

Supplementary Materials for

Improving oligo-conjugated antibody signal in multimodal single-cell analysis

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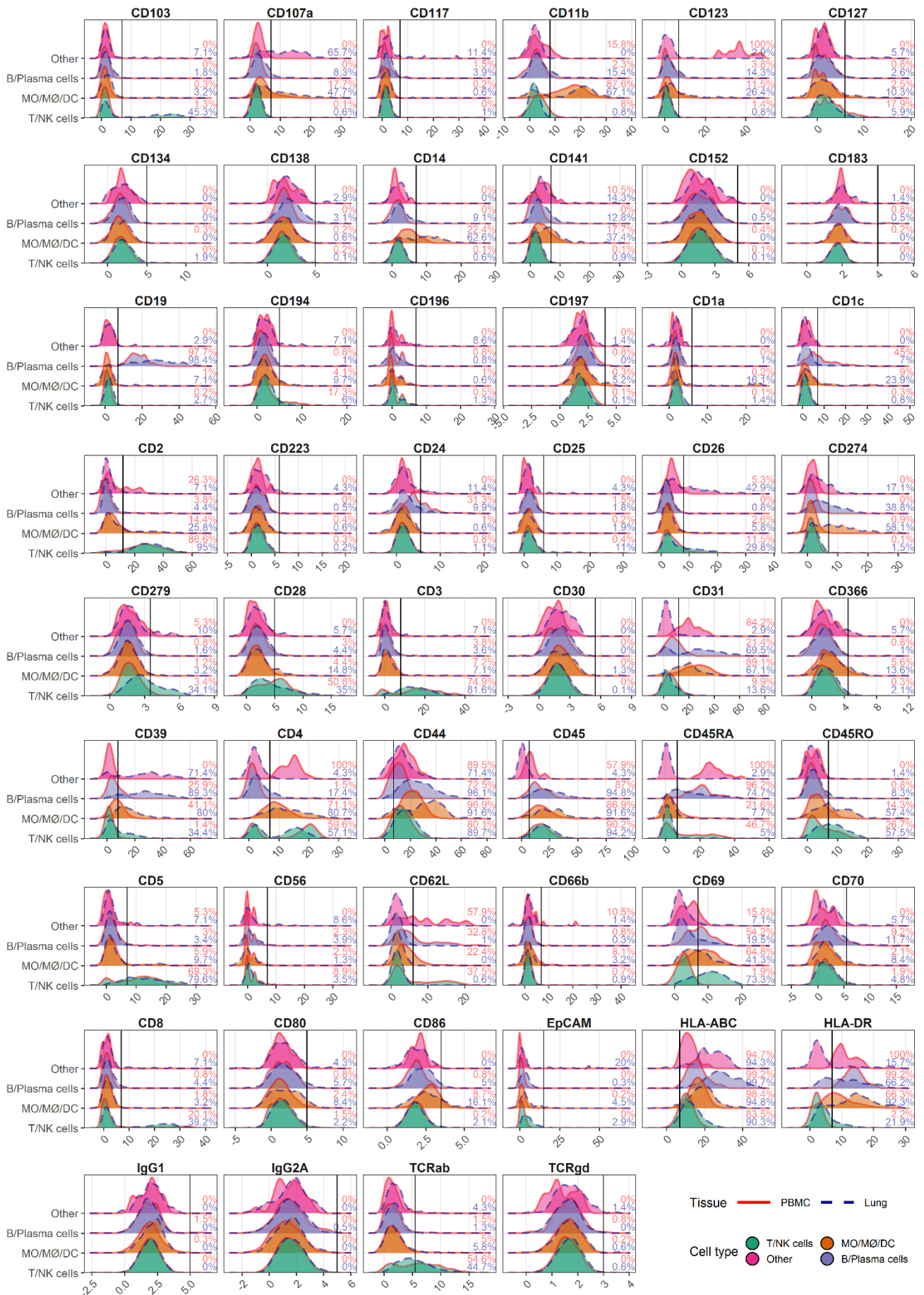
Supplementary Table S1: Oligo-conjugated antibody panel for 5'-CITE-seq

Marker	Alias	Clone	Isotype (Mouse)	Vendor	TotalSeq C Tag	Cat#	DF1 conc. (µg/ml)	DF4 conc. (µg/ml)	Optimized conc. (µg/ml)	Optimized conc. (DF1 fold change)
CD44		BJ18	IgG1	BioLegend	0125	338827	0.050	0.013	0.025	2.00
CD2		TS1/8	IgG1	BioLegend	0367	309231	0.100	0.025	0.100	1.00
CD31	PECAM-1	WM59	IgG1	BioLegend	0124	303139	0.100	0.025	0.100	1.00
CD45		HI30	IgG1	BioLegend	0391	304068	0.100	0.025	0.100	1.00
CD123		6H6	IgG1	BioLegend	0064	306045	0.500	0.125	0.500	1.00
CD3		UCHT1	IgG1	BioLegend	0034	300479	0.500	0.125	0.500	1.00
CD1c		L161	IgG1	BioLegend	0160	331547	0.500	0.125	1.000	0.50
EpCAM		9C4	IgG2b	BioLegend	0123	324247	0.625	0.156	0.063	10.00
CD8		RPA-T8	IgG1	BioLegend	0080	301071	0.625	0.156	0.078	8.00
CD11b		ICRF44	IgG1	BioLegend	0161	301359	0.625	0.156	0.125	5.00
CD5		UCHT2	IgG1	BioLegend	0138	300637	0.625	0.156	0.156	4.00
CD69		FN50	IgG1	BioLegend	0146	310951	0.625	0.156	0.156	4.00
CD39		A1	IgG1	BioLegend	0176	328237	0.625	0.156	0.625	1.00
CD45RA		HI100	IgG2b	BioLegend	0063	304163	0.625	0.156	0.625	1.00
CD70		113-16	IgG1	BioLegend	0027	355119	0.625	0.156	1.250	0.50
CD196	CCR6	G034E3	IgG2b	BioLegend	0143	353440	0.625	0.156	3.125	0.20
CD19		H1B19	IgG1	BioLegend	0050	302265	1.000	0.250	0.100	10.00
HLA-ABC		W6/32	IgG2a	BioLegend	0058	311449	1.000	0.250	0.125	8.00
CD4		RPA-T4	IgG1	BioLegend	0072	300567	1.000	0.250	0.500	2.00
CD14		M5E2	IgG2a	BioLegend	0081	301859	1.000	0.250	2.000	0.50
CD66b		6/40c	IgG1	BioLegend	0166	392909	1.000	0.250	2.000	0.50
CD223		11C3C65	IgG1	BioLegend	0152	369335	1.000	0.250	4.000	0.25
CD56		5.1H11	IgG1	BioLegend	0047	362559	1.000	0.250	4.000	0.25
CD103		BerACT8	IgG1	BioLegend	0145	350233	1.250	0.313	0.125	10.00
CD62L		DREG-56	IgG1	BioLegend	0147	304851	1.250	0.313	0.125	10.00
TCRab		IP26	IgG1	BioLegend	0224	306743	1.250	0.313	0.625	2.00
CD194	CCR4	L291H4	IgG1	BioLegend	0071	359425	1.250	0.313	1.250	1.00
CD26	DPP4	BA5b	IgG2a	BioLegend	0396	302722	1.250	0.313	1.250	1.00
CD274	B7-H1 (PD-L1)	29E.2A3	IgG2a	BioLegend	0007	329751	1.250	0.313	1.250	1.00
CD28		CD28.2	IgG1	BioLegend	0386	302963	1.250	0.313	1.250	1.00
CD127	IL7Ralpha	A019D5	IgG1	BioLegend	0390	351356	1.250	0.313	2.500	0.50
CD141	Thrombomodulin	M80	IgG1	BioLegend	0163	344125	1.250	0.313	2.500	0.50
CD25		BC96	IgG1	BioLegend	0085	302649	1.250	0.313	2.500	0.50
CD45RO		UCHL1	IgG2a	BioLegend	0087	304259	1.250	0.313	2.500	0.50
CD80	B7-1	2D10	IgG1	BioLegend	0005	305243	1.250	0.313	2.500	0.50
HLA-DR		L243	IgG2a	BioLegend	0159	307663	1.650	0.413	0.330	5.00
CD117	C-kit	104D2	IgG1	BioLegend	0061	313243	2.500	0.625	0.625	4.00
IsoIgG1		MOPC-21		BioLegend	0090	400187	2.500	0.625	1.000	2.50
IsoIgG2A		MOPC-173		BioLegend	0091	400293	2.500	0.625	1.000	2.50
CD107a	LAMP1	H4A3	IgG1	BioLegend	0155	328649	2.500	0.625	2.500	1.00
CD366		F38-2E2	IgG1	BioLegend	0169	345049	2.500	0.625	2.500	1.00
CD138		DL-101	IgG1	BioLegend	0831	352327	3.000	0.750	3.000	1.00
CD24		ML5	IgG2a	BioLegend	0180	311143	3.300	0.825	3.300	1.00
CD134	OX40	Ber-ACT35	IgG1	BioLegend	0158	350035	5.000	1.250	1.000	5.00
CD1a		HI149	IgG1	BioLegend	0402	300135	5.000	1.250	1.000	5.00
CD279	PD-1	EH12.2H7	IgG1	BioLegend	0088	329963	5.000	1.250	1.000	5.00
CD30		BY88	IgG1	BioLegend	0028	333919	5.000	1.250	1.000	5.00
CD183	CXCR3	G025H7	IgG1	BioLegend	0140	353747	10.000	2.500	0.500	20.00
TCRgd		B1	IgG1	BioLegend	0139	331231	10.000	2.500	0.500	20.00
CD86	B7-2	IT2.2	IgG2b	BioLegend	0006	305447	10.000	2.500	0.667	15.00
CD152	CTLA4	BNI3	IgG2a	BioLegend	0151	369621	10.000	2.500	1.000	10.00
CD197	CCR7	G043H7	IgG2a	BioLegend	0148	353251	10.000	2.500	1.000	10.00

