbca_c147	16,396	27,843	1,823	456,518,118	86.1%	26,602	5,523
hbca_c148	12,133	35,379	1,981	429,254,174	84.1%	26,238	5,549
hbca_c149	15,047	28,209	1,653	424,470,724	86.6%	25,315	4,938
hbca_c150	11,377	33,789	1,917	384,419,538	85.7%	26,167	5,802
hbca_c151	11,573	35,975	1,930	416,343,438	84.7%	25,467	6,096
hbca_c152	10,921	37,854	1,979	413,405,270	81.3%	25,501	6,532
hbca_c153	13,262	33,068	1,834	438,549,472	82.0%	26,101	5,593
hbca_c154	9,473	46,895	1,672	444,236,569	91.4%	25,798	5,613
hbca_c155	13,146	45,395	1,522	596,766,041	86.9%	26,009	4,901
hbca_c156	11,052	56,779	1,591	627,526,390	87.4%	26,396	4,740
hbca_c157	7,219	46,745	2,441	337,456,663	87.8%	25,875	8,255
hbca_c158	9,460	44,674	1,754	422,618,255	78.6%	25,371	5,368
hbca_c159	7,262	63,461	2,021	460,857,056	90.7%	25,728	6,736
hbca_c160	7,091	56,563	1,789	401,089,051	90.1%	25,372	5,509
hbca_c161	7,291	45,513	2,108	331,836,547	91.7%	24,847	6,949
hbca_c162	9,054	57,130	1,980	517,263,400	91.5%	26,008	6,515
hbca_c163	10,161	41,428	1,496	420,957,053	92.6%	25,836	4,356
hbca_c164	9,297	53,506	1,774	497,447,029	91.0%	25,713	5,818
hbca_c165	7,339	99,427	1,723	729,696,394	73.2%	25,658	6,325
hbca_c166	9,116	38,452	1,871	350,528,531	90.7%	25,478	5,551
hbca_c167	8,053	48,291	2,392	388,888,979	79.0%	25,689	7,740

## Supplementary Table 2 – Single Cell RNA-seq Quality Control Metrics

This table lists the quality control metrics for the fresh breast tissue samples sequenced with single cell RNA sequencing using the 10X Genomics Chromium Platform before any filtering was applied. The columns listed from left to right indicate the Sample ID, Number of Cells, Mean Reads per Cell, Median Genes per Cell, Number of Reads, Fraction Reads in Cells, Total Genes Detected, Median UMI Counts per Cell.



