

Supplementary Materials for Diwanji et al.

Supplementary tables

Supplementary Table S1. T cell FACS panel for humanized BLT mouse models.

Marker	Fluorochrome	Clone	Dilution	Company	Catalog no.
CD45	FITC	HI30	1:50	BioLegend	304038
CD69	PerCp	FN50	1:50	BioLegend	310928
CD73	BV421	AD2	1:50	BioLegend	344008
CD8	BV510	SK1	1:50	BioLegend	344732
TIM-3	BV605	F38-2E2	1:50	BioLegend	345018
CD56	BV650	HCD56	1:50	BioLegend	318343
CD62L	BV711	SK11	1:50	BD	565040
CCR7	BV786	3D12	1:50	BD	536710
CD3	BUV395	SK7	1:50	BD	564001
CD4	BUV496	SK3	1:50	BD	564651
CD25	PE	BC96	1:50	eBioscience	12-0259-42
FoxP3	PeCy7	236A/E7	1:50	Invitrogen	25-4777-42
CD39	APC	A1	1:50	BioLegend	328210
CD19	A700	SJ25C1	1:50	BioLegend	363034
Live/Dead	APC-Cy7	N/A	1:1000	Thermo	L34976

Supplementary Table S2. Myeloid cell FACS panel for humanized BLT mouse models.

Marker	Fluorochrome	Clone	Dilution	Company	Catalog no.
CD45	FITC	HI30	1:50	BioLegend	304038
CD14	PercpCy5.5	HCD14	1:50	BioLegend	325622
CD80	BV421	2D10	1:50	BioLegend	305222
HLA-DR	BV510	L243	1:50	BioLegend	307646
CD15	BV650	W6D3	1:50	BioLegend	323033
CD206	BV711	15-2	1:50	BioLegend	321135
CD141	BV785	M80	1:50	BioLegend	344116
CD163	BUV395	GHI/61	1:50	BD	745572
CD16	BUV496	3G8	1:50	BD	612944
CD86	BUV737	2331	1:50	BD	564428
CD11b	PE	ICRF44	1:50	BioLegend	301306
CD68	PeCy7	Y1/82A	1:50	BioLegend	333815
TIM-3	APC	F38-2E2	1:50	BioLegend	345012
CD11c	A700	Bu15	1:50	BioLegend	337220
Live/Dead	APC-Cy7	N/A	1:1000	Thermo Fisher Scientific	L34976

Supplementary Table S3. T cell FACS panel for syngeneic mouse models.

Marker	Fluorochrome	Clone	Dilution	Company	Catalog no.
CD45	BV510	30-F11	1:400	BioLegend	103137
Live/Dead	APC-Cy7	Na	1:1000	eBioscience	65-0865-14
CD4	BUV395	GK1.5	1:200	BD	563790
CD8	BV650	53-6.7	1:200	BioLegend	100742
TIM-3	PE	5D12	1:100	NIBR	
CD11b/ CD19	BUV737	M1/70 1D3	1:200	BD	564443 612781
CD39	Pe-CF594	Duha59	1:200	BioLegend	143811
CD73	BV421	TY/11.8	1:200	BioLegend	127217
PD-1	Percp-Cy5.5	29F.1A12	1:200	BioLegend	135207
iCOS	BV785	C398.4A	1:50	BioLegend	313533
TOX	eFluor 660 (APC)	TXRX10	1:50	Thermo Fisher Scientific	50-6502-82
FoxP3	AF488	FJK-16s	1:100	eBioscience	53-5773-82
Ki-67	BV605	16A8	1:100	BioLegend	652413
Helios	PE-Cy7	22F6	1:300	BioLegend	137236

Supplementary Table S4. Myeloid cell FACS panel for syngeneic mouse models.