Supplementary Table S2: Five categories of response to four-fold dilution

Category	Response to 4-fold dilution	Markers	Considerations
Category A (Fig. 2A)	Antibodies where the positive signal is full saturated (without response to four-fold dilution), absent, or obscured within high background signal and do not respond to dilution.	<i>CD1a, CD30, CD86, CD134, CD138, CD152, CD183, CD197, CD279, CD336, IgG1, IgG2A and TCRgd</i>	Reducing antibody concentration is always the right choice. These antibodies sequester a large amount of UMIs without any biological information. Reducing concentration may reveal a true positive population obscured by the background signal.
Category B (Fig. 2B)	Antibodies that respond by a reduction in signal but without hampering the ability to distinguish positive and negative fractions.	<i>CD4, CD5, CD8, CD11b, CD19, CD62L, CD69, CD103, CD107a, CD194, CD274, EpCAM, HLA-DR and TCRab</i>	Reducing antibody concentrations will be economically beneficial with minimal loss of biological information. For instance, In the Lung at DF1, HLA-DR uses 9% of the total UMI counts within cell-containing droplets and can be reduced at least four-fold without any apparent change in ability to discriminate between positive and negative cells.
Category C (Fig. 2C)	Antibodies that respond by a reduction in both signal and changes the ability to distinguish positive from negative cells or bring the cutoff value for positive cells down to only a few UMIs.	<i>CD1c, CD2, CD3, CD14, CD25, CD26, CD28, CD31, CD39, CD45RA, CD45RO, CD141,</i>	Reducing antibody concentration will reduce biological information as cells expressing the targeted epitopes may not exhibit sufficient signal. If only cells expressing high levels of the marker need to exhibit signal, these can be slightly reduced.
Category D (Fig. 2D)	Antibodies targeting (almost) ubiquitously expressed markers.	<i>CD44, CD45 and HLA-ABC</i>	These can be reduced if all cells exhibit high UMI counts. Unless these markers have a clear purpose, most experiments will benefit from dropping them from the panel as they tend to sequester a large proportion of total sequencing reads with little biological information.
Category E (Fig. 2E)	Antibodies that do not show a convincing positive population due to either lack of epitopes (no positive cells in either tissue) or lack of antibody binding (non-functional antibody).	<i>CD24, CD56, CD66b, CD70, CD80, CD117, CD123, CD127, CD196 and CD223</i>	Should be evaluated individually. Do you have prior information indicating that this marker is expressed by cells in these types of samples? Do any cells in the sample express high levels of the gene encoding the targeted protein? If so, increasing the concentration of the antibody or trying a different clone may yield better signal.

Supplementary Figure S1

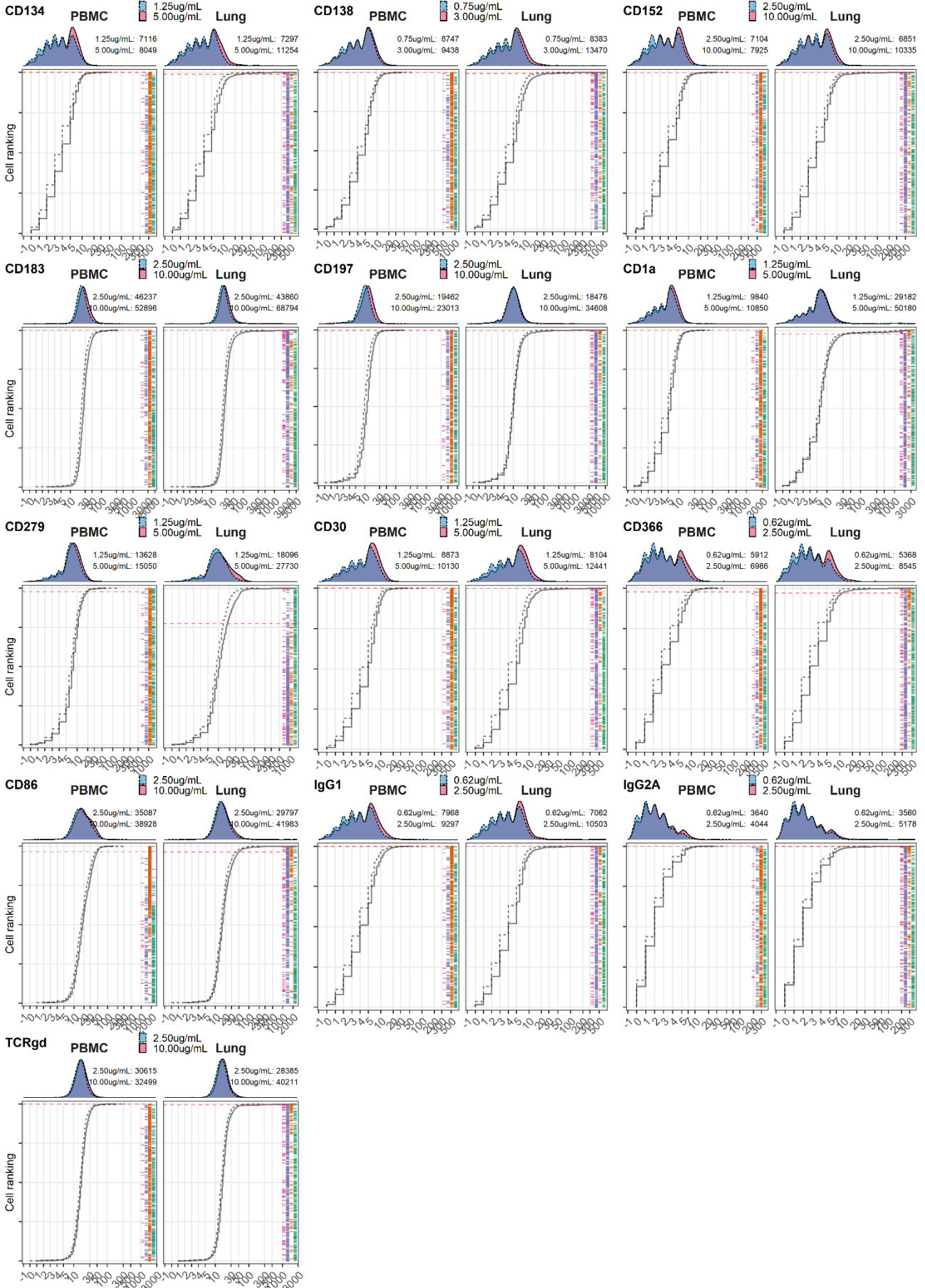


Supplementary Figure S1: Gating positive cells based on ADT signal at dilution factor 1 (DF1).

Histograms for normalized ADT expression of each marker within major cell types in PBMC (red) and Lung (blue). Gating threshold indicated by vertical line and numbers denote percent positive within each cell type of each tissue. MO/MØ/DC: monocyte, macrophage or dendritic cell.