Cell Type	Gene	Average LogFC	pct1	pct2	Cell Type	Gene	Average LogFC	pct1	pct2
Fibroblasts	DCN	3.847	0.991	0.251	T cells	IL7R	3.143	0.780	0.023
	APOD	3.458	0.962	0.289		CCL5	2.796	0.659	0.014
	CFD	3.454	0.789	0.236		PTPRC	2.674	0.940	0.051
	TNFAIP6	2.918	0.823	0.085		CXCR4	2.559	0.913	0.098
	LUM	2.886	0.939	0.077		GNLY	2.408	0.180	0.007
	COL1A2	2.622	0.839	0.095		CD2	1.995	0.700	0.003
	COL1A1	2.481	0.693	0.083		SRGN	1.985	0.961	0.279
	COL3A1	2.481	0.613	0.074		NKG7	1.970	0.323	0.005
	MMP3	2.427	0.203	0.044		KLRB1	1.890	0.442	0.002
	GSN	2.287	0.966	0.539		ARHGDI1	1.816	0.829	0.204
	FBLN1	2.166	0.855	0.064		CD3D	1.803	0.671	0.003
	CCDC80	2.151	0.820	0.114		CREM	1.787	0.744	0.322
	MEG3	2.107	0.861	0.111		TRBC2	1.785	0.593	0.006
	SFRP2	2.107	0.698	0.037		LEPROTL1	1.736	0.712	0.143
	COL6A2	1.980	0.943	0.217		CD7	1.731	0.570	0.014
	IGFBP6	1.899	0.684	0.094		SARAF	1.724	0.905	0.594
	IGF1	1.896	0.669	0.040		CD52	1.688	0.594	0.024
	C1S	1.894	0.895	0.098		SYTL3	1.680	0.591	0.020
	COL6A3	1.872	0.847	0.056		CNOT6L	1.680	0.710	0.161
	C1R	1.868	0.871	0.152		CST7	1.669	0.505	0.011
Lymphatic	CCL21	4.375	0.909	0.016	Myeloid	HLA-DRA	3.553	0.933	0.200
	TFF3	2.945	0.893	0.093		IL1B	3.125	0.524	0.020
	MMRN1	2.928	0.910	0.010		HLA-DPA1	3.112	0.870	0.149
	CAVIN2	2.259	0.758	0.094		HLA-DPB1	3.014	0.855	0.212
	CLDN5	2.109	0.874	0.108		HLA-DRB1	2.935	0.894	0.195
	LYVE1	2.108	0.689	0.019		CD74	2.828	0.941	0.331
	TFPI	2.027	0.971	0.404		CCL3	2.733	0.461	0.033
	PPFIBP1	1.915	0.893	0.278		HLA-DQA1	2.686	0.743	0.052
	GNG11	1.879	0.904	0.269		C1QB	2.611	0.710	0.012
	ECSCR	1.774	0.759	0.075		C1QA	2.570	0.724	0.013
	ANGPT2	1.717	0.647	0.151		RNASE1	2.539	0.466	0.077
	PROX1	1.679	0.636	0.039		FCER1G	2.528	0.921	0.024
	CD9	1.667	0.933	0.506		TYROBP	2.528	0.943	0.021
	FABP4	1.656	0.804	0.197		LYZ	2.527	0.696	0.011
	FABP5	1.635	0.809	0.229		CCL4	2.425	0.431	0.051
	AKAP12	1.503	0.838	0.319		HLA-DQB1	2.374	0.769	0.102
	RAMP2	1.440	0.668	0.152		GPR183	2.232	0.808	0.070
	CAV1	1.425	0.914	0.460		CTSB	2.205	0.874	0.338
	EFEMP1	1.412	0.695	0.227		CD163	2.203	0.646	0.009
	S100A10	1.316	0.984	0.796		C1QC	2.197	0.682	0.006

### Supplementary Table 3 – Top Marker Genes Expressed in Major Breast Cell Types

This table lists the top 20 marker genes expressed in the scRNA-seq data for each major cell type cluster based on average log fold-change and specificity of the gene expressed in the indicated cell type (pct1) compared to the other cell types (pct2). The columns listed (from left to right) indicate the name of the major cell type, the gene names, the average log-fold change (Average LogFC), the pct1 value indicating the fraction of cells within the cluster expressing the gene, the pct2 value indicating the fraction of cells in other clusters expressing the gene.





Patient ID	Number of spots under tissue	Number of reads	Median UMI per spot	Median Genes per spot	Total genes detected	Reads mapped to exonic regions	Reads mapped to intergenic regions	Reads mapped to intronic regions	Reads mapped to transcriptome
P10	2741	461115960	3031	1546	23050	0.836	0.022	0.032	0.816
P35	1896	478182981	2223	1184	22295	0.878	0.019	0.026	0.855
P46	3129	404340693	2348	1315	22442	0.823	0.026	0.052	0.803
P47	3449	362966308	1108	797	22121	0.837	0.027	0.06	0.817
P65	3226	404107425	2284	1035	23915	0.896	0.02	0.027	0.875
P67	3655	452452634	2666	1343	23688	0.899	0.018	0.026	0.879
P79	3571	438705200	2379	1279	24489	0.816	0.027	0.047	0.798
P82	3631	376273215	1512	841	23688	0.82	0.03	0.05	0.801
P85	3669	404861379	3570	1545	24788	0.845	0.027	0.038	0.826
P127	2854	423500797	2000	1024	23038	0.75	0.04	0.064	0.732

### Supplementary Table 5 – Quality Control Metrics for Spatial Transcriptomics Data

This table lists the quality control metrics for breast tissue samples sequenced with the Spatial Transcriptomics platform (Visium, 10X Genomics). The columns listed (from left to right) indicate the Patient ID, Number of spots under tissue, Number of reads, Median UMI per spot, Median Genes per spot, Total genes detected, Reads mapping rate (exonic, intergenic, intronic regions and transcriptome).