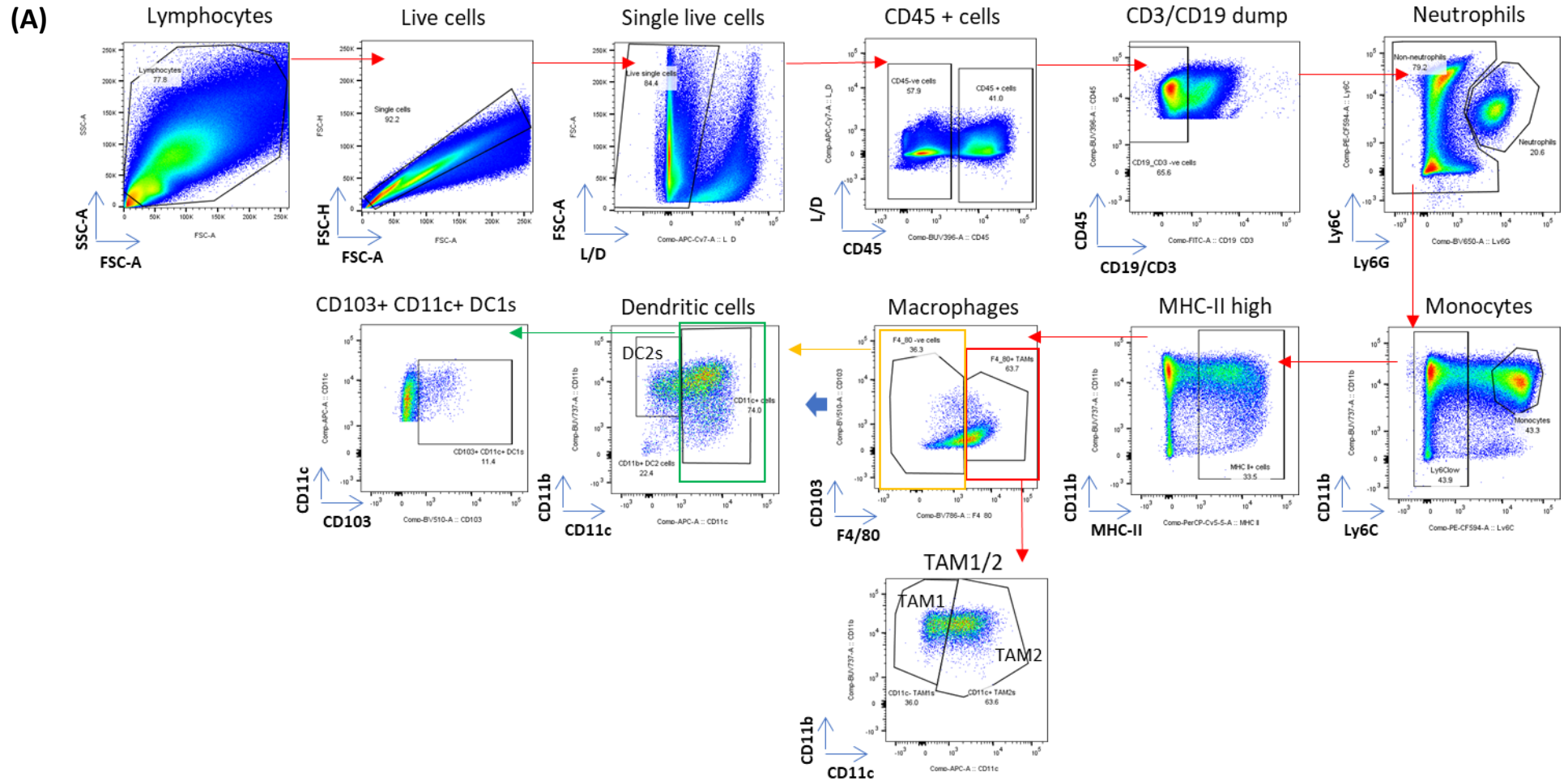
Marker	Fluorochrome	Clone	Dilution	Company	Catalog no.
CD45	PE-Cy7	30-F11	1:400	BioLegend	103114
Live/Dead	APC-Cy7	N/A	1:1000	eBioscience	65-0865-14
CD11c	APC	N418	1:200	eBioscience	17-0114-81
CD19	FITC	eBio1D3	1:200	eBioscience	11-0193-82
TIM-3	PE	5D12	1:100	NIBR	
CD103	BV510	2E7	1:200	BioLegend	121423
F4/80	BV785	BM8	1:200	BioLegend	123141
MHCII	PerCP-Cy5.5	M5/115.15.2	1:400	BioLegend	107626
CD11b	BUV737	M1/70	1:200	BD	564443
Ly6C	AF700	HK1.4	1:200	BioLegend	128024
Ly6G	BV650	1A8	1:200	BioLegend	127641
CD24	BV605	M1/69	1:200	BioLegend	101827