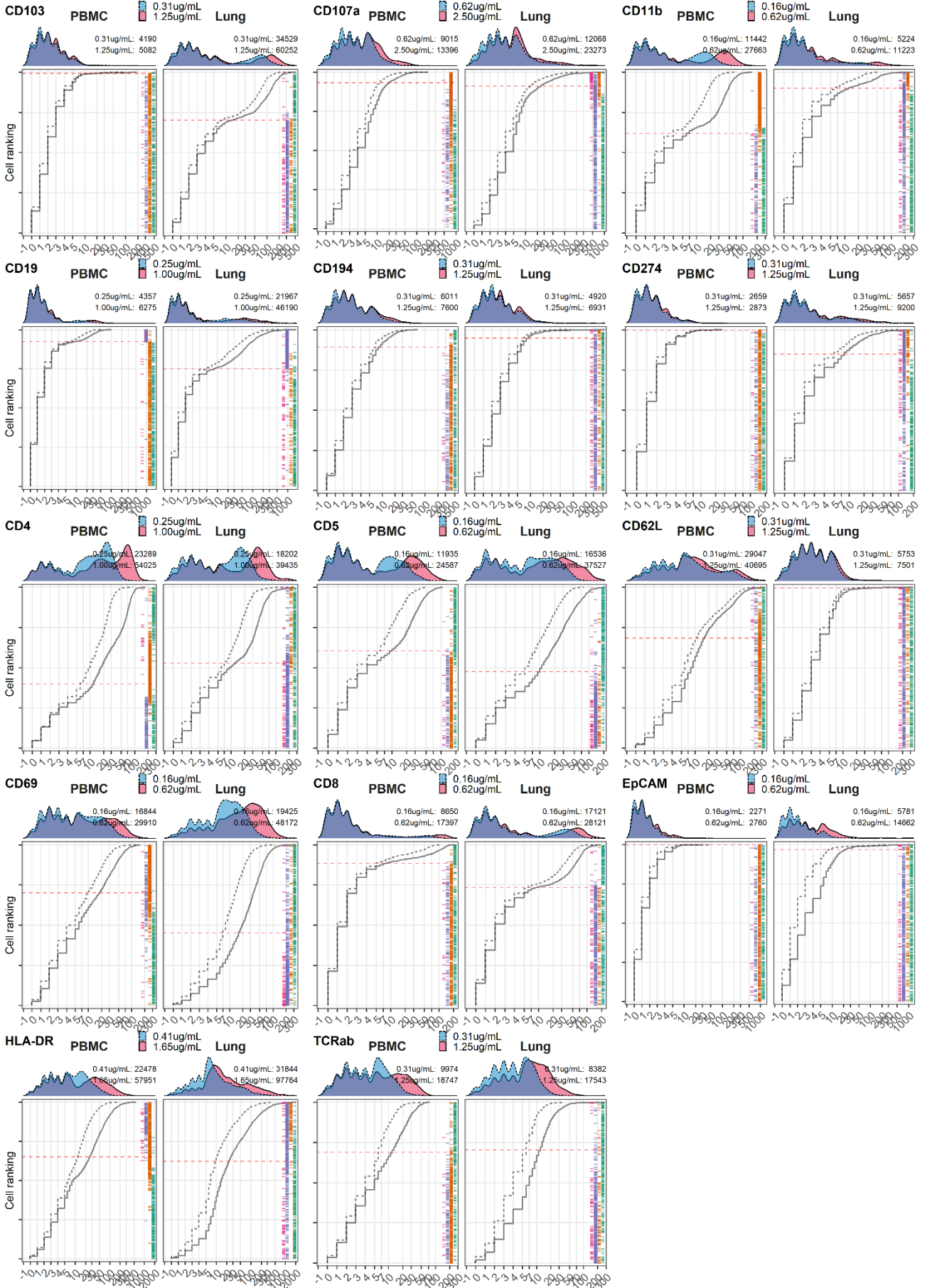
Supplementary Figure S2A: Category A

■ T/NK cells ■ MO/MØ/DC ■ B/Plasma cells ■ Other

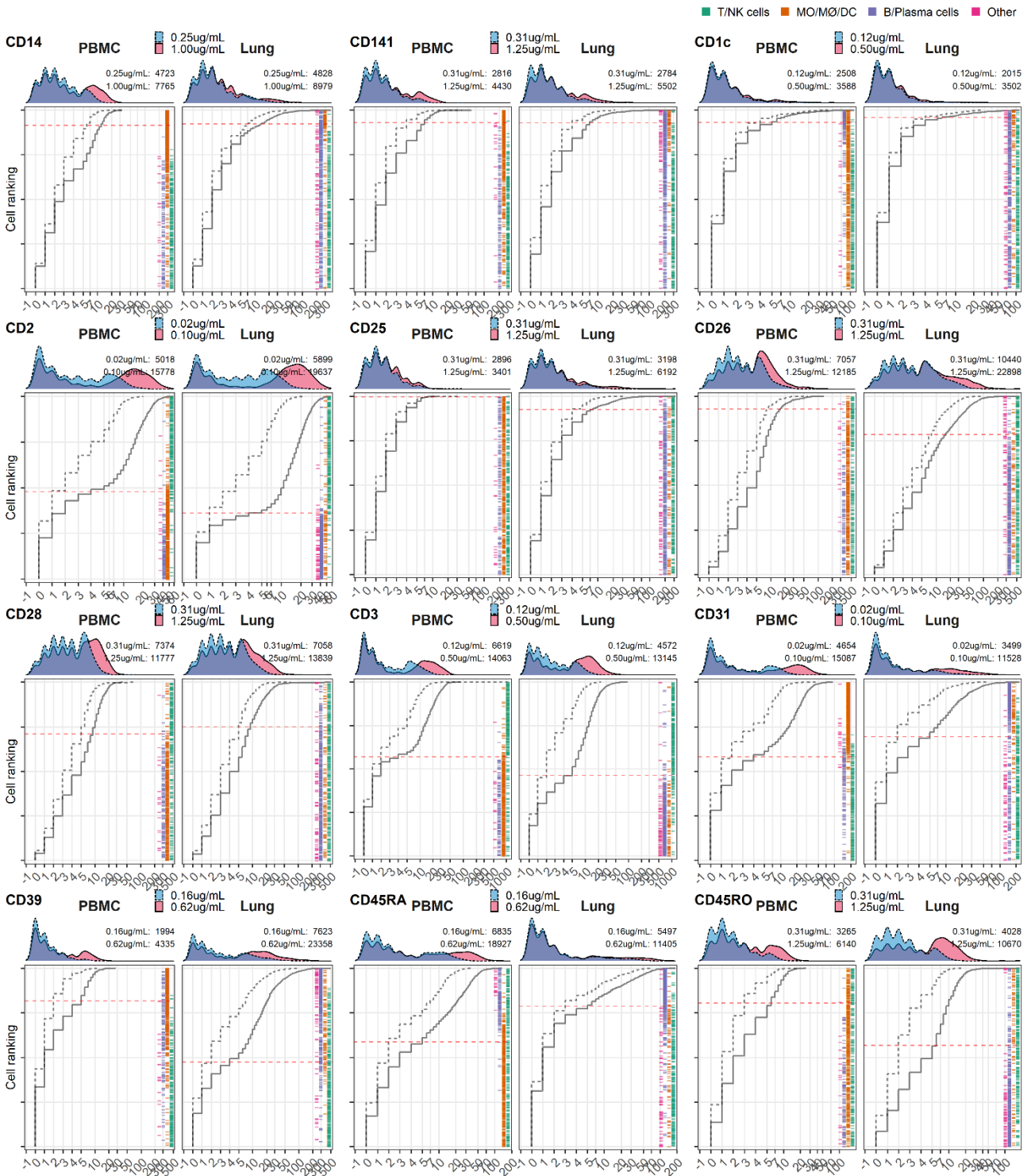


Supplementary Figure S2B: Category B

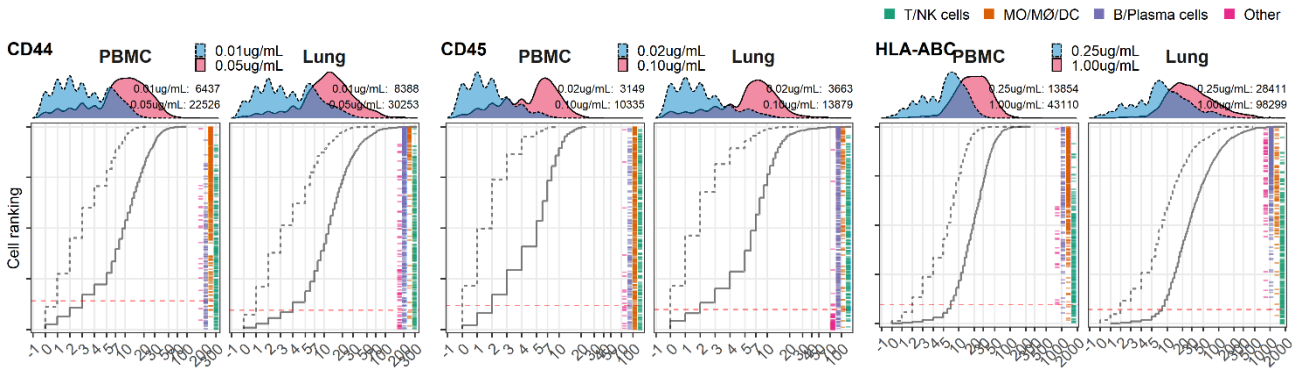
T/NK cells MO/MØ/DC B/Plasma cells Other