Cell Type	Gene	Cell Annotation (module score)	Cell Type	Gene	Cell Annotation (module score)
Basal	KRT5	1	Myeloid	CD14	1
	ACTG2	1		CD68	1
	TUBB2B	0		C1QA	1
	COL17A1	1		C1QB	1
	KRT6B	0		FCER1G	0
	LAMA3	1		C1QC	1
LumHR	AR	1		LYZ	1
	ESR1	1		CD163	1
	PGR	1		MSR1	1
	AREG	1		IL18R1	0
	OXTR	0		HPGD	0
	ANKRD30A	1		HDC	0
	AGR3	1		SLC18A2	0
	AGR2	1		CPA3	0
	TMC5	1		CD69	0
LumSec	DNAJC12	1		HPGDS	0
	RASEF	1		CD3D	0
	SLPI	1		CD3E	0
	LTF	1		CD4	1
	KRT15	1		CD8A	1
	MMP7	1	T cells	IL7R	1
Fibroblasts	CCL28	1		CCL5	1
	ALDH1A3	1		NKG7	1
	PIGR	1		GZMB	1
	LUM	1		CD2	1
	TNFAIP6	0		CD27	0
Pericytes	COL1A2	1		DERL3	0
	COL1A1	1		JCHAIN	1
	FBLN1	1		IGHM	1
	MMP2	1		TNFRSF17	0
	SERPINF1	1		MZB1	1
	MCAM	0		CD79A	0
Lymphatic	RGS5	1	Adipocytes	PLIN1	0
	GJA4	1		ADIPOQ	0
	NDUFA4L2	1		PLIN4	0
	SSTR2	1		GPD1	0
	AVPR1A	0		LPL	0
	PLN	1		LIPE	0
	EDNRA	1		EPCAM	0
	CCL21	1		PECAM1	0
	MMRN1	0	Epithelial	MKI67	0
Vascular	PROX1	1		PTPRC	0
	SCN3B	1		TRAC	0
	PKHD1L1	0		TP63	0
	TBX1	1		KRT19	0
	PGM5	1		IGHA2	Did not work
	VWF	0			
Basal	SELE	0			
	ACKR1	1			
	CSF3	0			
	ADGRL4	0			
	RBP7	1			
	PGF	1			
Luminal	AQP1	1			

### Supplementary Table 6 – Custom Targeted Gene Panel for smFISH (Resolve)

This table lists the top expressed genes selected for each breast cell type from the scRNA-seq data to generate a custom 100-gene panel for smFISH analysis (Resolve Biosciences). The entire panel of 100 genes was used to profile each of the breast tissue samples, to determine the spatial distribution of the breast cell types in the tissue sections. The table shows the cell type, the genes used for classification and cell annotation module score with values of 1 (Yes) or 0 (No).

<b>Section ID</b>	<b>Patient ID</b>	<b>Total Counts (raw)</b>	<b>Total Counts (post filter)</b>	<b>Total Cells (raw)</b>	<b>Filtered Cells (post filter)</b>
P46-S1	P46	1491813	1301494	11929	9879
P35-S1	P35	913325	702059	7604	6683
P47-S1	P47	667314	615735	6872	6050
P69-S1	P69	491862	330047	4276	3390
P69-S2	P69	580993	157526	4754	4018
P46-S2	P46	460881	319725	5847	3961
P128-S1	P128	113031	75978	1244	868
P69-S3	P69	1549406	853581	5441	4591
P46-S3	P46	1991213	1018173	11788	8989
P46-S4	P46	703202	343803	3635	2906
P128-S2	P128	208133	95192	1290	928
P128-S3	P128	1019583	545389	4597	3372

### **Supplementary Table 7 – Quality Control Metrics for smFISH Data**

The table lists the quality control metrics for the 12 breast tissue samples profiled with the custom 100-gene panel for smFISH analysis (Resolve Biosciences). The columns listed (from left to right) indicate the Section ID, Patient ID, the number of transcripts captured under tissue, the number of transcripts after filtering, the number of cells detected and the number of cells after filtering.

