Immune responses in pancreatic cancer may be restricted by prevalence of activated regulatory T-cells, dysfunctional and senescent CD8+ T-cells

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Supplementary Data

Tables

Table 1: Patient data.

Patient	Sex	Age	TNM stage	Size of primary	Background pancreas related pathology	Stent before surgery	Other co-morbidities	Baseline ca19.9
1	Male	71	pT3 N2 (11/52) L1 V1 R1(Posterior, Medial)	45mm	PanIn	No	No	N/A
2	Male	72	pT2 N1 (3/36) L1 V1 R0	28mm	PanIn	Yes	Diabetes mellitus, Acute kidney injury (Stage 1), Ex-smoker.	N/A
3	Female	51	pT2 N2 (12/66) M0 L1 V1 R2(Medial)	26mm	IPMN	No	Diabetes mellitus.	Ca19.9 - 18 IU/mL
4	Male	80	pT3 N0(0/22 + 0/1 = 0/23) M0 L0 V0 Pn1 R0	15mm	No	No	Cancer of prostate.	Ca19.9 - 1,356 U/mL
5	Female	73	R1 (medial x 2 & pancreatic RM) pT3 L1 V0 Pn1 N2 (16/37)	42mm	No	Yes	No	N/A
6	Female	67	pT3, N2 (10/68 M0,L1,V1,R1 anterior medial)	28mm	PanIn	No	Inactive: Ex-smoker	Ca19.9 - 147 U/mL
7	Male	67	R1 (posterior) pT2 L0 V1 Pn1 N1(1/19)	24mm	PanIn	No	Smoker.	N/A
8	Male	69	pT2 N2 (9/18) M0 L1 V1 R0	28mm	PanIn	No	Diabetes mellitus, Ex-smoker.	N/A

Table 2: CYTOF panel

Antigen	Metal Tag	Clone	Vendor
CD45	89 Y	HI30	Fluidigm
CD14 Qdot	Qdot655 (114 Cd)	TuK4	ThermoFisher

EpCAM	141 Pr	9C4	Fluidigm
CD19	142 Nd	HIB19	Fluidigm
CD127	143 Nd	A019D5	Fluidigm
HLA-A,B,C	144 Nd	W6/32	Fluidigm
CD4	145 Nd	RPA-T4	Fluidigm
CD8a	146 Nd	RPA-T8	Fluidigm
CD39	147 Sm	A1	Biolegend
ICOS	148 Nd	C398.4A	Fluidigm
CD25	149 Sm	2A3	Fluidigm
OX40	150 Nd	ACT35	Fluidigm
CD103	151 Eu	BerACT8	Fluidigm
CD66b	152 Sm	80H3	Fluidigm
Tigit	153 Eu	MBSA43	Fluidigm
Tim-3	154 Sm	F382E2	Fluidigm
CD27	155 Gd	L128	Fluidigm
PD-L1	156 Gd	29E.2A3	Fluidigm
CD33	158 Gd	WM53	Fluidigm
GITR	159 Tb	621	Fluidigm
CD28	160 Gd	CD28.2	Fluidigm
CTLA-4	161 Dy	14D3	Fluidigm
FoxP3	162 Dy	PCH101	Fluidigm
CD56	163 Dy	NCAM16.2	Fluidigm
CD15	164 Dy	W6D3	Fluidigm
Lag-3	165 Ho	11C3C65	Fluidigm
CCR7	167 Er	G043H7	Fluidigm
CD40L	168 Er	2431	Fluidigm
CD45RA	169 Tm	HI100	Fluidigm
CD3	170 Er	UCHT1	Fluidigm
Granzyme B	171 Yb	GB11	Fluidigm
Ki67	172 Yb	B56	Fluidigm
4-1BB	173 Yb	4B4-1	Fluidigm
HLADR	174 Yb	L243	Fluidigm
PD-1	175 Lu	EH12.2H7	Fluidigm
CD57	176 Yb	HCD57	Fluidigm
CD16	209 Bi	3G8	Fluidigm

Supplemental Data File 1: Top differentially expressed genes by 75th percentile and means for Exhausted, Senescent and T-reg metaclusters.
Supplemental Data File 2: Differential expression analysis for Exhausted, Senescent and T-reg metaclusters.
Supplemental Data File 3: Reference pancreas and immune gene lists
Supplemental Data File 4: T-cell complete gene list

Supplementary Figures

Figure S1: Individual patients' immune populations. Expression of clustering markers on top of a flowsom tree showing the relationship between metaclusters, for the pooled single cell data (top). Population frequency is presented in the pie chart in each circle along the tree, the background colour represents the metacluster identity. (Bottom) Flowsom trees showing the individual metacluster frequency for each patient. The size of the pie chart corresponds to the frequency relative frequency. (Expression profile: from low (Dark blue) to high (Deep red)).

Figure S2. Individual patients' NK populations. Expression of clustering markers on top of a flowsom tree showing the relationship between metacluster, for the pooled single-cell data (top; ~2,500 cells). Population frequency is presented in the pie chart in each circle along the tree, the background colour represents the metacluster identity. (Bottom) Flowsom trees showing the individual metacluster frequency for each patient. The size of the pie chart corresponds to the frequency relative frequency. (Expression profile: from low (Dark blue) to high (Deep red)).

Figure S3. Individual patients' Granulocyte populations. Expression of clustering markers on top of a flowsom tree showing the relationship between metacluster, for the pooled single-cell data (top; ~36,000 cells). Population frequency is presented in the pie chart in each circle along the tree, the background colour represents the metacluster identity. (Bottom) Flowsom trees showing the individual metacluster frequency for each patient. The size of the pie chart corresponds to the frequency relative frequency. (Expression profile: from low (Dark blue) to high (Deep red)).

Figure S4. Individual patients' Mononuclear Phagocytes populations. Expression of clustering markers on top of a flowsom tree showing the relationship between metacluster, for the pooled single-cell data (top; ~20,260 cells). Population frequency is presented in the pie chart in each circle along the tree, the background colour represents the metacluster identity. (Bottom) Flowsom trees showing the individual metacluster frequency for each patient. The size of the pie chart corresponds to the frequency relative frequency. (Expression profile: from low (Dark blue) to high (Deep red)).

Figure S5. Immune-complexes and MDSC observed in peripheral blood of PDAC patients. (A) 124,000 $CD45^{+}$ cells pooled from 8 patients and viSNE analysis using main cell lineage markers was performed to identify the main immune cell populations. The main immune populations coloured and labelled by Flowsom. Bar plots of metacluster frequencies in each patient. Inset shows the lower frequency metaclusters. Heatmap of Flowsom metaclusters of $CD45^{+}$ cells; rows represent metaclusters from combined single cells across patients. $CD66^{+}$ represent low density neutrophils and $CD14^{+}CD3^{+}$ are T-cell/monocyte complexes. (B) ~12,750 NK cells were pooled and 13 different metaclusters identified with Flowsom. Bar plots show metacluster frequencies. Inset shows the lower frequency metaclusters. Expression profile is presented in the heatmap. Most metacluster are Granzyme B^{+}, indicative of cytolytic capacity. (C) ~42,700 Mononuclear Phagocyte cells were pooled and 13 different

metaclusters identified with Flowsom. Bar plots show metacluster frequencies. Inset shows the lower frequency metaclusters. Expression profile is presented in the heatmap. The main metacluster appear to be MDSCs. All bar plots are median and the individual dots are individual patients. Heatmaps are normalised for each marker.

Figure S6. Individual patients' CD8+ T-cells populations. Expression of clustering markers on top of a flowsom tree showing the relationship between metacluster, for the pooled single-cell data (top). Population frequency is presented in the pie chart in each circle along the tree, the background colour represents the metacluster identity. (Bottom) Flowsom trees showing the individual metacluster frequency for each patient. The size of the pie chart corresponds to the frequency relative frequency. (Expression profile: from low (Dark blue) to high (Deep red)).