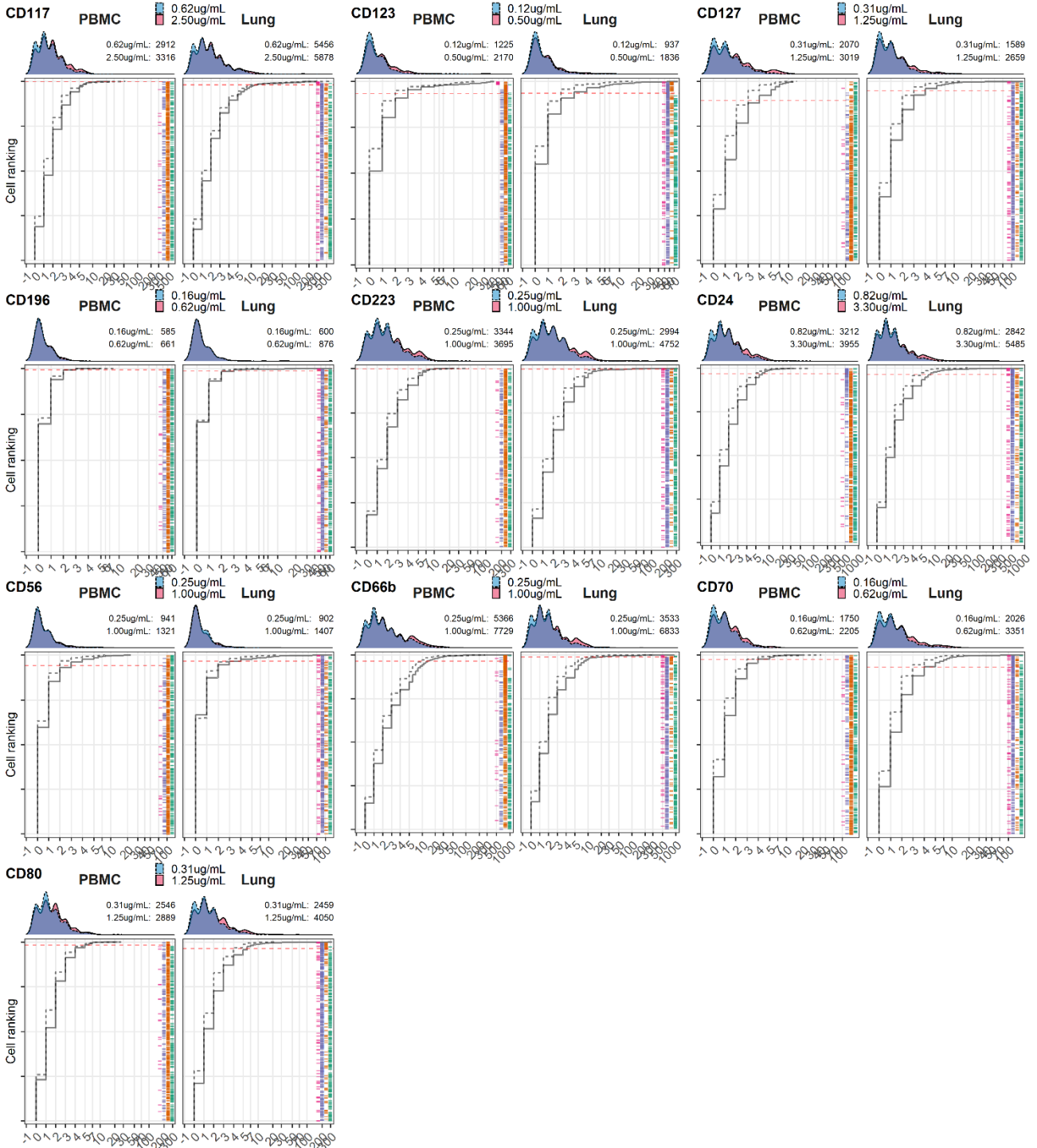
Supplementary Figure S2C: Category C



Supplementary Figure S2D: Category D



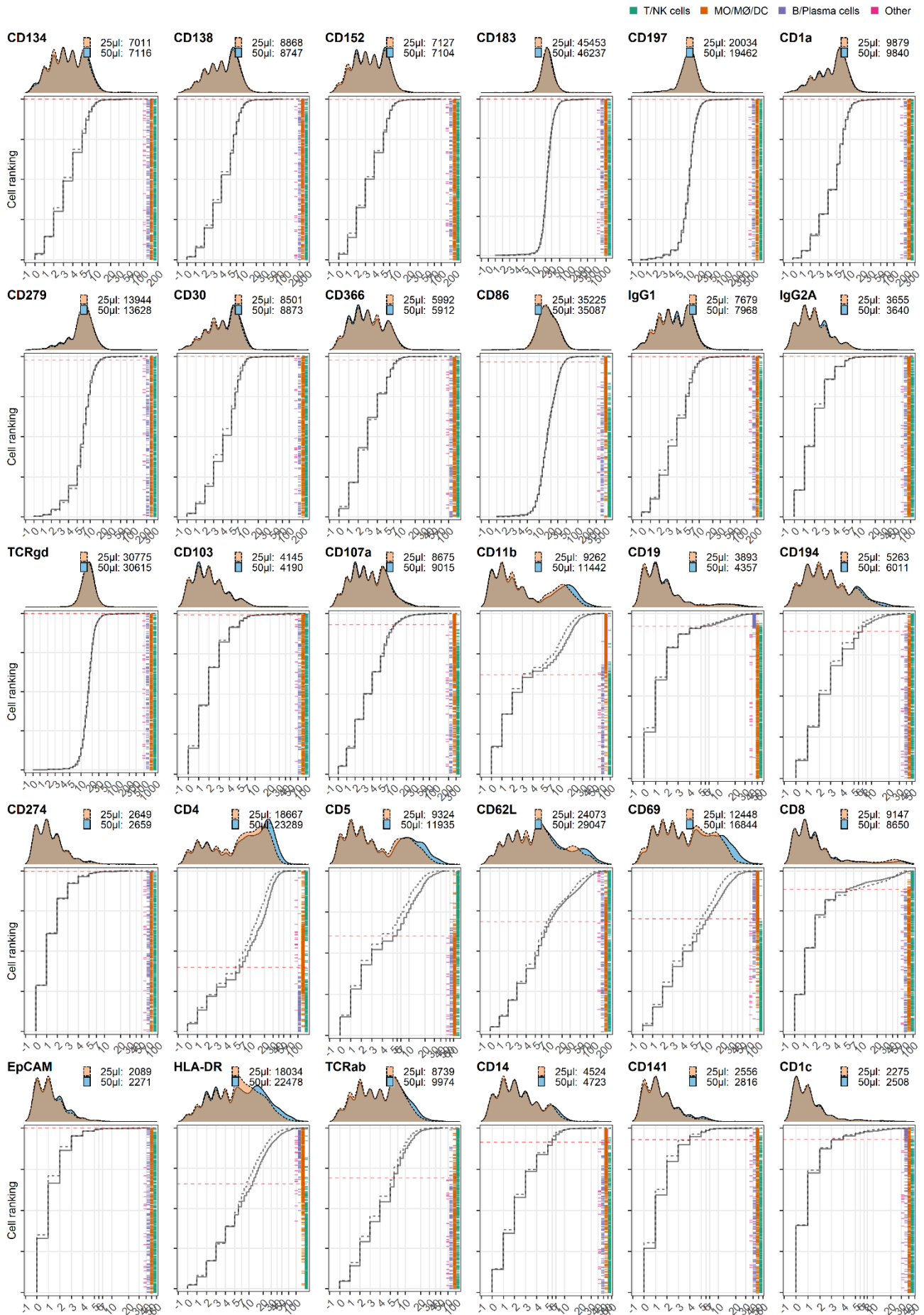
Supplementary Figure S2E: Category E



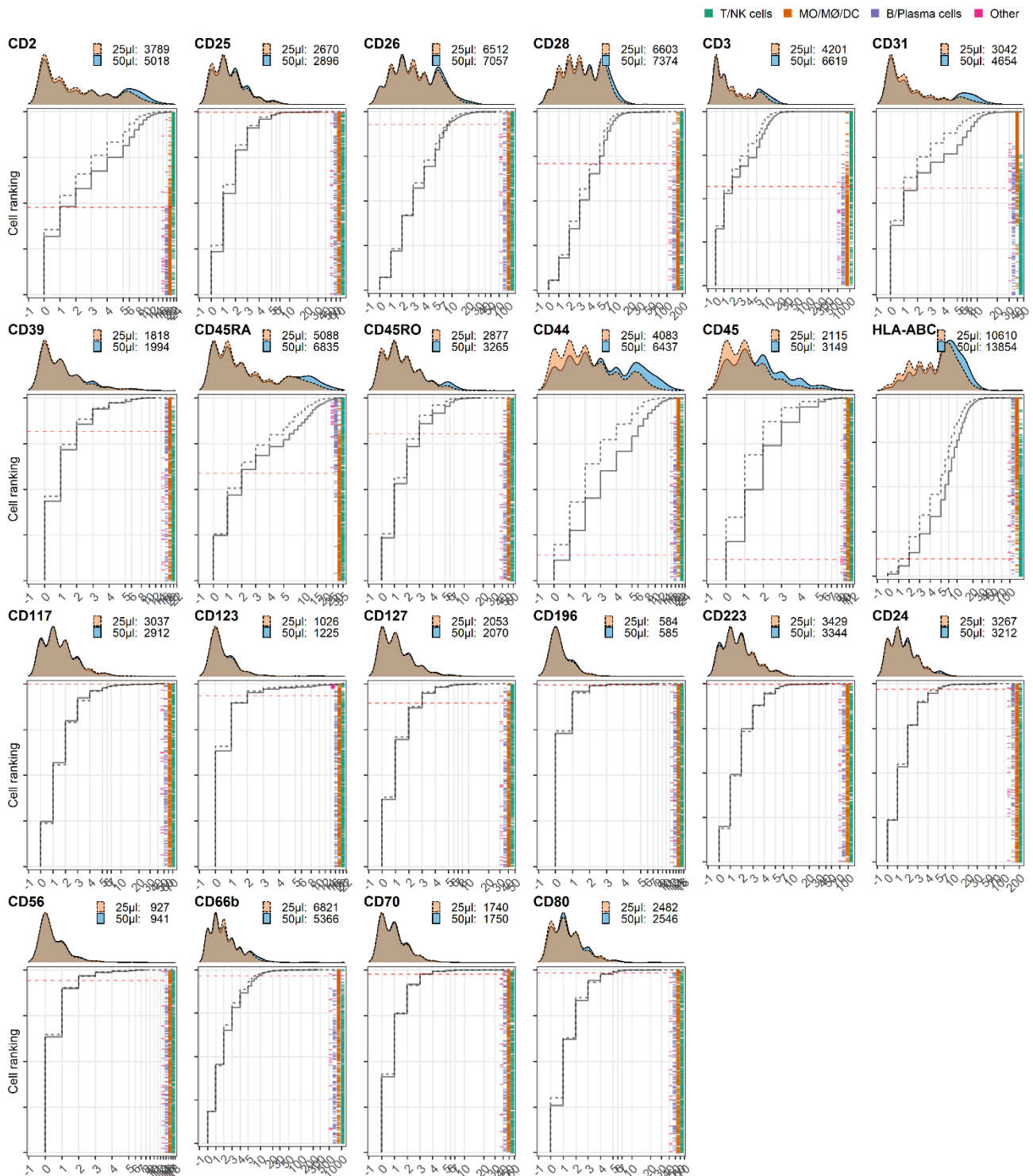
Supplementary Figure S2: Response of individual antibodies to four-fold reduction in concentration in PBMCs and Lung tumor immune cells.

“Titration plots” (Marker UMI count vs. Normalized cell rank) for response to reduction in antibody concentration from dilution factor 1 (DF1; solid line) to DF4 (dashed line). Histogram depicts distribution