<b>Sample</b>	<b>Patient ID</b>	<b>Cells profiled</b>
P129_s1	P129	78219
P130_s1	P130	62302
P131_s1	P131	40781
P132_s1	P132	49985
P123_s1	P123	21585
P125_s1	P125	5557
P119_s1	P119	18144
P114_s1	P114	10468

#### **Supplementary Table 8 - Breast Tissue Samples for CODEX**

This table lists the 8 breast tissue samples that were used for CODEX analysis, along with the clinical metadata for each woman, and total number of cells that were profiled and analyzed. Columns from left to right are Sample Section, Patient ID, number of cells profiled.

Supplementary Table 9 – CODEX 34-Antibody Panel Design

Cycle #	Antibody	Clone		Reporter	Ratio	Ab vendor	Ab Catalog number
Cycle1	blank						
	blank						
	blank						
Cycle2	Keratin19	A53-B/A2	BX025	RX025-A750	1:50	Biolegend	628502
	CD8	C8/144B	BX026	RX026-ATTO	1:200	Akoya	4250012
	PCNA	PC10	BX020	RX020-Cy5	1:50	Biolegend	307902
Cycle3	Vimentin	RV202	BX034	RX034-A750	1:50	BD	550513
	CD31	EP3095	BX001	RX001-ATTO	1:200	Akoya	(custom, ab226157 from abcam)
	CD3e	EP449E	BX045	RX045-Cy5	1:150	Akoya	(custom, ab271850 from abcam)
Cycle4	Keratin7	W16155A	BX019	RX019-A750	1:50	Biolegend	601602
	Ki67	B56	BX047	RX047-ATTO	1:200	Akoya	4250019
	CD4	EPR6855	BX003	RX003-Cy5	1:200	Akoya	(custom, ab181724 from abcam)
Cycle5	CD227	HMPV	BX004	RX004-A750	1:50	BD	555925
	Perlecan	5D7-2E4	BX017	RX017-ATTO	1:50	BD	565781
	CD45	2D1	BX021	RX021-Cy5	1:100	Akoya	from Novus
Cycle6	Keratin17	W16131A	BX022	RX022-A750	1:50	Biolegend	W16131A
	Podoplanin	NC-08	BX023	RX023-ATTO	1:200	Akoya	4250004
	CD68	KP1	BX015	RX015-Cy5	1:200	Akoya	(custom; MA5-13324 frm ThermoFisher
Cycle7	Empty						
	CD14	EPR3653	BX037	RX037-ATTO	1:50	Abcam	ab226121
	CollagenIV	EPR20966	BX042	RX042-Cy5	1:50	Abcam	ab226485
Cycle8	Keratin18	DA-7	BX049	RX049-A750	1:50	Biolegend	628402
	HLA-DPB1	EPR11226	BX035	RX035-ATTO	1:50	Abcam	ab157210
	CD11c	118/A5	BX024	RX024-Cy5	1:200	Akoya	Biological NBP2-
Cycle9	Empty						
	E-Cadherin	4A2C7	BX014	RX014-ATTO	1:200	Akoya	4250021
	PR	KMC912	BX007	RX007-Cy5	1:50	ThermoFis	14-9764-82
Cycle10	Keratin8	1E8	BX040	RX040-A750	1:50	Biolegend	697902
	Keratin14	Poly19053	BX032	RX032-ATTO	1:300	Akoya	(custom; 905304 from Biolegend)
	TP63	W15093A	BX033	RX033-Cy5	1:50	Biolegend	W15093A
Cycle11	Empty						
	Empty						
	SMA	1A4	BX030	RX030-Cy5	1:50	Abcam	ab7817
Cycle12	Empty						
	Empty						
	Runx3	R3-5G4	BX036	RX036-Cy5	1:50	Biolegend	R3-5G4