Figure S7. Individual patients' CD4+ T-cells populations. Expression of clustering markers on top of a flowsom tree showing the relationship between metacluster, for the pooled single-cell data (top). Population frequency is presented in the pie chart in each circle along the tree, the background colour represents the metacluster identity. (Bottom) Flowsom trees showing the individual metacluster frequency for each patient. The size of the pie chart corresponds to the frequency relative frequency. (Expression profile: from low (Dark blue) to high (Deep red)).

Figure S8. Individual patients' Treg populations. Expression of clustering markers on top of a flowsom tree showing the relationship between metacluster, for the pooled single-cell data (top). Population frequency is presented in the pie chart in each circle along the tree, the background colour represents the metacluster identity. (Bottom) Flowsom trees showing the individual metacluster frequency for each patient. The size of the pie chart corresponds to the frequency relative frequency. (Expression profile: from low (Dark blue) to high (Deep red)).

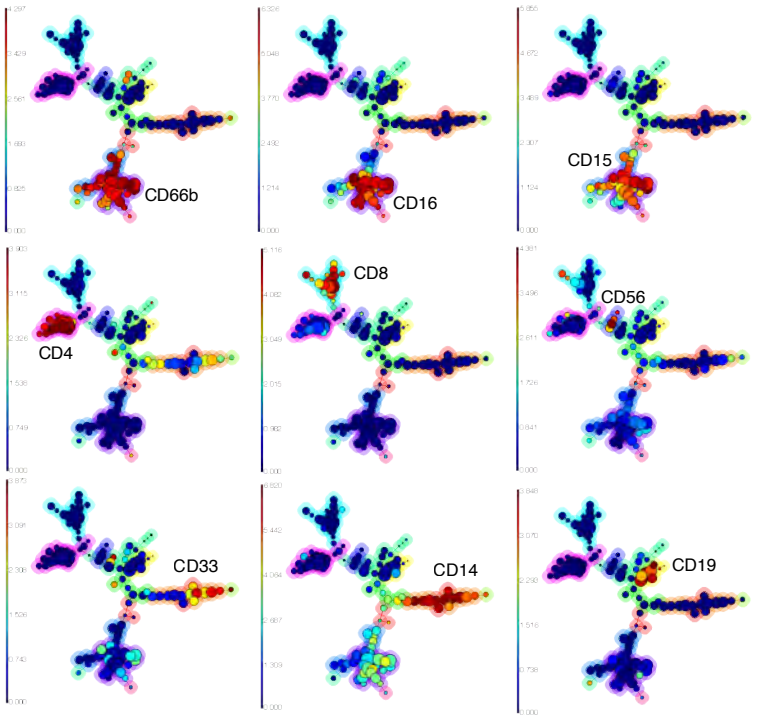
Figure S9. Similar T-cell signatures of senescence and suppression to tumours are observed in peripheral blood. (A) ~18,270 CD8+ T-cells were pooled and 13 metaclusters identified with Flowsom and visualised on viSNE plot. Metaclusters' relative abundance is shown in the bar plot. Inset shows the lower frequency metaclusters. The heatmaps show the expression profile of immune checkpoints in the different metaclusters. Note that the major CD8+ populations are senescent and central memory cells. (B) ~38,350 CD4+ T-cells were pooled and 14 metaclusters identified with Flowsom and visualised on viSNE plot. Metaclusters' relative abundance is shown in the bar plot. Inset shows the lower frequency metaclusters. The heatmaps show the expression profile of immune checkpoints in the different metaclusters. The major populations are effector and central memory cells, followed by regulatory (Foxp3+) T-cells. Two patients also show a signature of senescent cells in the periphery. (C) ~3,600 CD4+ regulatory T-cells were pooled and 10 metaclusters identified with Flowsom and visualised on viSNE plot. Metaclusters' relative abundance is shown in the bar plot. Inset shows the lower frequency metaclusters. The heatmaps show the expression profile of immune checkpoints in the different metaclusters. There is a noticeable naïve metacluster, and the activated metacluster with similar charactertics to the tumour is observed at lower frequency. Bar plots are medians and each dot represents a patient.

Heatmaps were normalised across all T-cell populations per marker. Hierarchal clustering of heatmaps was done in Morpheus.

Figure S10. Multiplex cell annotation scheme. (A) Cancer region showing H&E (left), fluorescence image (middle) and cell annotation from HALO (right). Cyan- epithelium; magenta- αSMA, green- $CD4_+$, orange- $CD8_+$, yellow- $CD3_+$, purple- $Foxp3_+$, blue- $DAPI_+$. (B) Distribution of T-cells across stroma regions with different αSMA densities, where high was defined as the intensity from a blood vessel ($CD8_+$ T-cells, left; $CD4_+$ T-cells middle and Tregs- right).

Granolocytes
Myeloid
B cells
CD4 T-cells
CD8 T-cells
NK cells
MDSC
CD4-CD8- T-cells