of UMIs at each condition. Numbers in histograms denote total UMI count within cell-containing droplets at each condition. “Barcode plot” to the right depicts cell type occurrence at the corresponding rank to visualize cell specificity of the antibody. Horizontal red lines depicts cell rank cutoff for “positive” cells (shown in Suppl. Fig S1).

Supplementary Figure S3A



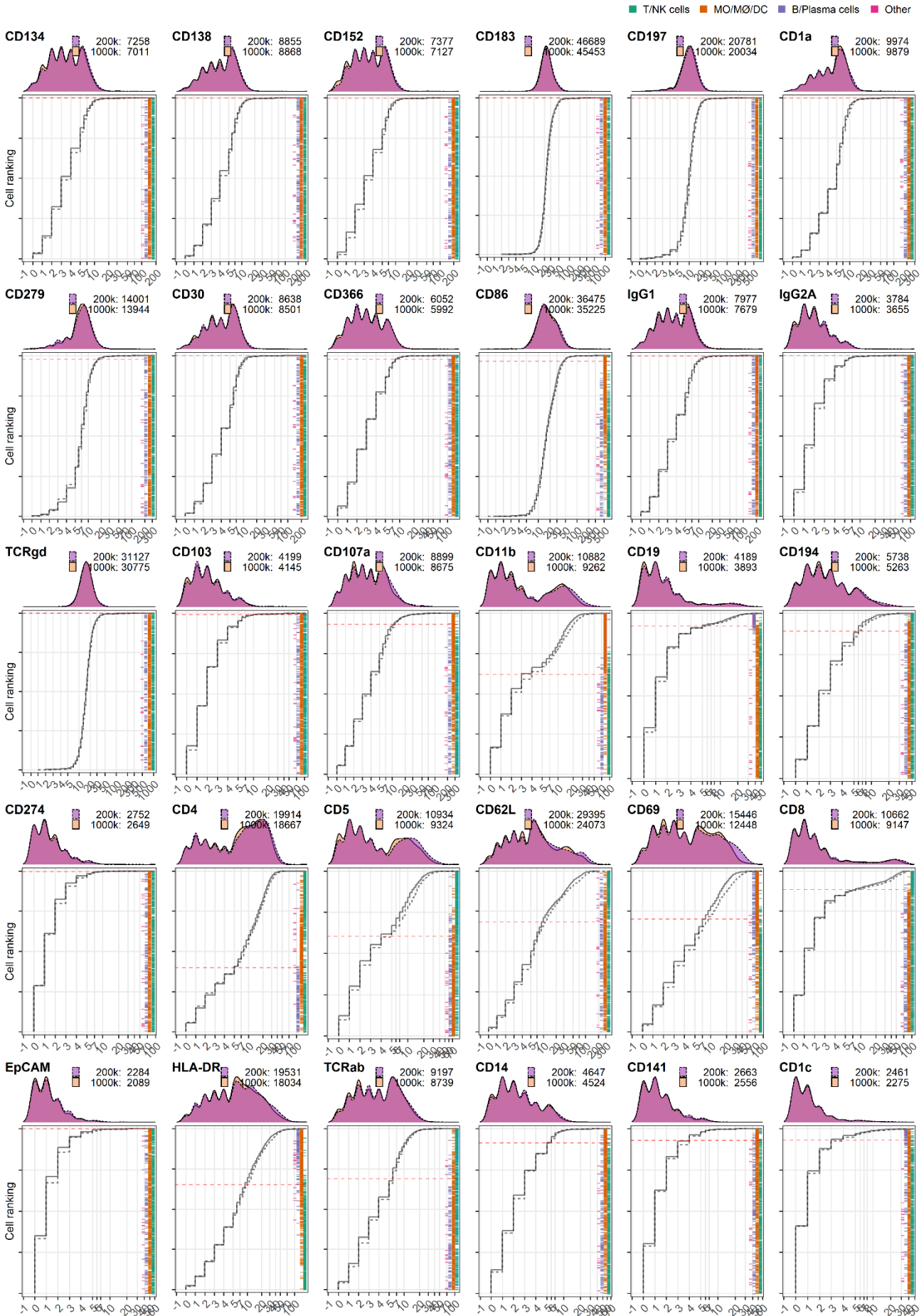
Supplementary Figure S3B



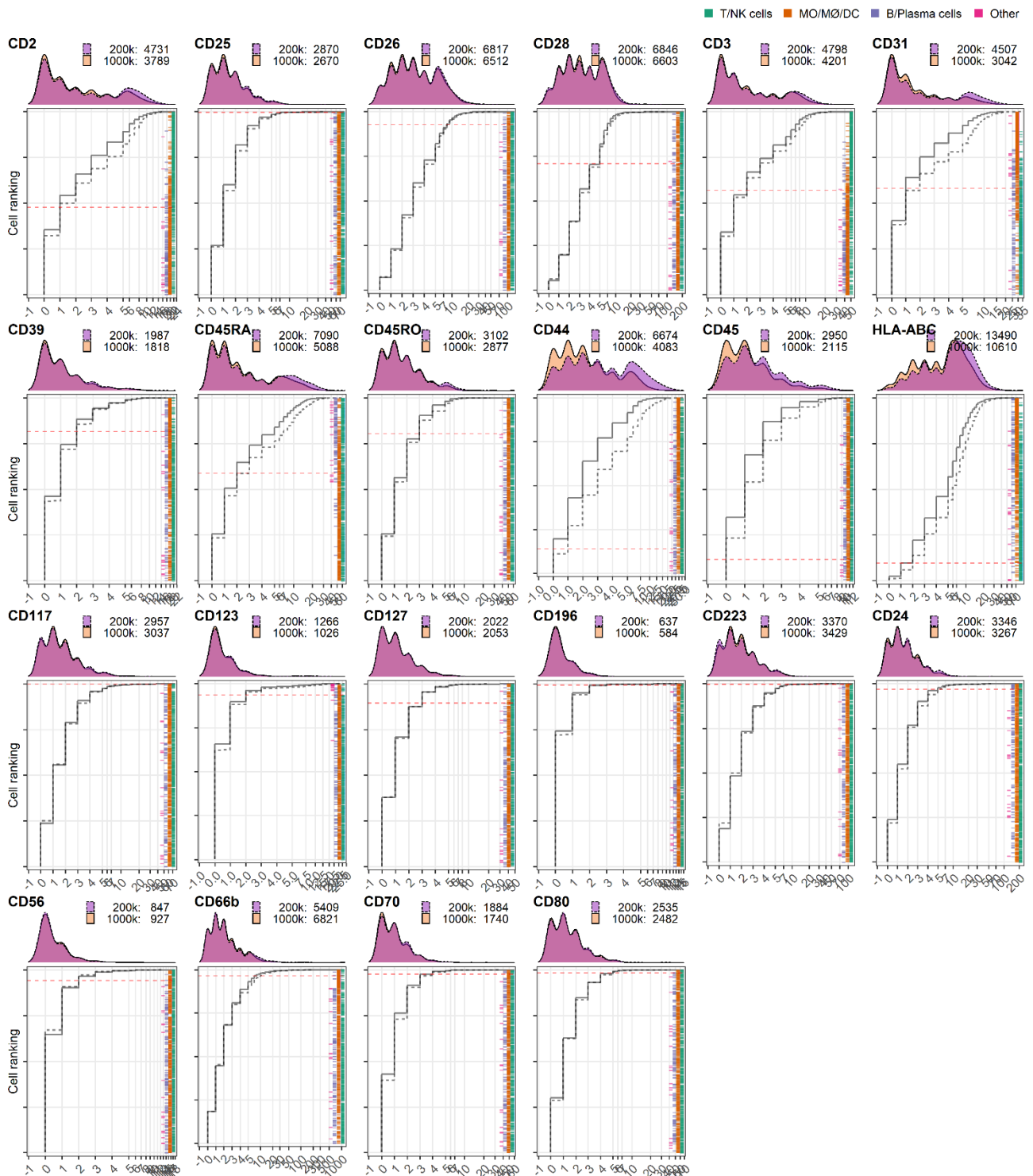
Supplementary Figure S3: Response of individual antibodies to reduction in staining volume.