Cycle #	Antibody	Clone		Reporter	Ratio	Ab vendor	Ab Catalog number
Cycle13	Empty						
	Empty						
	CD66e	BSB-13	BX016	RX016-Cy5	1:50	Biolegend	847002
Cycle14	Empty						
	Empty						
	BCL6	K112-91	BX041	RX041-Cy5	1:50	BD	561520
Cycle15	Empty						
	Empty						
	Foxp3	259D/C7	BX027	RX027-Cy5	1:40	BD	560044
Cycle16	Empty						
	Empty						
	LIF	M1506B09	BX006	RX006-Cy5	1:200	Akoya	(custom; 674702 from Biolegend)
Cycle17	Empty						
	Empty						
	GranzymeB	D6E9W	BX046	RX046-Cy5	1:50	CST	79903SF
Cycle18	Empty						
	Empty						
	BCL2	N46-467	BX029	RX029-Cy5	1:50	BD	N46-467
Cycle19	Empty						
	Empty						
	Keratin5	Poly19055	BX005	RX005-Cy5	1:50	Biolegend	905504
Cycle20	blank						
	blank						
	blank						

#### Supplementary Table 9 – CODEX 34-Antibody Panel Design

This table lists the cycle order and description of the 34 antibodies run in the CODEX panel. The columns listed (from left to right) indicate, the cycle, the antibody, the clone, the reporter, the dilution ratio use, antibody vendor and antibody catalog number.





















Patient ID	Total Counts (raw)	Total Counts (post filter)	Total Cells (raw)	Filtered Cells (post filter)
P91	18962267	17504490	230073	93859
P96	18597651	18187226	218464	93687
P101	12129915	11587548	175447	73242

#### Supplementary Table 11 - Quality Control Metrics for smFISH (MERFISH) Data

The table lists the quality control metrics for the 3 breast tissue samples profiled with the custom 300-gene panel for smFISH analysis (Merscope). The columns listed (from left to right) indicate the Patient ID, the number of transcripts captured under tissue, the number of transcripts after filtering, the number of cells detected and the number of cells after filtering.

Supplementary Table 12 – Custom Targeted Gene Panel for smFISH (MERFISH)

CellType	CellState	Gene	Cell Type Annotation	Cell State Annotation
adipocytes	adipocytes	ADIPOQ	0	0
adipocytes	adipocytes	GPD1	1	0
adipocytes	adipocytes	LPL	1	0
adipocytes	adipocytes	PDE3B	1	0
adipocytes	adipocytes	PLIN1	1	0
B-cells	b_naive	CD37	0	1
B-cells	b_naive	CD52	0	0
B-cells	b_naive	LTB	0	1
B-cells	B-cells	CREB3	0	0
B-cells	B-cells	TFEB	0	0
B-cells	bmem_unswitched	BCL2A1	0	0
B-cells	bmem_unswitched	CD27	0	0
B-cells	bmem_unswitched	EGR3	0	0
B-cells	bmem_unswitched	IGHD	0	1
B-cells	bmem_unswitched	MYC	0	0
B-cells	cannonical	CD79A	1	0
B-cells	cannonical	IGHM	1	1
B-cells	cannonical	MZB1	1	1
B-cells	plasma_IgA	IGHA2	0	0
B-cells	plasma_IgA	JCHAIN	1	1
B-cells	plasma_IgG	IGHG1	0	0
B-cells	plasma_IgG	IGHG3	1	1
B-cells	plasma_IgG	IGHG4	1	1
basal	basal	CARMN	0	0
basal	basal	COL17A1	1	0
basal	basal	KLF16	0	0
basal	basal	LINC01060	0	0
basal	basal	SOX9	0	0
basal	basal	TEAD3	0	0
basal	basal	TFAP2C	1	0
basal	cannonical	ACTG2	1	1
basal	cannonical	KRT14	1	0
basal	cannonical	KRT5	1	0
basal	cannonical	LAMA3	0	0
basal	cannonical	TUBB2B	0	0
epithelial	cannonical	EPCAM	0	0
epithelial	cannonical	KRT19	0	0
epithelial	cannonical	KRT8	0	0
epithelial	cannonical	TP63	1	0
fibro	cannonical	COL1A1	1	0
fibro	cannonical	FBLN1	1	0
fibro	cannonical	LUM	0	0
fibro	cannonical	TNFAIP6	0	0