MC1 MC7
MC2 MC8
MC3 MC9
MC4 MC10
MC5 MC11
MC6 MC12



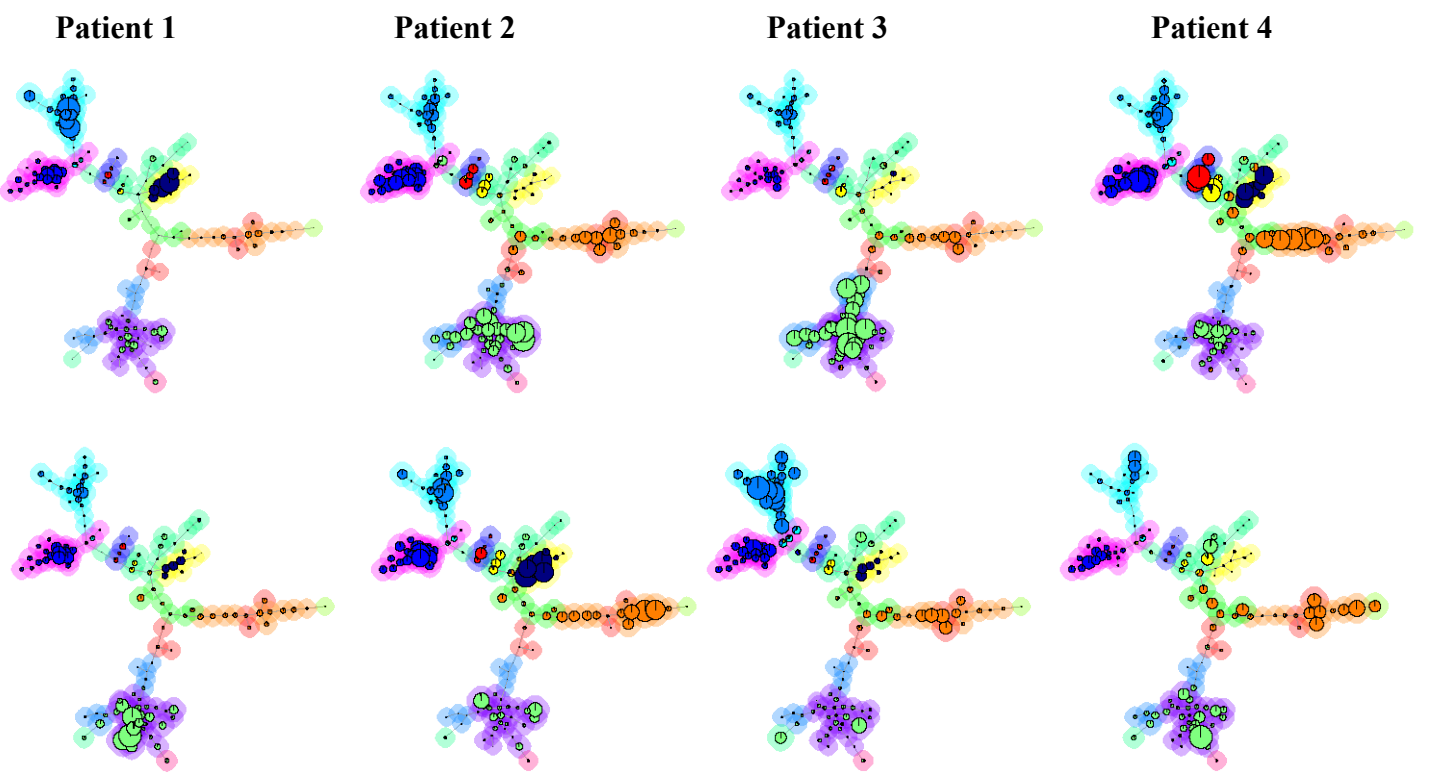


Figure S1. Sivakumar and Abu-Shah et al

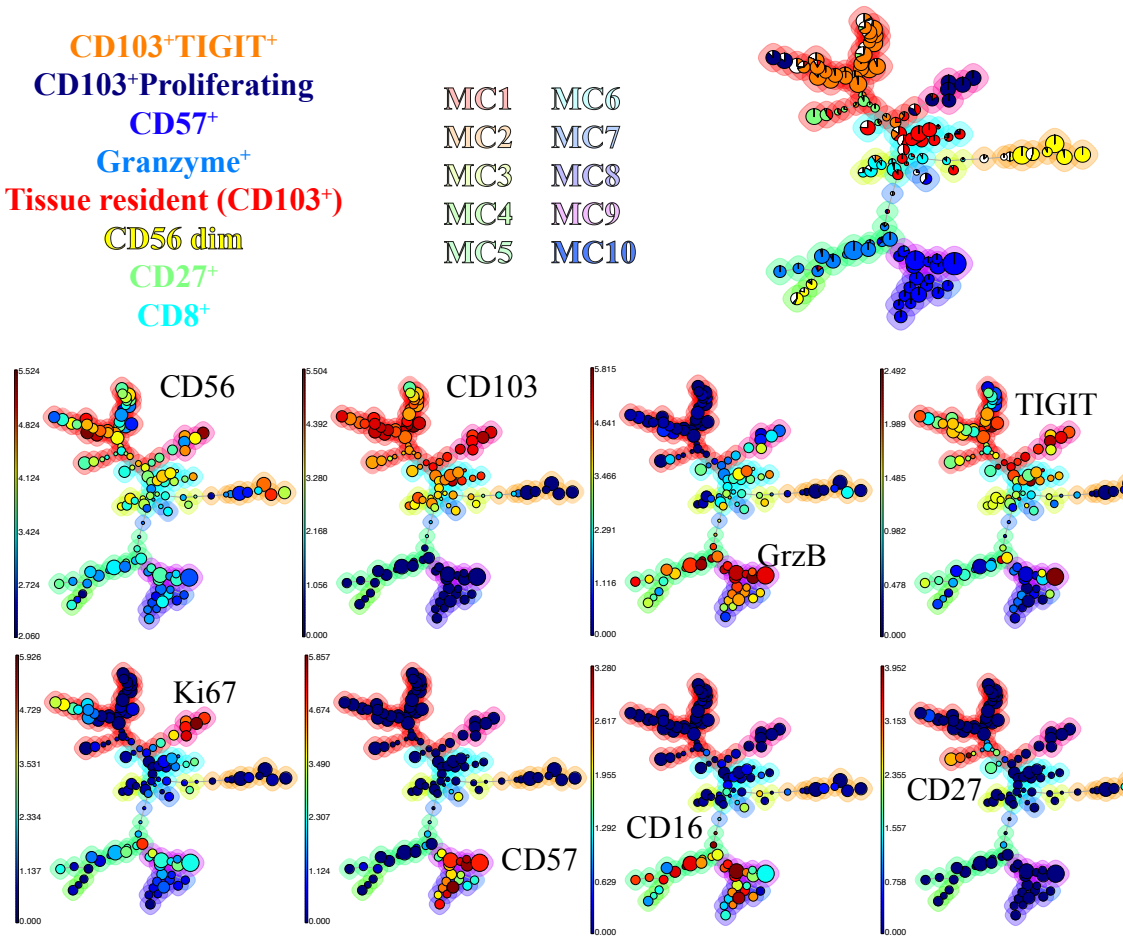
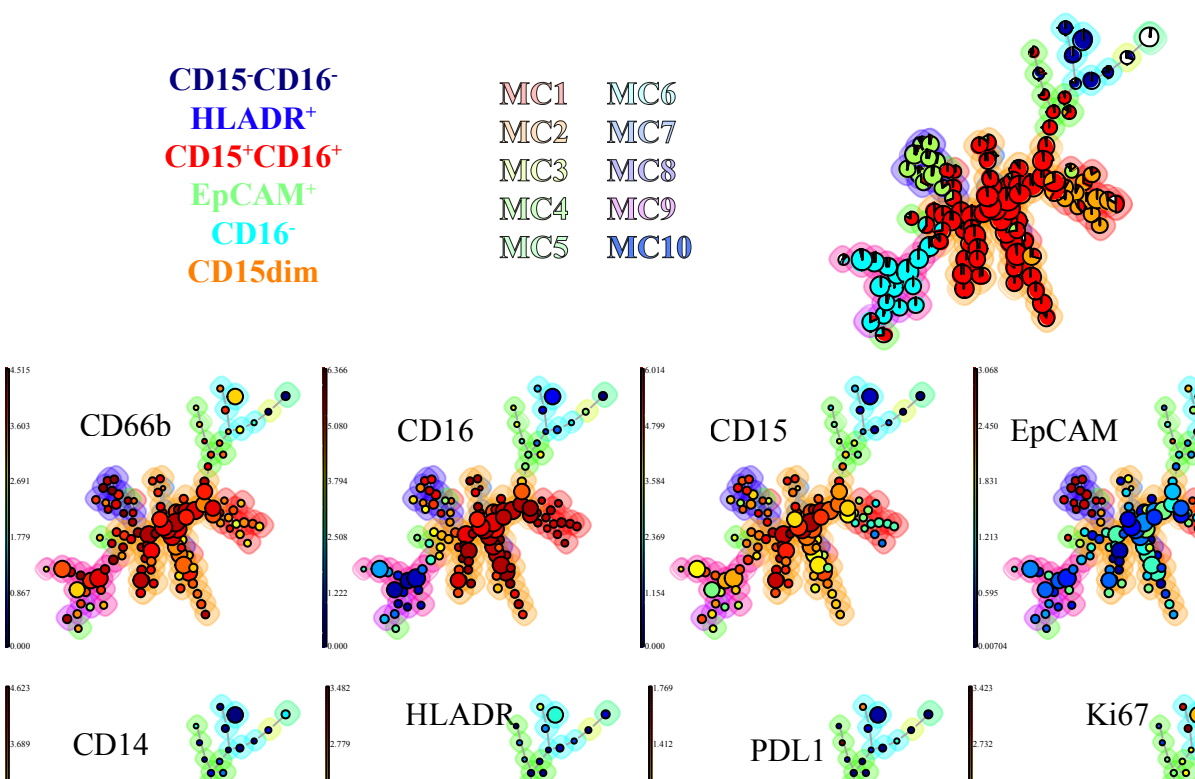
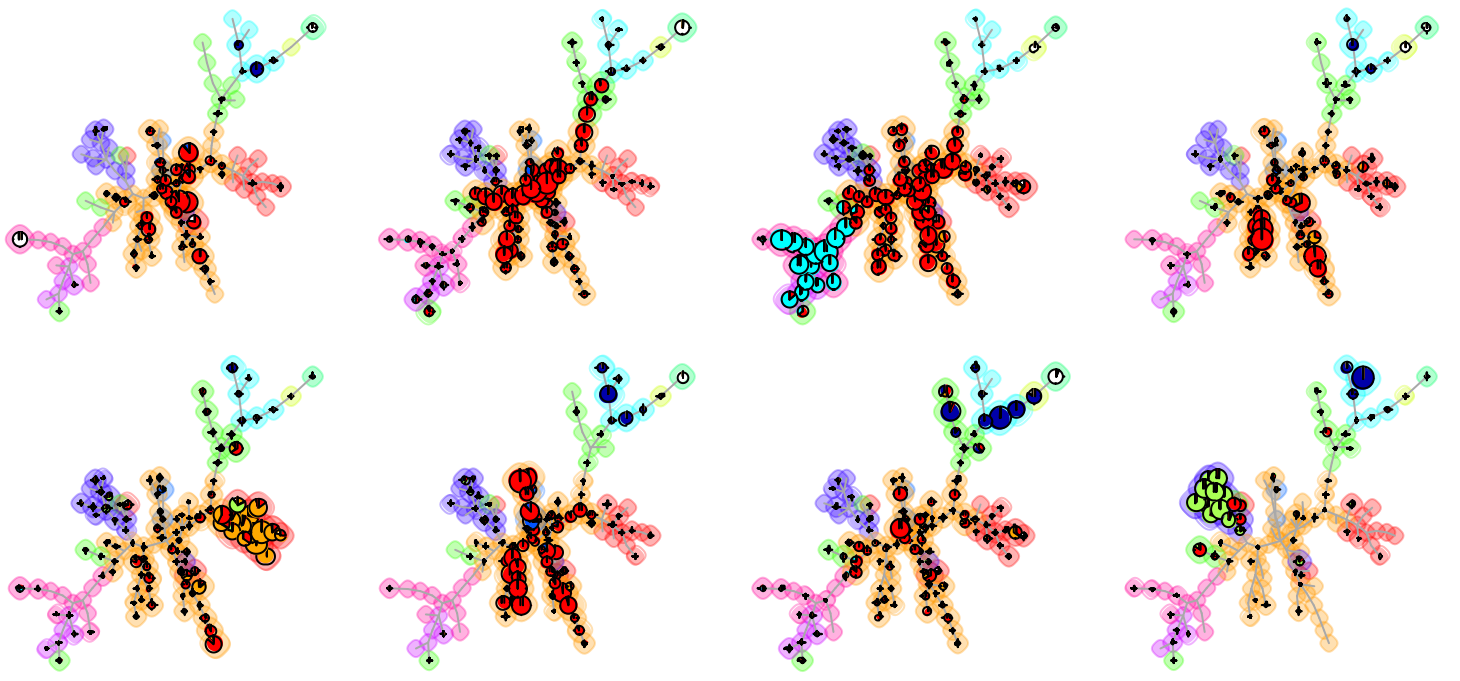