“Titration plots” (Marker UMI count vs. Normalized cell rank) for CD31 signal response to reduction in staining volume from 50 µl (solid line) to 25 µl (dashed line). Histogram depicts distribution of UMIs at each condition. “Barcode plot” to the right depict cell type occurrence at the corresponding rank to visualize cell specificity of the antibody. Numbers in legend denote total UMI count within cell-containing droplets from each sample.

Supplementary Figure S4A



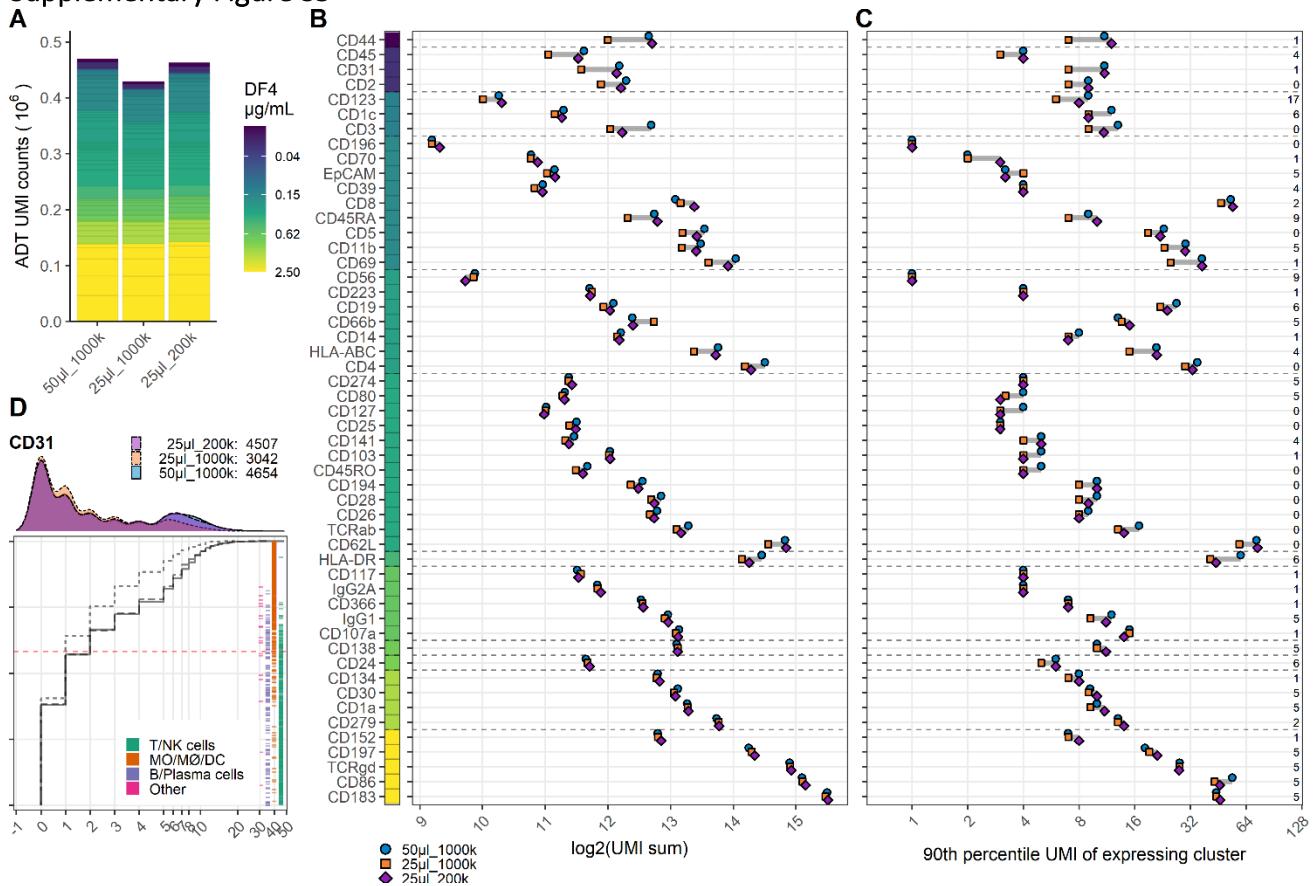
Supplementary Figure S4B



Supplementary Figure S4: Response of individual antibodies to reduction in cell numbers at staining.

“Titration plots” (Marker UMI count vs. Normalized cell rank) for CD31 signal response to reduction in cell numbers at staining from 1×10^6 (solid line) to 0.2×10^6 cells (dashed line). Histogram depicts distribution of UMIs at each condition. “Barcode plot” to the right depict cell type occurrence at the corresponding rank to visualize cell specificity of the antibody. Numbers in legend denote total UMI count within cell-containing droplets from each sample.

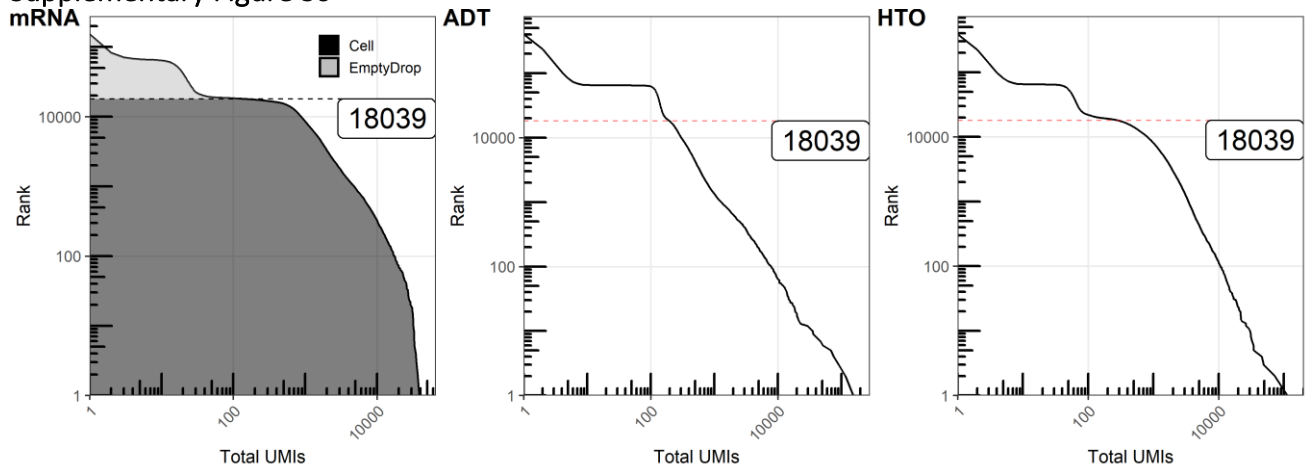
Supplementary Figure S5



Supplementary Figure S5: 5-fold reduction in cell density mitigates but does not supersede 2-fold reduction in staining volume.