CellType	CellState	Gene	Cell Type	Cell State
fibro	fibro	CREB3L1	0	0
fibro	fibro	LAMA2	1	0
fibro	fibro	LINC02511	0	0
fibro	fibro	NEGR1	0	0
fibro	fibro	PLAGL1	1	0
fibro	fibro	TWIST2	0	0
fibro	fibro_col1a1	POSTN	0	0
fibro	fibro_col1a1	TAC1	0	0
fibro	fibro_col1a1	TGFBI	0	0
fibro	fibro_col1a1	WNT2	0	0
fibro	fibro_pcolce2	CCN5	0	0
fibro	fibro_pcolce2	PCOLCE2	0	0
fibro	fibro_pcolce2	PDK4	0	0
fibro	fibro_pcolce2	RAMP2	0	0
fibro	fibro_pcolce2	SVEP1	0	0
fibro	fibro_pdpn	CXCL1	0	0
fibro	fibro_pdpn	CXCL2	0	0
fibro	fibro_pdpn	CXCL3	0	0
fibro	fibro_pdpn	CXCL8	0	0
fibro	fibro_pdpn	IL6	0	0
Immune	cannonical	PTPRC	0	0
lumhr	cannonical	AGR2	0	0
lumhr	cannonical	AGR3	0	0
lumhr	cannonical	ANKRD30A	1	0
lumhr	cannonical	AREG	1	0
lumhr	cannonical	DNAJC12	1	0
lumhr	cannonical	RASEF	1	0
lumhr	cannonical	TMC5	0	0
lumhr	lumhr	AR	0	0
lumhr	lumhr	BATF	1	0
lumhr	lumhr	ESR1	0	0
lumhr	lumhr	GATA3	1	0
lumhr	lumhr	PGR	0	0
lumhr	lumhr	TBX3	0	0
lumhr	lumhr	TTC6	0	0
lumhr	lumhr_egln3	EGLN3	0	0
lumhr	lumhr_fasn	CA2	0	0
lumhr	lumhr_fasn	FASN	0	0
lumhr	lumhr_fasn	MYBPC1	0	0
lumhr	lumhr_pip	SERPINA1	0	1
lumhr	lumhr_pip	TFPI2	0	1

CellType	CellState	Gene	Cell Type	Cell State
lumsec	canonical	ALDH1A3	1	1
lumsec	canonical	CCL28	1	0
lumsec	canonical	KRT15	1	0
lumsec	canonical	LTF	1	1
lumsec	canonical	MMP7	0	0
lumsec	canonical	PIGR	1	0
lumsec	canonical	SLPI	1	1
lumsec	lumsec	EHF	0	0
lumsec	lumsec	ELF5	1	1
lumsec	lumsec	GABRP	1	1
lumsec	lumsec	GRHL1	0	0
lumsec	lumsec	NR2F6	0	0
lumsec	lumsec_hla	AQP3	0	1
lumsec	lumsec_hla	CCL20	0	1
lumsec	lumsec_hla	RBP1	0	1
lumsec	lumsec_KIT	HES1	0	0
lumsec	lumsec_KIT	KIT	0	0
lumsec	lumsec_krt23	KRT23	0	1
lumsec	lumsec_lactation	CSN1S1	0	0
lumsec	lumsec_lactation	CSN3	0	0
lumsec	lumsec_lactation	LALBA	0	0
lumsec	lumsec_prol	STMN1	0	1
lumsec	lumsec_prol	TYMS	0	1
lymph	canonical	MMRN1	1	0
lymph	canonical	PECAM1	0	0
lymph	canonical	PGM5	1	0
lymph	canonical	PROX1	1	0
lymph	canonical	SCN3B	1	0
lymph	canonical	TBX1	1	0
lymph	lymph	HMGB3	0	0
lymph	lymph	KLHL4	0	0
lymph	lymph	LINC02147	0	0
lymph	lymph	NR2F2	0	0
lymph	lymph	PKHD1L1	0	0
lymph	lymph	RHOJ	0	0
lymph	lymph-immune	ACKR4	0	0
lymph	lymph-immune	PTGS2	0	0
lymph	lymph-immune	TGM2	0	0
lymph	lymph-major	FABP4	0	0
lymph	lymph-major	LYVE1	0	0
lymph	lymph-valve	CLDN11	0	1
lymph	lymph-valve	MYLIP	0	1
lymph	lymph-valve	SCG3	0	1