Figure S2. Sivakumar and Abu-Shah et al





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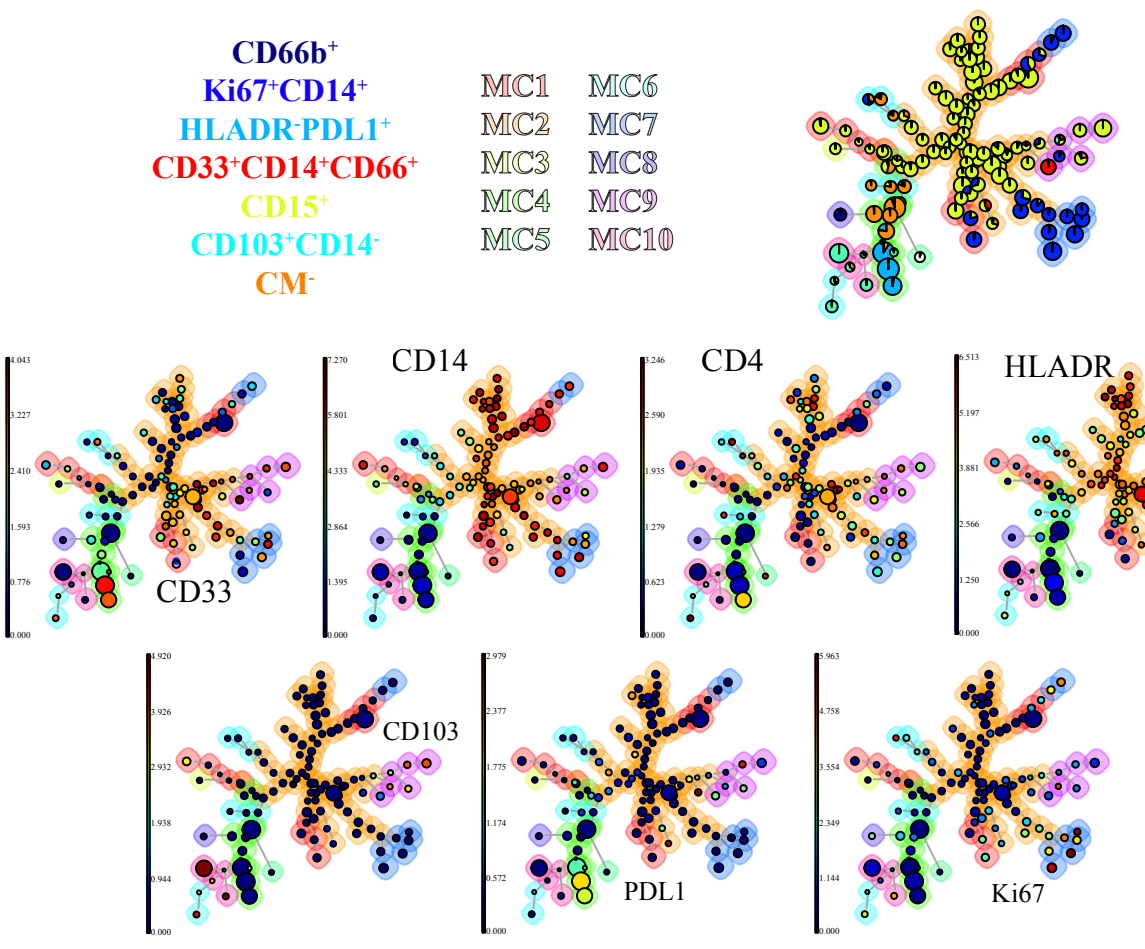


Figure S4. Sivakumar and Abu-Shah et al

Figure S5. Sivakumar and Abu-Shah et al

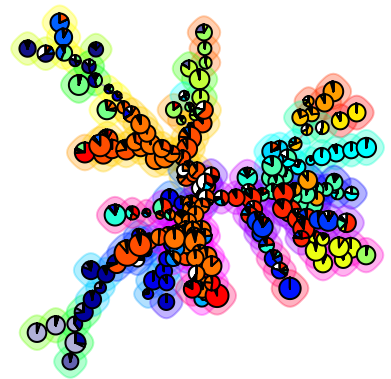
Foxp3+
CD57dimCD28-CD27dim
41BB+Ki67+DR+
exhausted
CD56+
CD56+GrzB+
Naive infeltrating
Central Memory Infiltraiting
GITR+ effector memory

GITR+ central memory
Central memory Tissue res
CD56+ Infiltrting
CD27-GrzB+ EMRA
GrzB- EMRA
GrzB+ EMRA
HLADR+
CD57+CD28-CD27+

CD57+CD28-CD27-GrzB+
(senescent)
CD57+CD28-CD27+GrzB+
Senescent EMRA
Senescent
CD27+ senescent
CD127+ effector memory
CD127 Effector mem resident

Metaclusters

1	11	21
2	12	22
3	13	23
4	14	24
5	15	25
6	16	26
7	17	27
8	18	28
9	19	29
10	20	30



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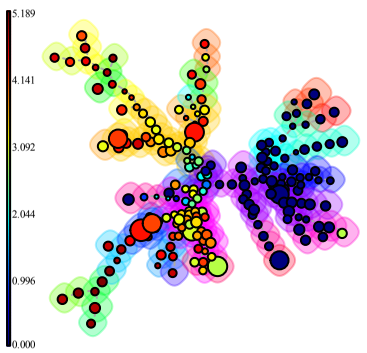