Comparison of PBMC samples stained in 50 μl , 25 μl and 25 μl antibody staining solution at dilution factor 4 (DF4) at cell densities: 1×10^6 (50 μl _1000k), 1×10^6 (25 μl _1000k) or 0.2×10^6 (25 μl _200k) cells, respectively. **A**. Summarized UMI counts within cell-containing droplets segmented by the individual antibodies colored by their concentration. **B, C**. ADT signal for each antibody at each condition. Individual antibodies are colored by their concentration. Quantified by **(B)** sum of UMIs within cell-containing droplets assigned to each antibody and **(C)** 90th percentile UMI count within cell cluster with most abundant expression (the assayed cluster is annotated by numbers inside the). **D**. "Titration plot" (Marker UMI count vs. Normalized cell rank) for CD31 signal at the three conditions. Histogram depicts distribution of UMIs at each condition. "Barcode" to the right depict cell type occurrence at the corresponding rank to visualize cell specificity of the antibody. Numbers in legend denote total UMI count assigned to CD31 within cell-containing droplets from each sample.

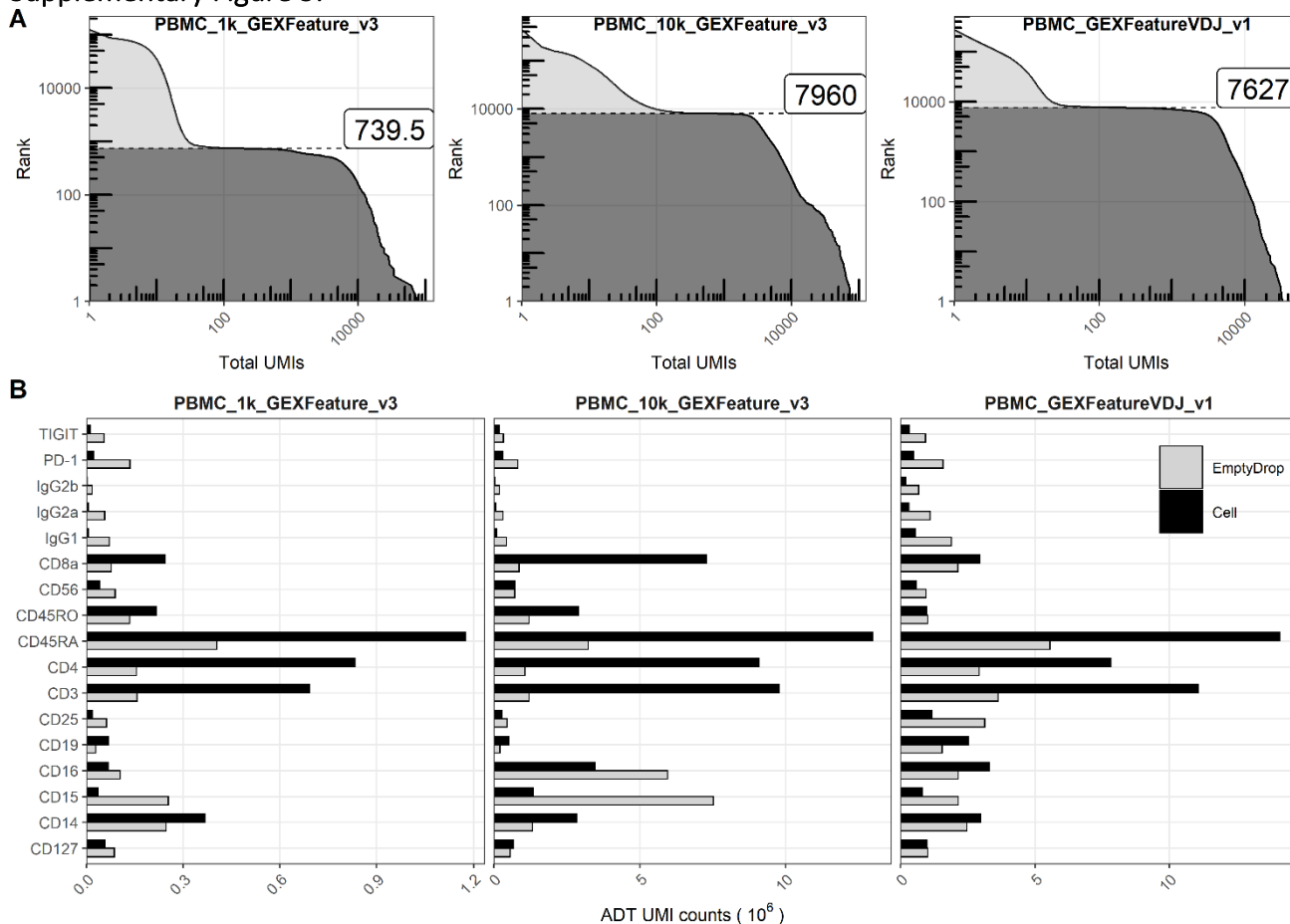
Supplementary Figure S6



Supplementary Figure S6: Quantifying UMIs within cells and empty droplets of ADT and HTO.

“Knee plots” (Barcode rank vs. Total UMIs within barcode) for mRNA, antibody-derived tag (ADT) and hashtag-oligo (HTO) libraries. Cell-containing droplets were filtered based on the total UMI count at the inflection point in the mRNA plot. Red lines depict the position of rank 18039, largely corresponding to the cutoff for cell-containing droplets.