CellType	CellState	Gene	Cell Type	Cell State
mast	canonical	CD69		0
mast	canonical	CPA3		0
mast	canonical	HPGD		0
mast	canonical	HPGDS		0
mast	canonical	TPSB2		0
myeloid	canonical	C1QA		1
myeloid	canonical	C1QB		1
myeloid	canonical	C1QC		1
myeloid	canonical	CD14		1
myeloid	canonical	CD163		1
myeloid	canonical	CD68		0
myeloid	canonical	LYZ		1
myeloid	canonical	MSR1		0
myeloid	cDC1	CLEC9A		0
myeloid	cDC1	WDFY4		0
myeloid	cDC1	XCR1		0
myeloid	cDC2	CCL21		0
myeloid	cDC2	CD1C		0
myeloid	cDC2	CLEC10A		0
myeloid	cDC2	FCER1A		1
myeloid	macro_ifn_m2	IFIT1		0
myeloid	macro_ifn_m2	STAT1		0
myeloid	macro_ifn_m2	XAF1		0
myeloid	macro_lipo	APOC1		1
myeloid	macro_lipo	SPP1		0
myeloid	macro_lipo	TREM2		1
myeloid	macro_m1	APOE		0
myeloid	macro_m1	C3		1
myeloid	macro_m1	RGS1		1
myeloid	macro_m2	MRC1		1
myeloid	macro_m2	RNASE1		1
myeloid	macro_m2	SELENOP		1
myeloid	mast	HDC		0
myeloid	mast	IL18R1		0
myeloid	mast	NTM		0
myeloid	mast	SYTL3		0
myeloid	mDC	CCL19		0
myeloid	mDC	CCL22		0
myeloid	mDC	LAMP3		0
myeloid	mono_active	CXCL5		0
myeloid	mono_active	SERPINB2		0
myeloid	mono_naive	TESC		0

CellType	CellState	Gene	Cell Type	Cell State
myeloid	Myeloid	ARID3A	0	0
myeloid	Myeloid	MAF	0	0
myeloid	Myeloid	MAFB	1	0
myeloid	pDC	SPIB	0	0
myeloid	pDC	TPM2	0	0
myeloid	pDC	TSPAN13	0	0
peri	cannonical	AVPR1A	1	0
peri	cannonical	EDNRA	1	0
peri	cannonical	MCAM	1	1
peri	cannonical	PLN	0	0
peri	cannonical	RGS5	1	1
peri	cannonical	SSTR2	0	0
peri	peri	ADGRL3	0	0
peri	peri	COL25A1	1	1
peri	peri	RGS6	1	1
peri	peri	SOX5	0	0
peri	peri	TBX2	1	0
peri	peri_CREM	CREM	0	0
peri	peri_CREM	SLC25A4	0	0
peri	peri_CREM	SYNM	0	1
peri	peri_CREM	ZNF331	0	0
peri	peri_imm	EMP3	0	0
peri	peri_imm	GYPC	0	0
peri	peri_imm	PSME2	0	0
peri	peri_imm	SERPINE2	0	0
peri	peri_myo	EGR1	0	0
peri	peri_myo	PLAC9	0	0
peri	peri_myo	RGS16	0	0
prol	prol	MKI67	0	1
prol	prol	PCNA	0	1
prol	prol	TOP2A	0	1
T-cells	cannonical	CCL5	1	1
T-cells	cannonical	CD2	1	1
T-cells	cannonical	CD3D	1	1
T-cells	cannonical	CD3G	0	0
T-cells	cannonical	CD4	1	1
T-cells	cannonical	CD8A	1	1
T-cells	cannonical	CD8B	0	0
T-cells	cannonical	GZMB	0	0
T-cells	cannonical	IL7R	1	0
T-cells	cannonical	NKG7	1	0
T-cells	cannonical	TRBC1	0	0