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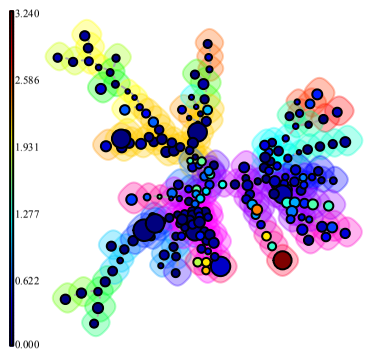

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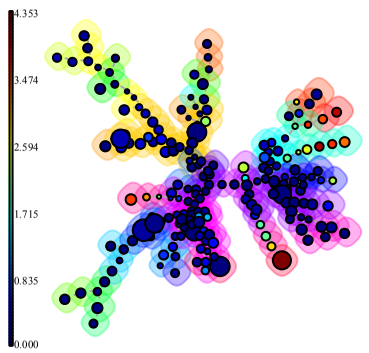

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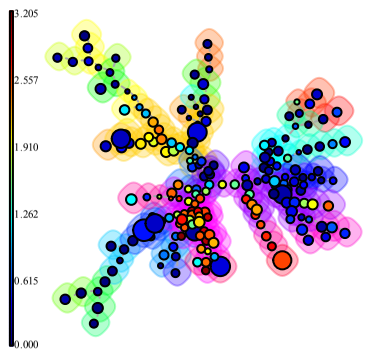

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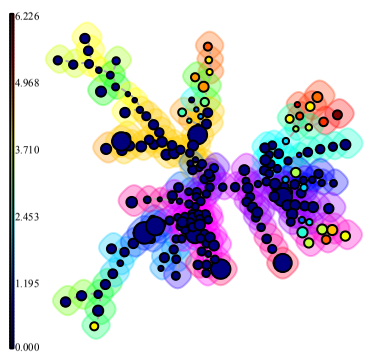

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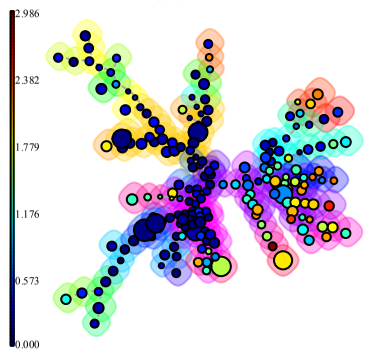

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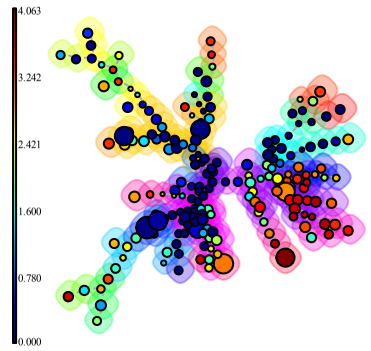

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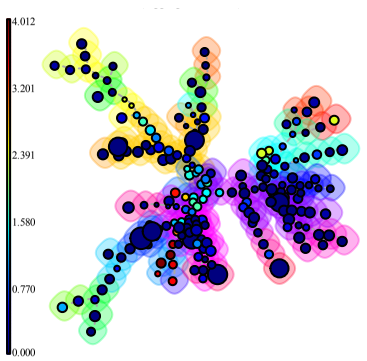
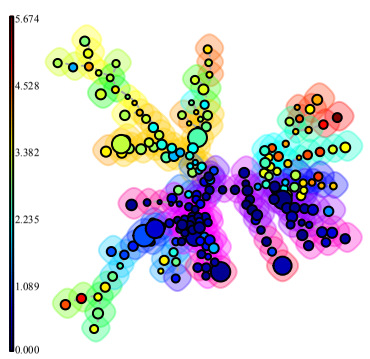


GranzymeB


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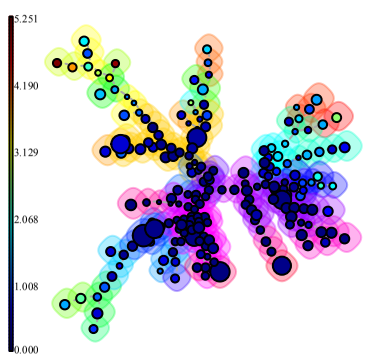
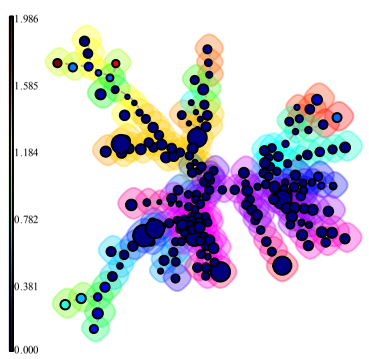


41BB


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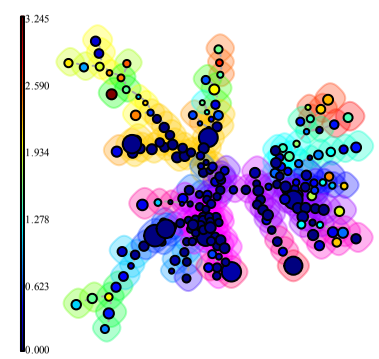

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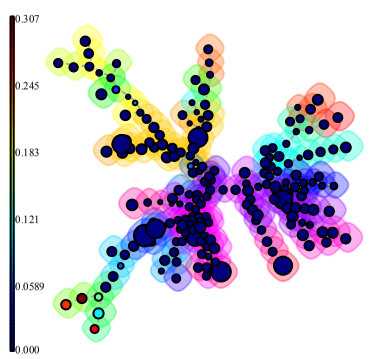

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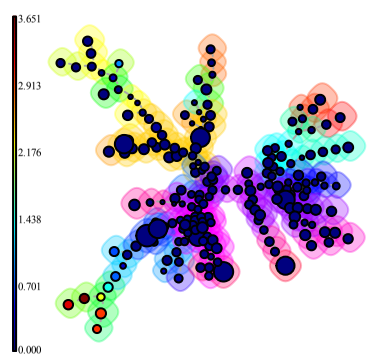

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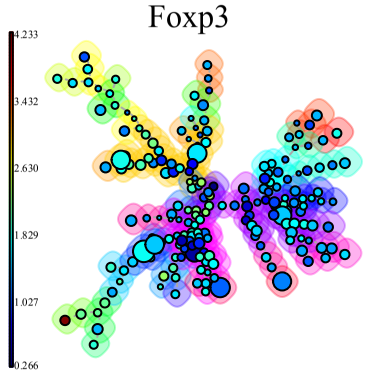

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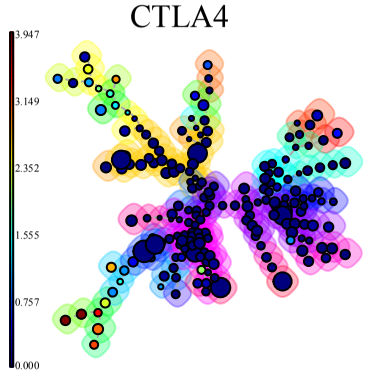

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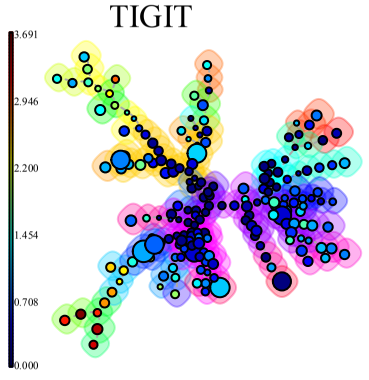

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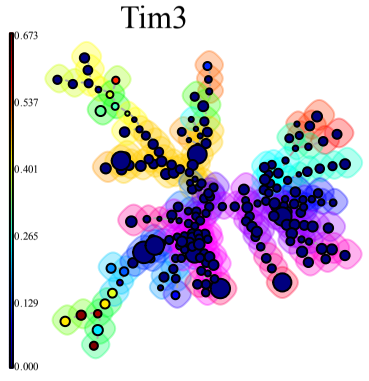