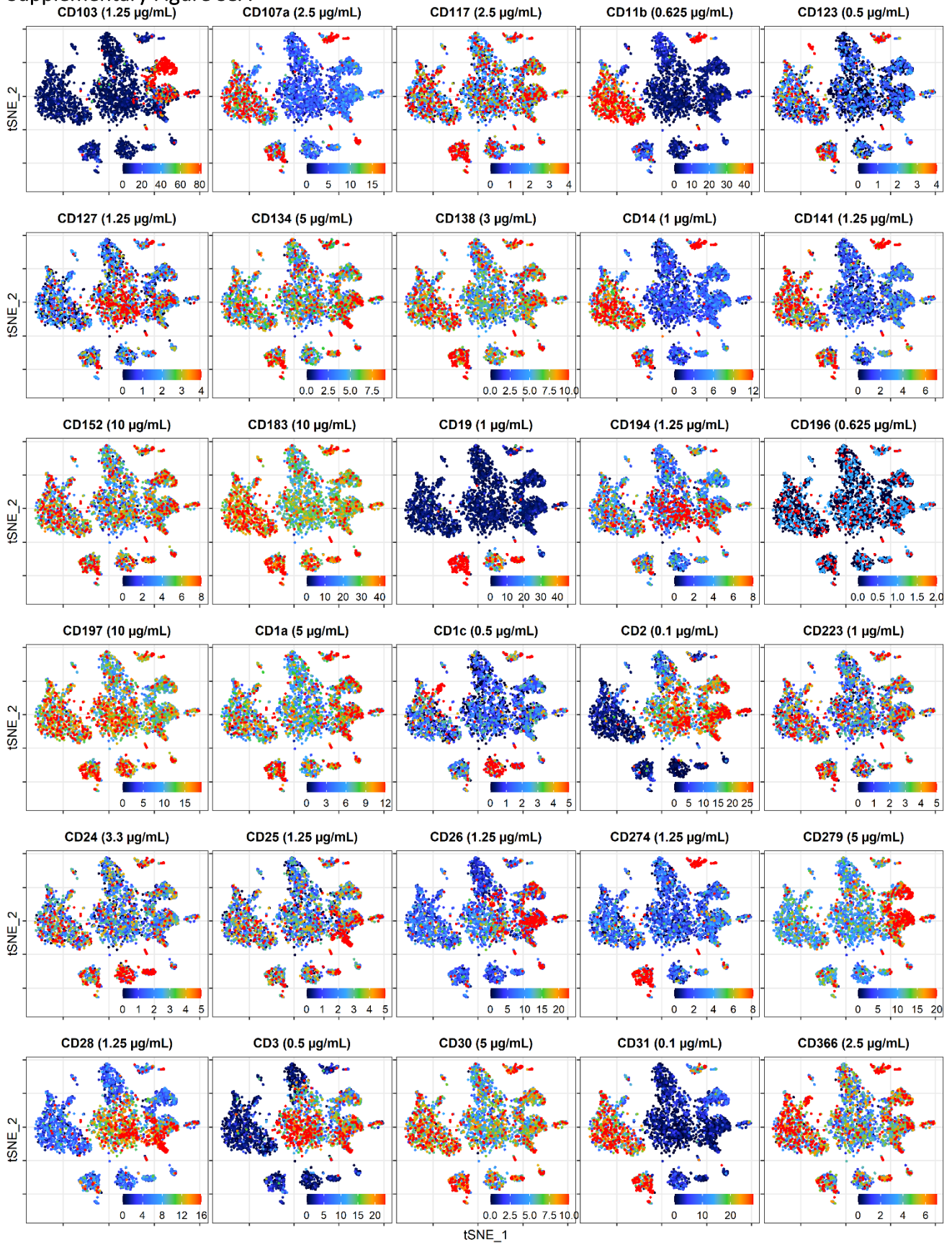
Supplementary Figure S7



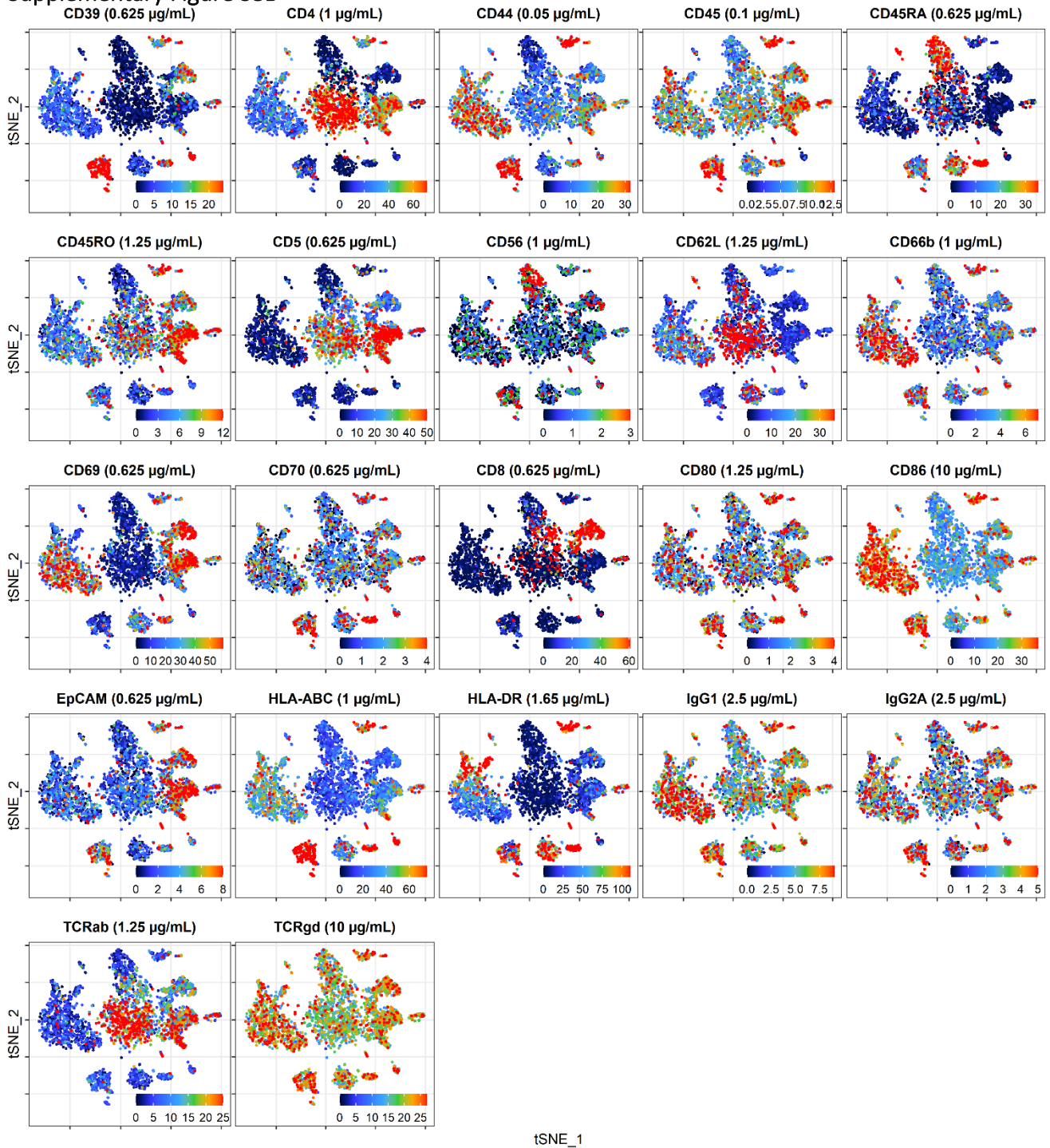
Supplementary Figure S7: Quantifying UMIs within cells-containing and empty droplets from public 10X datasets.

A. “Knee plots” (Barcode rank vs. Total UMIs within barcode) for mRNA libraries within three publicly available single-cell RNA sequencing runs (from the 10X Genomics website) showing filtering of cell-containing and empty droplets based on the total UMI count at the inflection point. **B.** Total UMI counts for the individual antibodies from each of the antibody-derived tag (ADT) libraries within cell-containing and empty droplets.

Supplementary Figure S8A



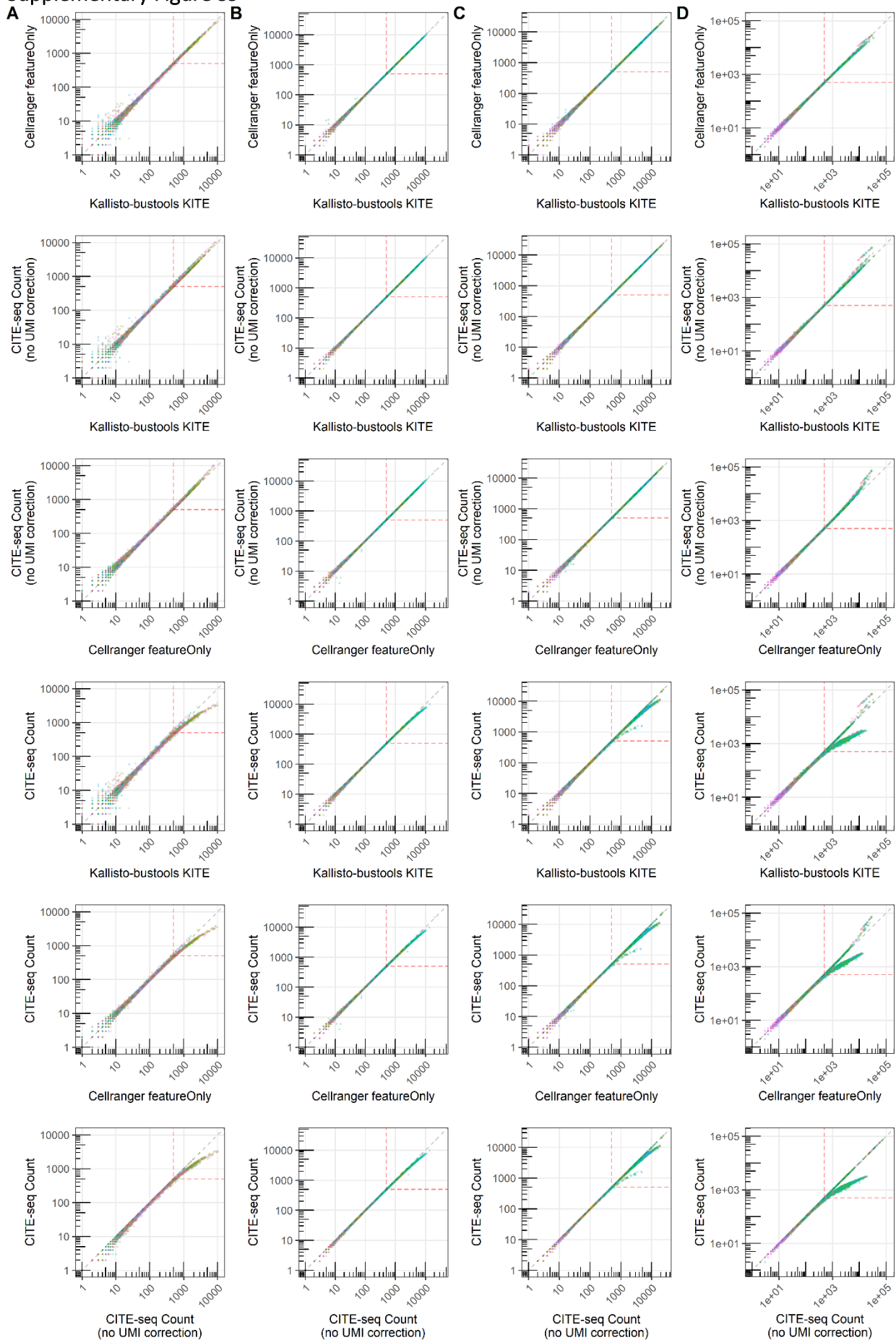
Supplementary Figure S8B



Supplementary Figure S8: Cellular distribution of raw UMI counts

tSNE plots displaying raw (unnormalized) UMI counts from the cells stained at dilution factor 1 (DF1) for each antibody. To make the color scale less sensitive to outliers, we set upper threshold at the 90% percentile.

Supplementary Figure S9



Supplementary Figure S9: UMI counts per marker per cell across counting methods.

Pair-wise concordance of UMI count per antibody per cell-containing droplet across (A) ADT library as well as publicly available 10X Genomics datasets: (B) PBMC_1k_GEXFeature_v3, (C) PBMC_10k_GEXFeature_v3 and (D) PBMC_GEXFeatureVDJ_v1. Dots are colored by antibody.