Supplementary Table S5. Stromal cell FACS panel for syngeneic mouse models.

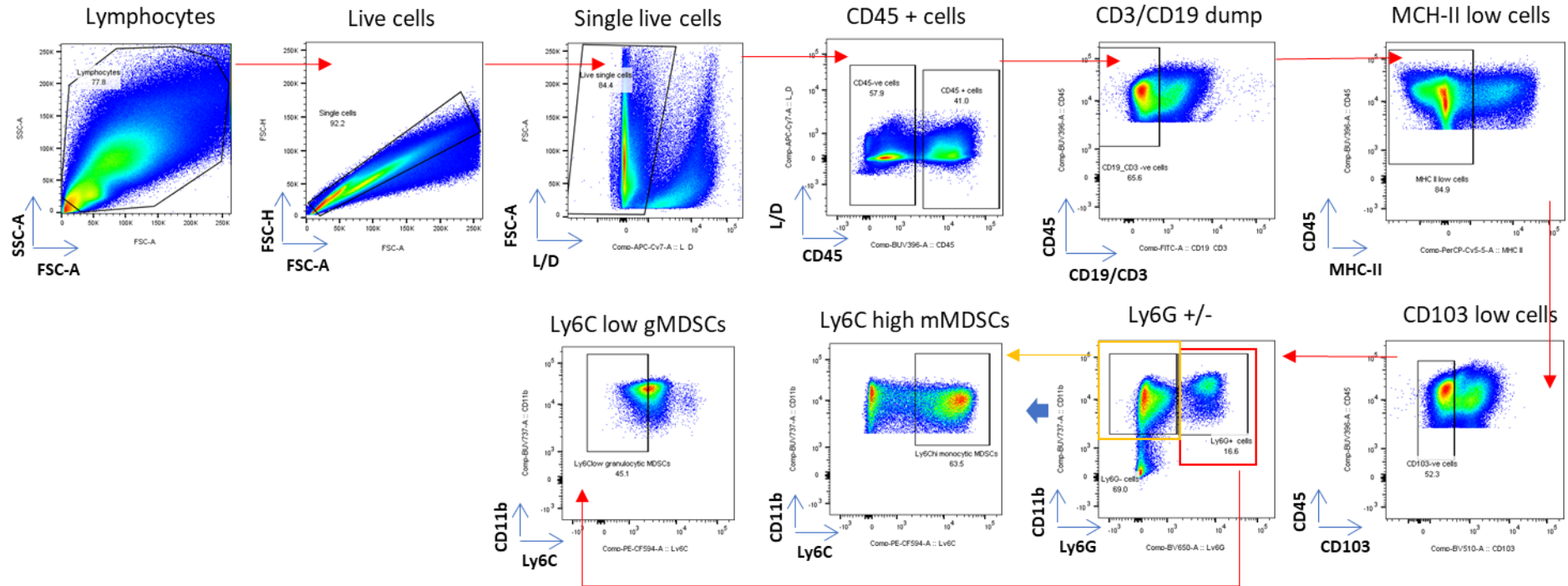
Marker	Fluorochrome	Clone	Dilution	Company	Catalog no.
CD31	FITC	MEC13.3	1:200	BioLegend	102506
α SMA	PE	1A4	1:100 (intracellular)	Sigma	C6198-2ML
CD39	PE-Cy7	24DMS1	1:100	eBioscience	25-0391-82
CD26	PerCP-Cy5.5	H194-112	1:100	eBioscience	45-0261-82
PDPN	APC	8.1.1	1:100	BioLegend	127410
CD73	PBlue/450	eBioTY/11.8	1:100	eBioscience	48-0731-82
CD90	BV605	53-2.1	1:100	BioLegend	140317
CD45	BUV395	30-F11	1:600	BD	564279
CD44	APC-Cy7/780	IM7	1:100	Invitrogen	4329926