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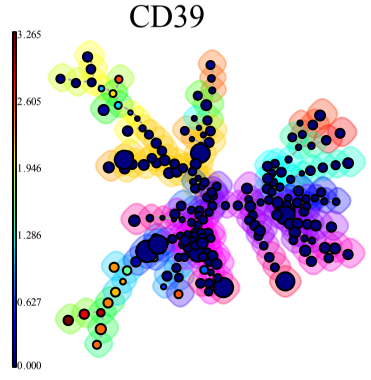

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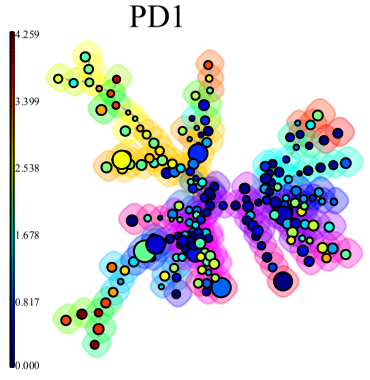

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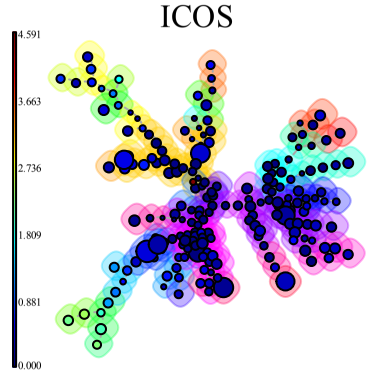

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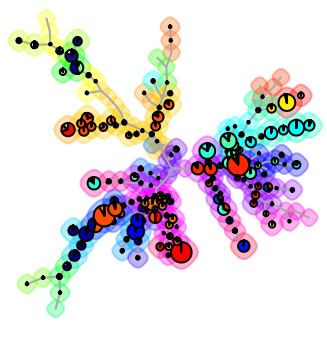

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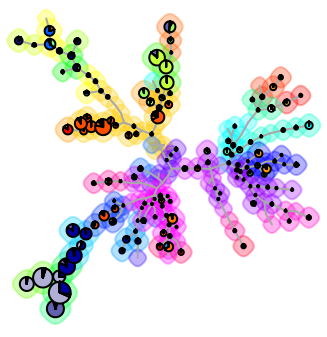

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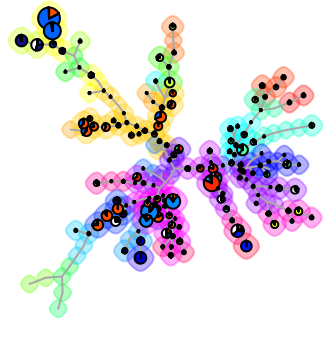

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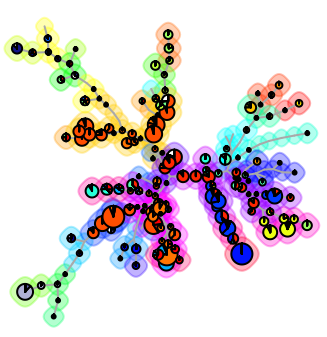

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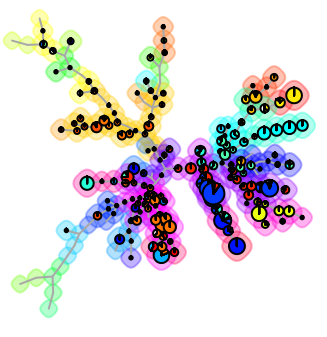

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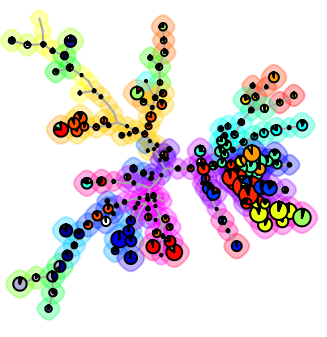


Patient 7

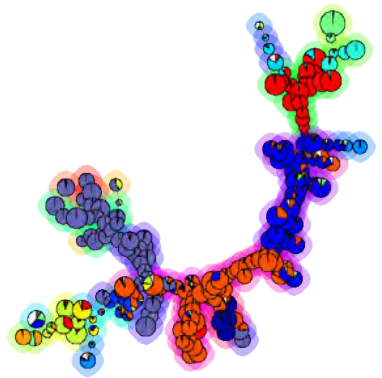

Patient 8


Figure S6. Sivakumar and Abu-Shah et al

Foxp3+
Naive
CD27- Effector memory
exhausted
Proliferating
Profilerating (resident)
Senescent (resident)

Senescent
CD27+ senescent
GrzB+ effector memory
GrzB+ senescent
CD27+ senescent
Effector memory (resident)

Metaclusters
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17



CD103 CCR7 CD45RA CD127 CD27 CD28 CD57

CD56 GranzymeB Ki67 41BB HLADR OX40 CD25

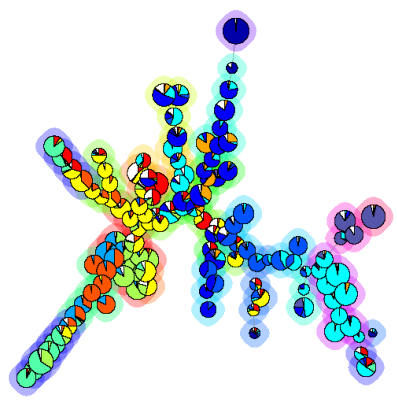
Foxp3 CTLA4 TIGIT Tim3 CD39 PD1 ICOS

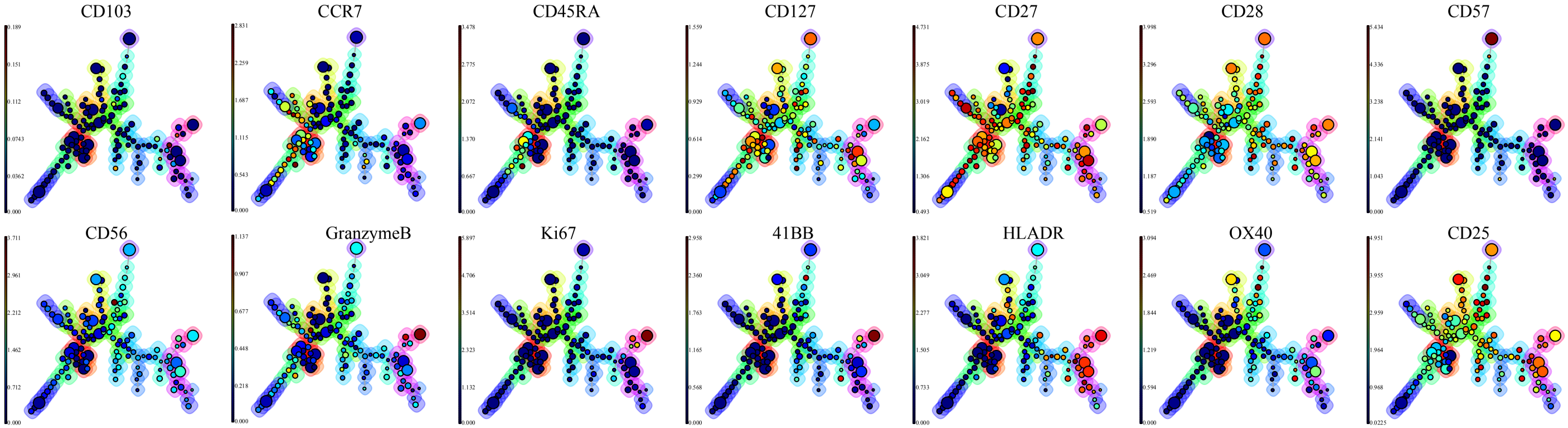
Patient 1 Patient 2 Patient 3 Patient 4 Patient 5 Patient 6 Patient 7 Patient 8