CellType	CellState	Gene	Cell Type	Cell State
T-cells	CD4_naive	CCR7	0	1
T-cells	CD4_naive	SELL	0	1
T-cells	CD4_PLCG2	CD40LG	0	0
T-cells	CD4_PLCG2	PLCG2	0	0
T-cells	CD4_Th1	CCR6	0	0
T-cells	CD4_Th1like	PGAP1	0	0
T-cells	CD4_Treg	CTLA4	0	0
T-cells	CD4_Treg	FOXP3	0	0
T-cells	CD4_Treg	TBC1D4	0	0
T-cells	CD8_TEM	CRTAM	0	0
T-cells	CD8_TEM	EOMES	0	1
T-cells	CD8_TEM	GZMK	0	1
T-cells	CD8_TEM	TNFRSF9	0	1
T-cells	CD8_TRM	AUTS2	0	0
T-cells	CD8_TRM	ITGA1	0	1
T-cells	CD8_TRM	KLRC1	0	1
T-cells	CD8_ZNF683	ZNF683	0	1
T-cells	GD	CCDC57	0	0
T-cells	GD	GPCPD1	0	0
T-cells	NK	FCER1G	0	1
T-cells	NK	KLRF1	0	1
T-cells	NK/ILCs	TNFRSF18	0	0
T-cells	NK/ILCs	TRDC	0	0
T-cells	NKT	GNLY	0	0
T-cells	NKT	KLRG1	0	0
T-cells	t_prol	CENPF	0	0
T-cells	t_prol	ZWINT	0	0
T-cells	T-cells	ARHGAP15	0	0
T-cells	T-cells	ELF1	0	0
T-cells	T-cells	IKZF1	0	0
T-cells	T-cells	PRDM1	0	0
T-cells	T-cells	PTPRC	0	0
T-cells	T-cells	RUNX2	0	0
T-cells	T-cells	SKAP1	0	0
T-cells	T-cells	TCF7L2	0	0
T-cells	T-cells	THEMIS	0	0
T-cells	T-cells	ZNF831	0	0
vas	cannonical	AQP1	1	0
vas	cannonical	CSF3	0	0
vas	cannonical	PGF	0	0
vas	vas	BTNL9	1	0
vas	vas	IRF7	1	0
vas	vas	MECOM	0	0
vas	vas	PTPRB	0	0
vas	vas	SMAD1	0	0

CellType	CellState	Gene	Cell Type	Cell State
vas	vas-arterial	GJA4	0	1
vas	vas-arterial	HEY1	1	1
vas	vas-arterial	IGFBP3	0	0
vas	vas-arterial	SOX17	1	1
vas	vas-capillary	CA4	0	1
vas	vas-capillary	RGCC	1	1
vas	vas-veinous	ACKR1	1	1
vas	vas-veinous	RAMP3	0	1
vas	vas-veinous	SELE	0	0
vas	vas-veinous	SELP	1	1

#### Supplementary Table 12 – Custom Targeted Gene Panel for smFISH (MERFISH)

This table lists the custom panel of 266 genes that were selected to distinguish a subset of cell types and cell states identified by top marker genes expressed in the scRNA-seq data. The table lists the cell types, cell state and gene name, and also shows which markers were used to identify cell types and cell states in the final annotation analysis after cell segmentation.