Supplementary figures

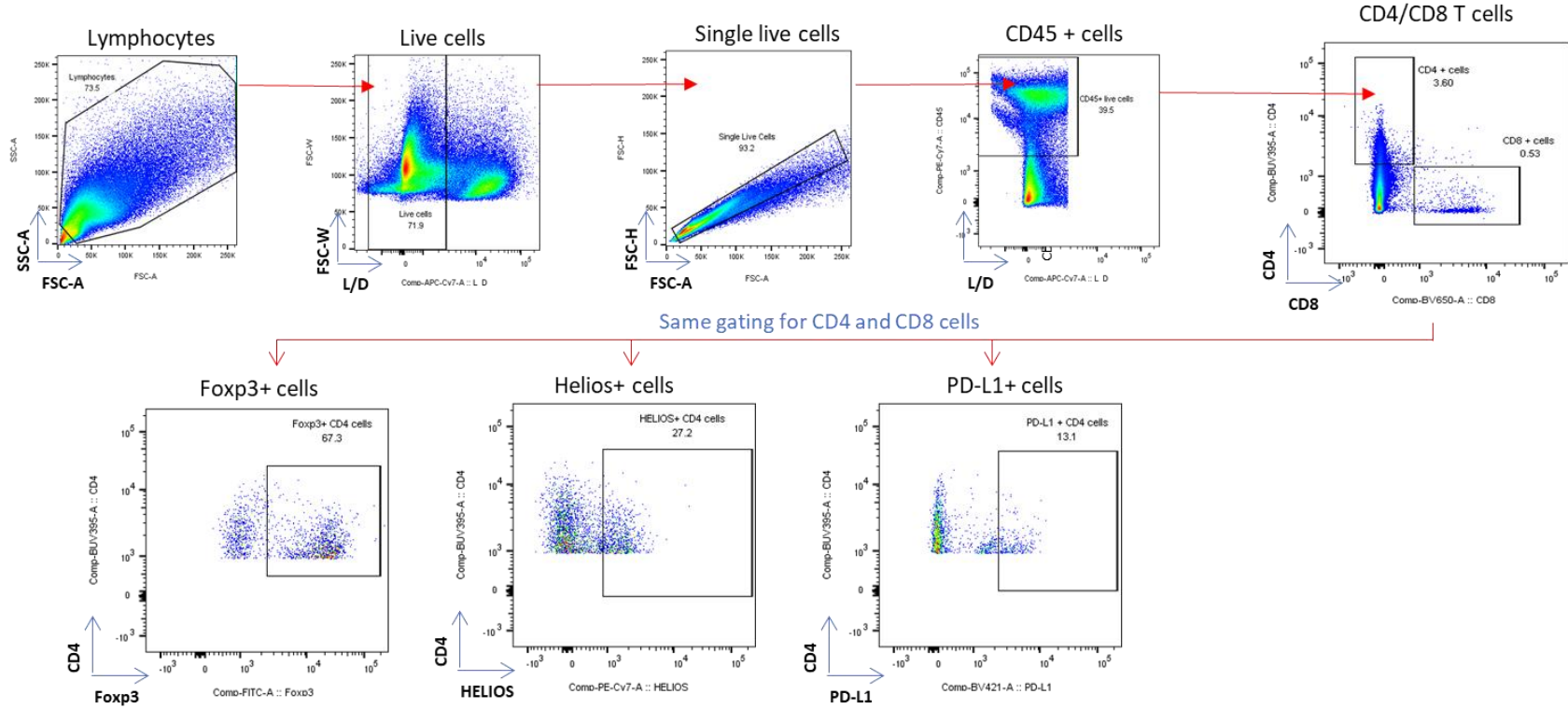
Supplementary Figure S1. Gating strategy for cell subpopulations. **A and B.** Myeloid panel gating overview. **C.** T-cell panel gating overview.