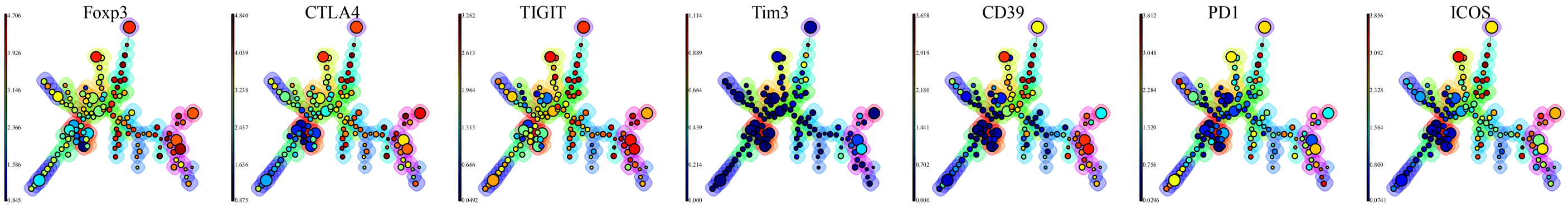
Figure S7. Sivakumar and Abu-Shah et al

Proliferating
CD57+
TIGIT++ ICOS++
TIGIT+ICOS+CD39-
CD45RA+
HLADR+CD39+ICOS+TIGIT+

PDI+
Foxp3 low
Foxp3lowCD27+
CD56+
Naive
TIGIT-ICOS-







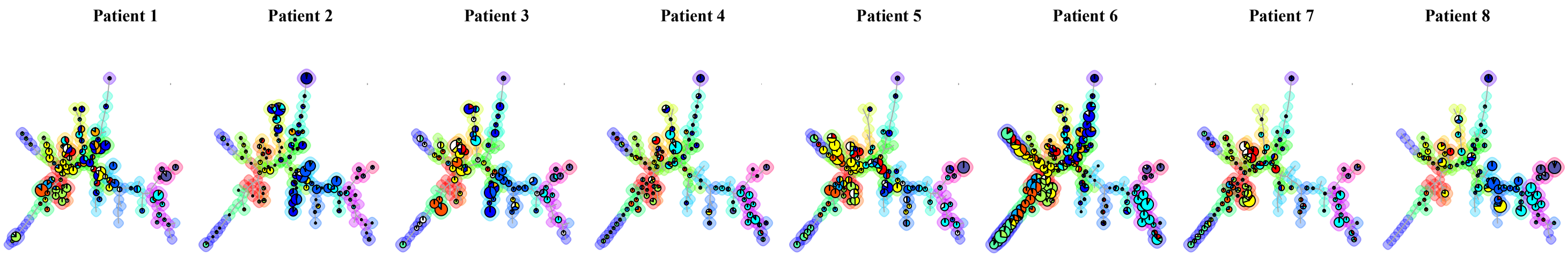
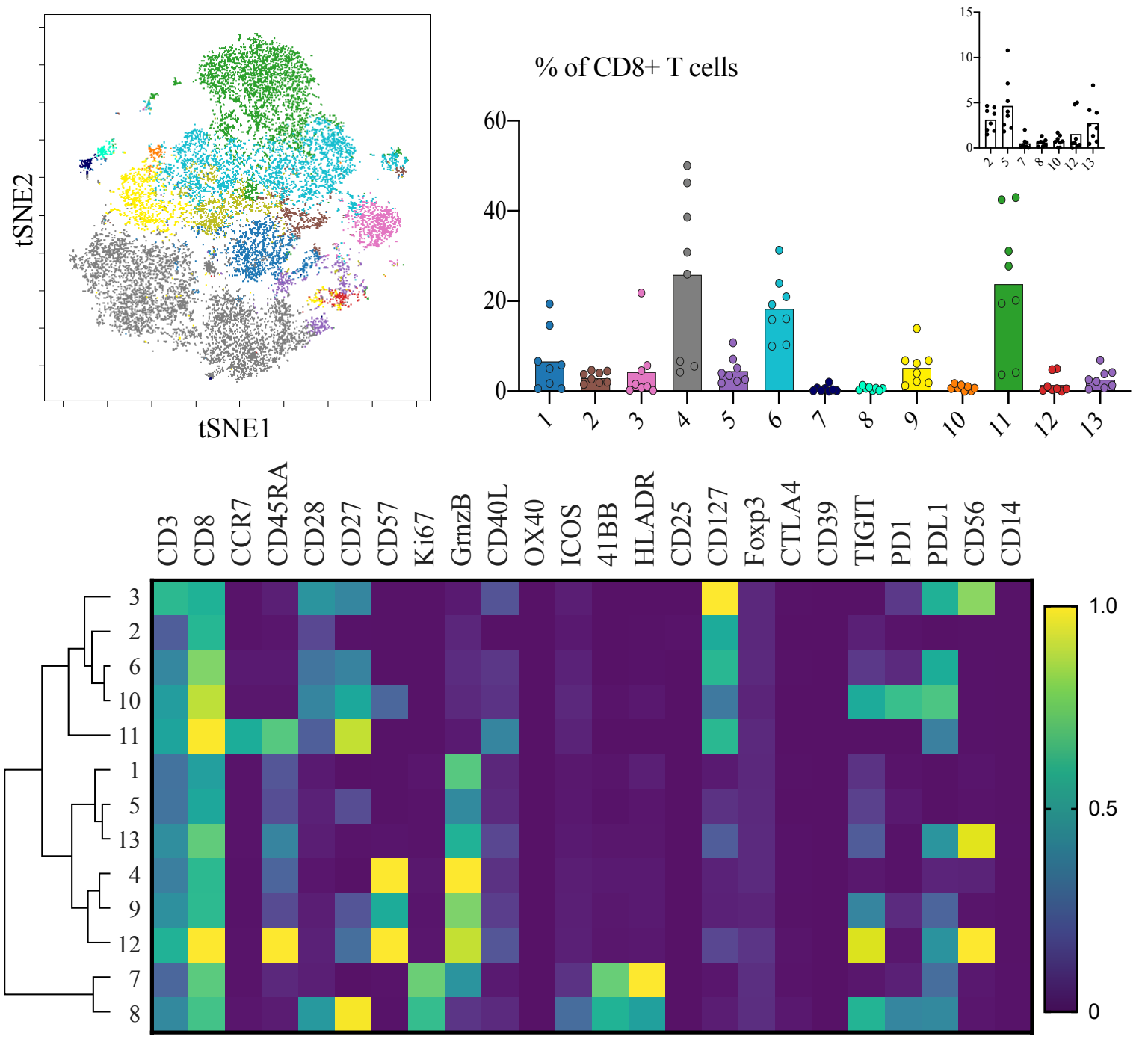
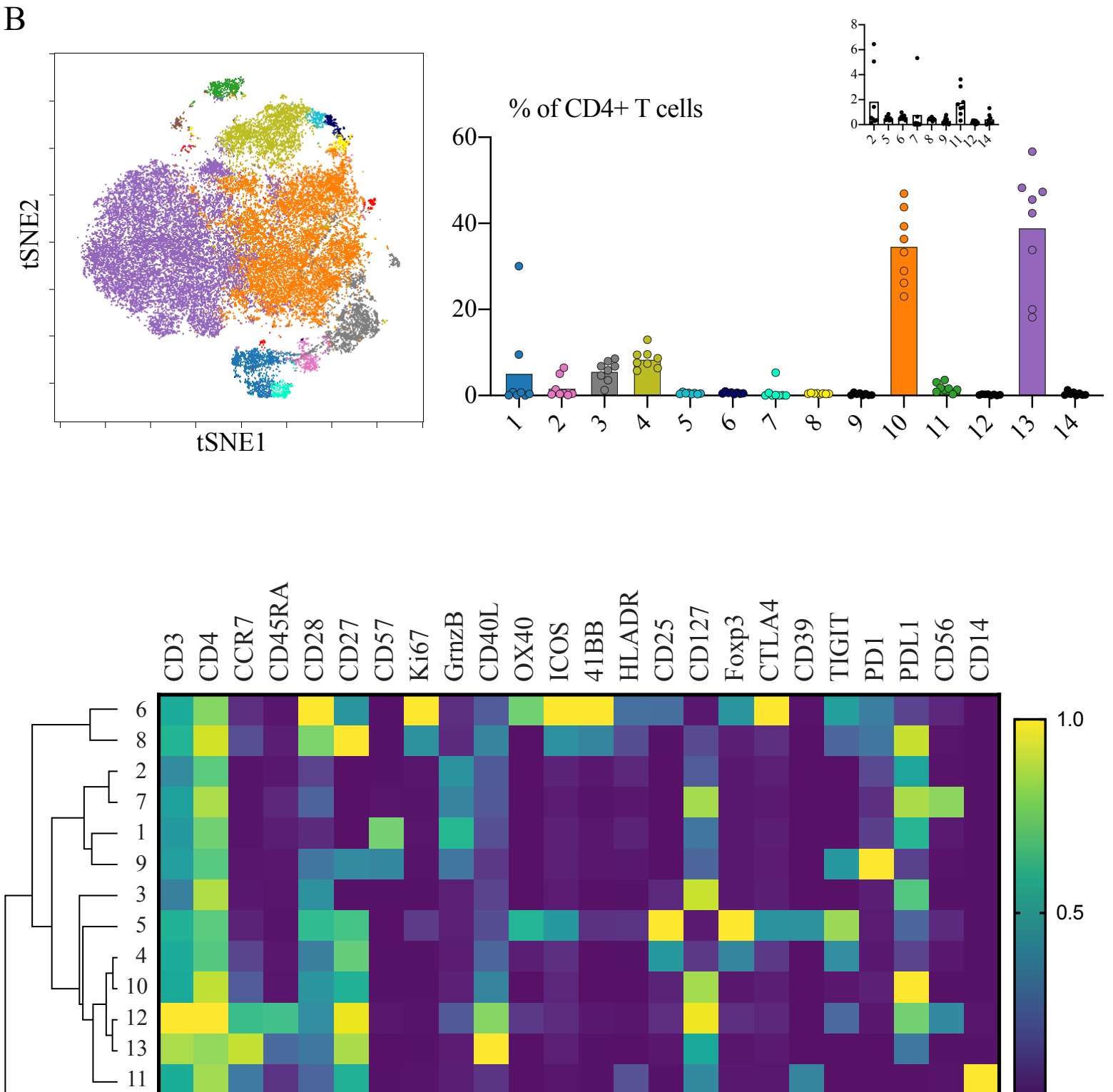