(B)



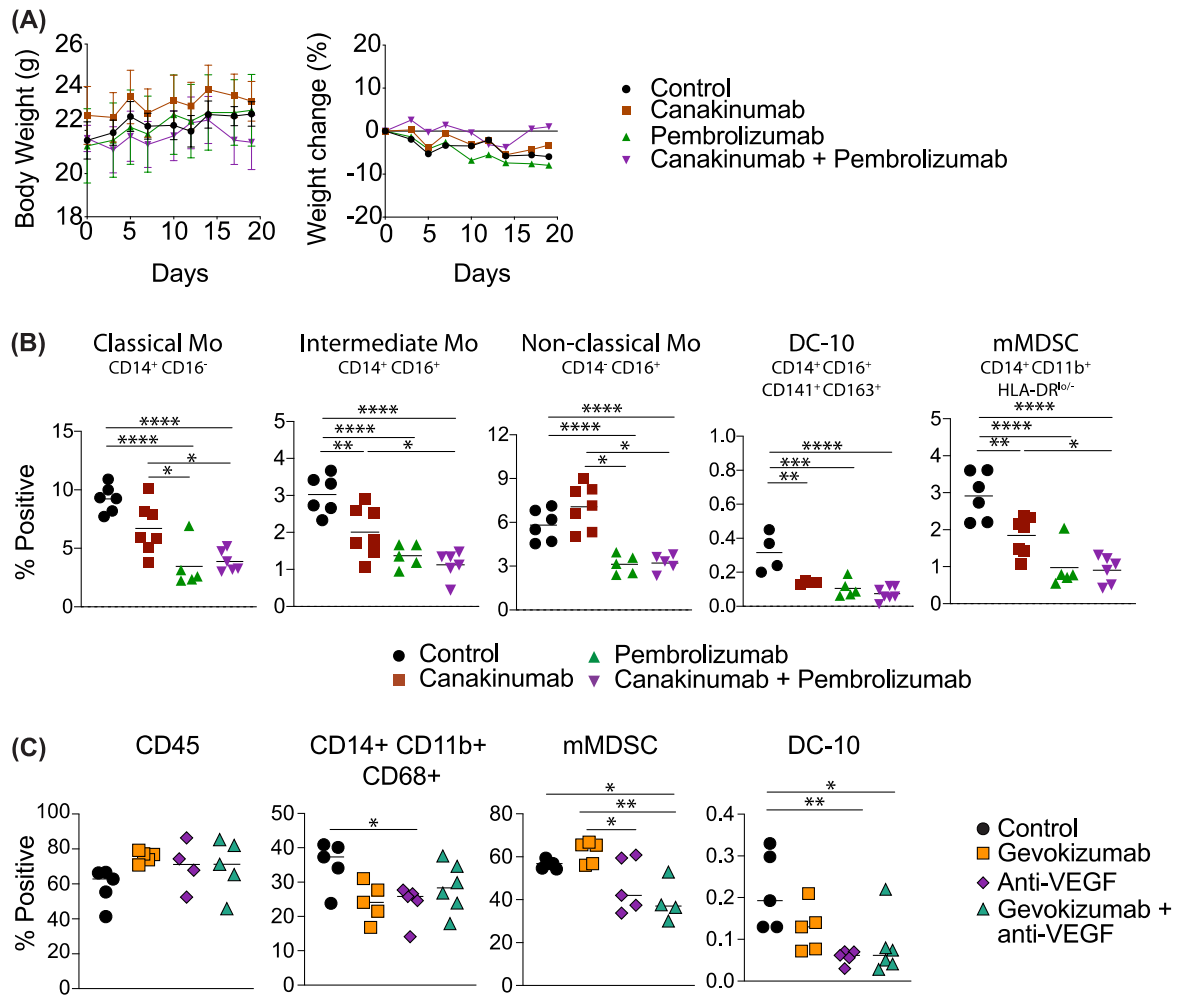
(c)