Figure S8. Sivakumar and Abu-Shah et al

A



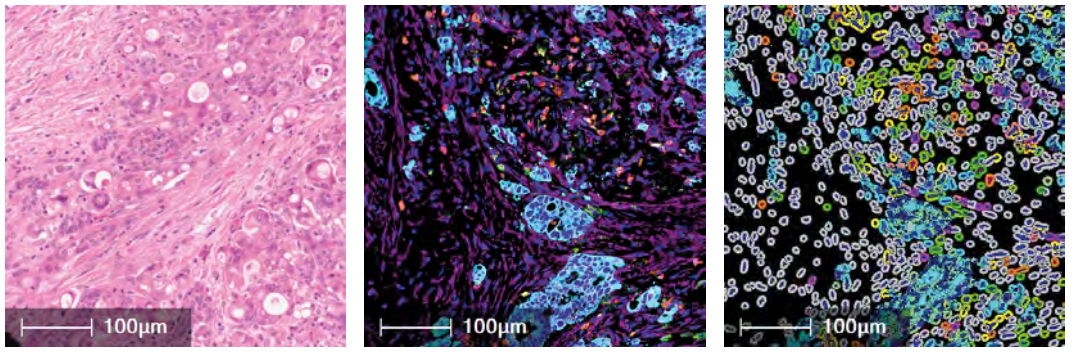
B



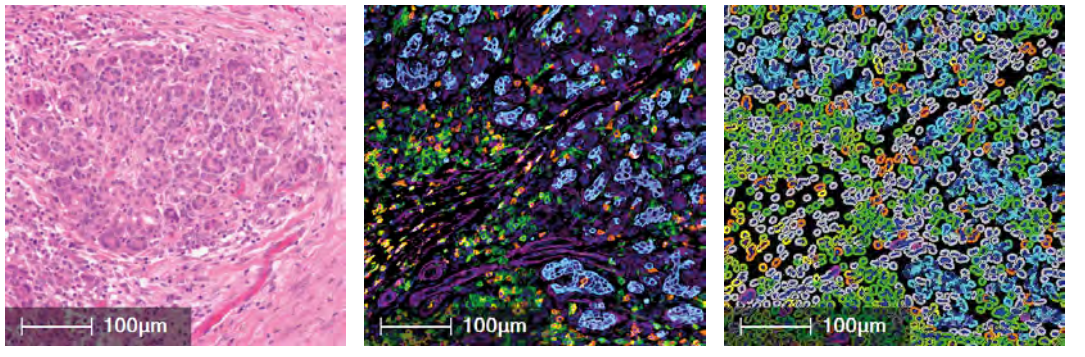
C

Figure S9, Sivakumar and Abu-Shah et al

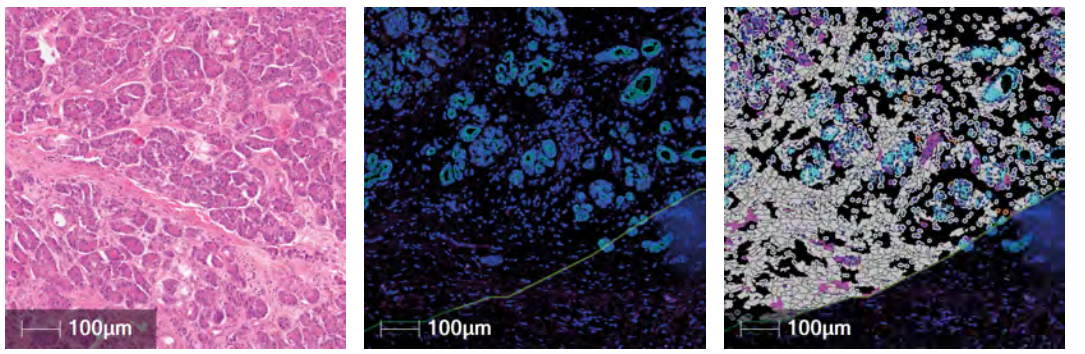
A



B



C



D

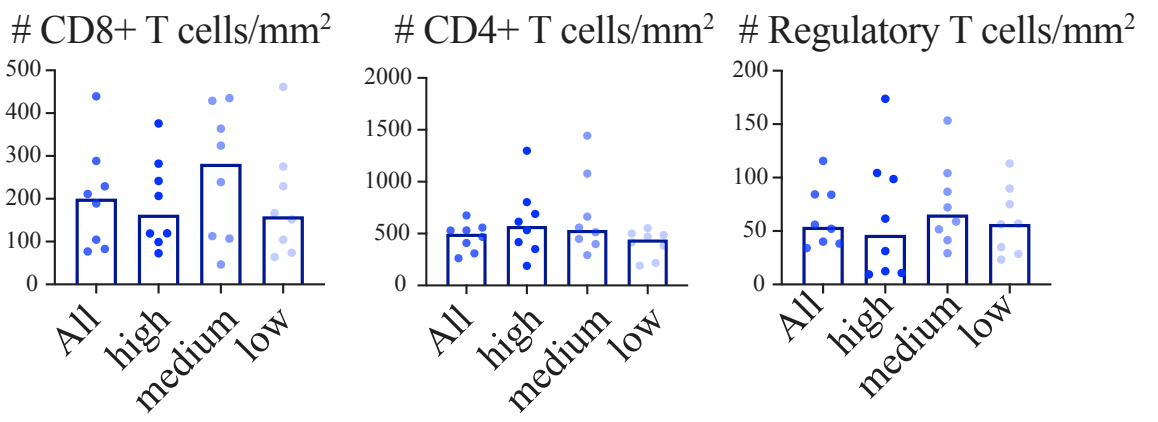


Figure S10, Sivakumar and Abu-Shah et al