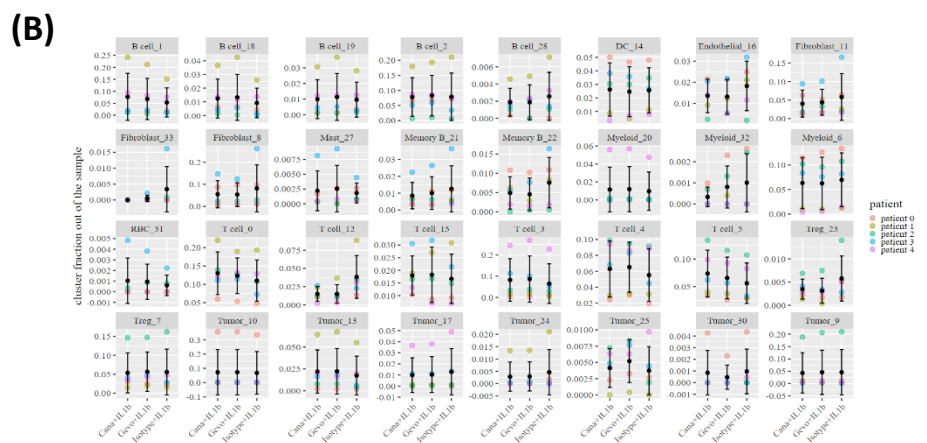
Supplementary Figure S2. A. Body weight in grams (left panel) and % change in body weight (right panel) in humanized BLT mice implanted with H538 tumors treated with isotype controls, canakinumab, pembrolizumab or a combination of canakinumab and pembrolizumab. Each line in the graph represents a treatment group, mean $\pm$ SEM is depicted in the left graph, mean in the right graph. N=15 mice per group, representative data from 2 independent experiments shown.

B. Effect of canakinumab alone or in combination with pembrolizumab on tumor-infiltrating immune cells in H538 tumors. Each dot represents an individual mouse, n=4–7 mice per group, representative data from 2 independent experiments shown. Line represents mean. *p* values were calculated by one-way ANOVA with Dunnett's post-test correction.

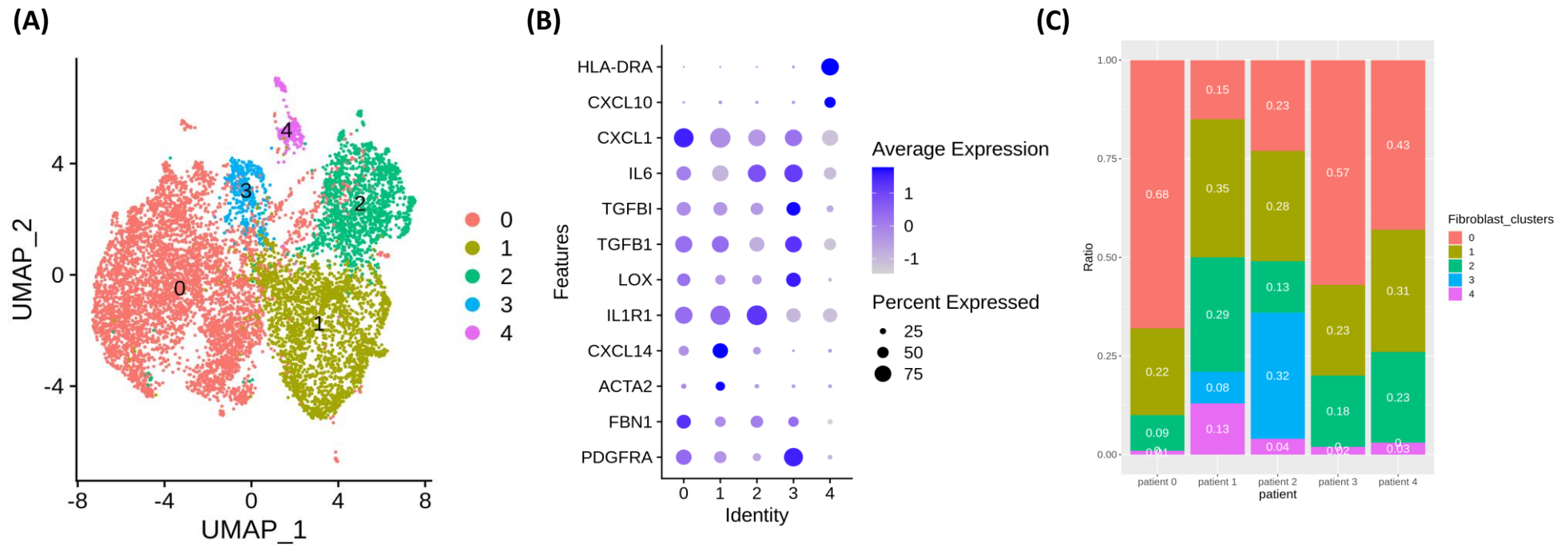
C. Effect of gevokizumab alone or in combination with anti-VEGF α on tumor-infiltrating immune cells in SW480 tumors. Each dot represents an individual mouse, n=4–5 mice per group, representative data from 2 independent experiments shown. Line represents mean. *p* values were calculated by one-way ANOVA with Dunnett's post-test correction. **p* $\leq$ 0.05; ***p* $\leq$ 0.01; ****p* $\leq$ 0.001; *****p* $\leq$ 0.0001 throughout. BLT, bone marrow-liver-thymus; SEM, standard error of the mean.



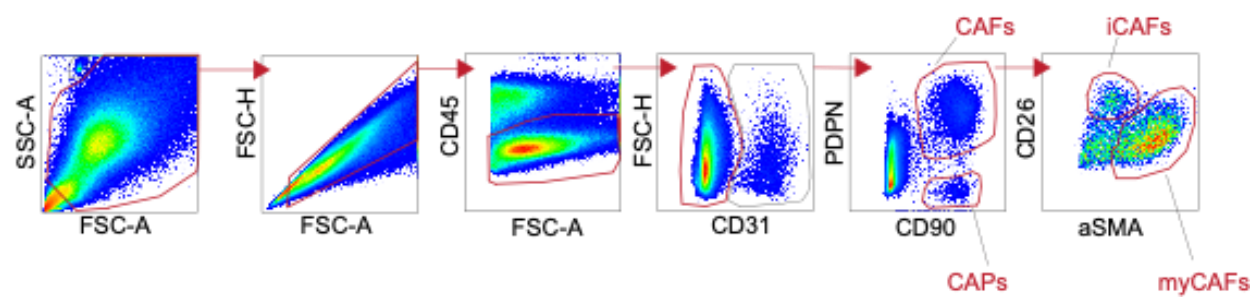
indicated by the black dot and error bar, respectively.



Supplementary Figure S4: Fibroblast subtyping analysis for human CRC sample experiment. **A.** UMAP plot of fibroblast cells colored by cluster ID. **B.** Dotplot of key canonical markers of fibroblast subtypes. **C.** Proportion of fibroblast subtypes across all five patients.

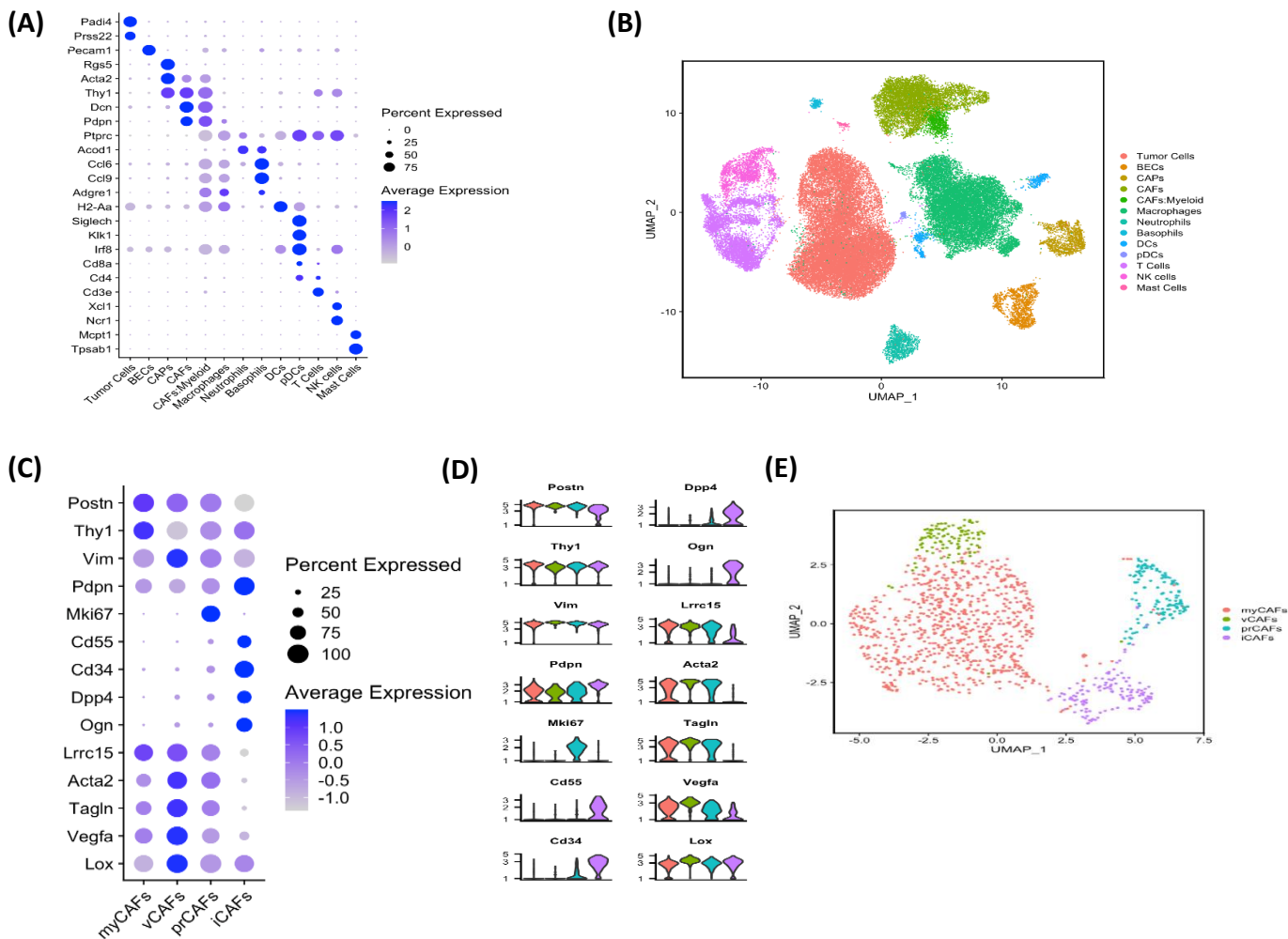


Supplementary Figure S5: Gating strategy for stromal CAF subpopulations.



Supplementary Figure S6. Characterization of tumor-infiltrating cells in 4T1 tumor models.

A. Key marker gene expression by immune cell type. **B.** UMAP visualization of different immune cell types. **C.** Key marker gene expression by CAF type. **D.** Violin plot visualization of CAF type distribution. **E.** UMAP visualization of CAF type distribution.



Supplementary Figure S7: IL-1 β blockade in combination with anti-TGF β affects PD-1 and PD-L1 expression in immune cells from the TME. Treatment with anti-mouse-IL-1 β and anti-TGF β modulates PD-1 and PD-L1 expression in immune cells infiltrating the TME in 4T1 tumor models. Each dot represents an individual mouse, n=10–15 mice per group, representative data from 2 independent experiments shown. Line represents median. *p* values were calculated by one-way ANOVA with Dunnett's post-test correction. **p* $\leq$ 0.05; ***p* $\leq$ 0.01; ****p* $\leq$ 0.001; *****p* $\leq$ 0.0001 throughout.

