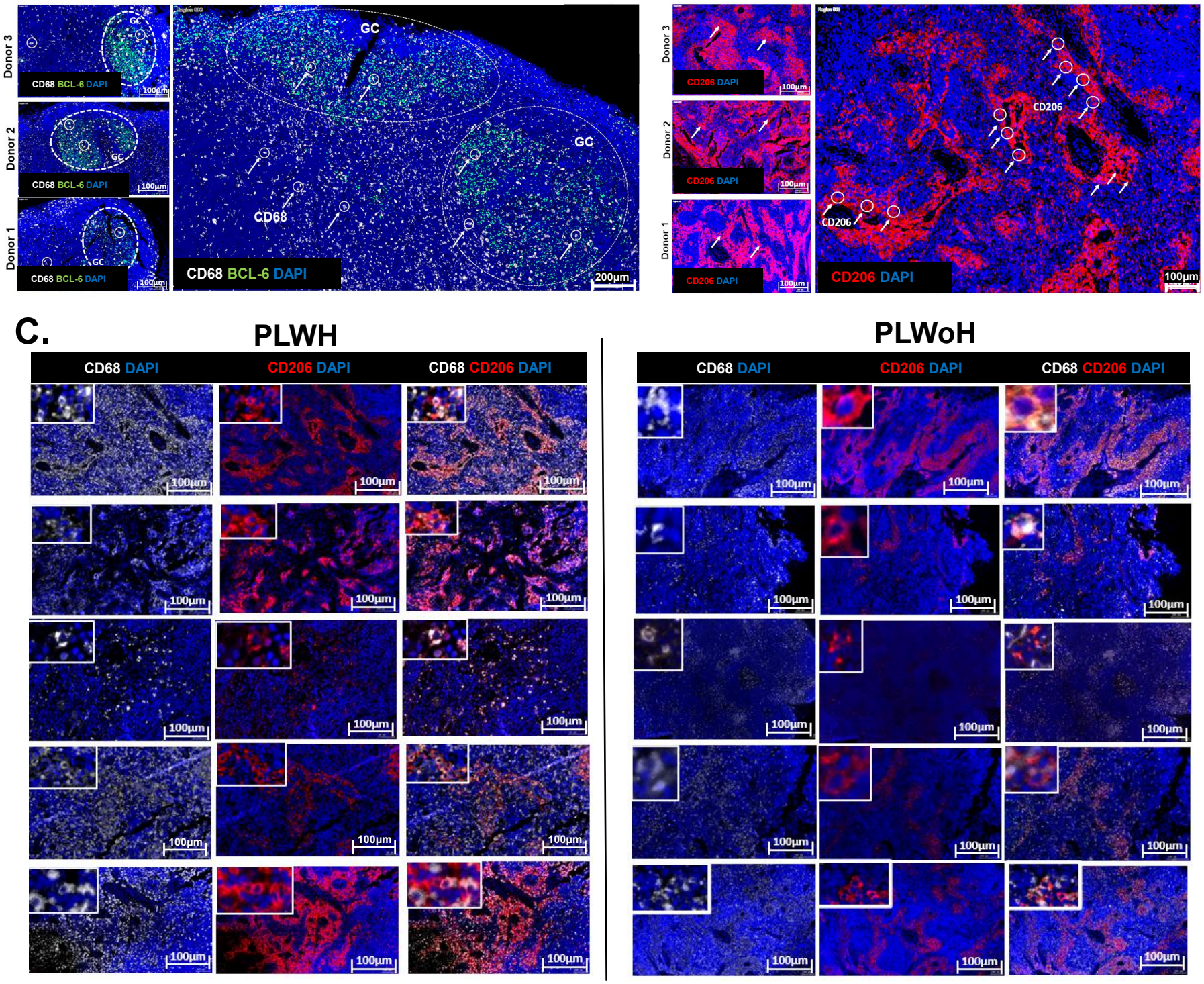
Supplementary Table 1. Participant demographics and clinical data. MID: participant ID uniquely assigned for this report to deidentify participants. PLWH: P; PLWoH: N. PLWH were classified by ART initiation status as UTx (Untreated): Not on ART at time of enrolment; acute: treated during Fiebig stages I-V; chronic: treated after Fiebig stage V and UN (unknown treatment status). n/a – not-applicable/ data not available.

MID	HIV status	Treatment status	Age	Gender	No. of days on treatment before LN excision	Plasma Viral load (cps/ml) nearest to LN excision	CD4 count (cells/ul) closest to LN excision	Figure 1 and Supplentary Fig. S1&S3	Figure 2 and Supplementary Fig. S4-S6	Figure 3 and Supplementary Fig.S7	Figure 4 and Supplementary Fig. 8	Figure 5 and Supplementary Fig. 8
M01	N	n/a	20	F	n/a	n/a	1143	X				
M02	N	n/a	24	F	n/a	n/a	928	X				
M03	N	n/a	25	F	n/a	n/a	667	X				
M04	N	n/a	22	F	n/a	n/a	874	X		X		
M05	N	n/a	21	F	n/a	n/a	1039	X				
M06	N	n/a	22	F	n/a	n/a	659	X				
M07	N	n/a	24	F	n/a	n/a	704	X				
M08	N	n/a	20	F	n/a	n/a	964	X				
M09	N	n/a	22	F	n/a	n/a	670			X		
M10	N	n/a	19	F	n/a	n/a	984			X		
M11	N	n/a	20	F	n/a	n/a	794				X	
M12	N	n/a	19	F	n/a	n/a	765				X	
M13	N	n/a	21	F	n/a	n/a	1304		X		X	
M14	N	n/a	23	F	n/a	n/a	1241				X	
M15	N	n/a	24	F	n/a	<20	1221		X			
M16	N	n/a	23	F	n/a	<20	760		X			
M17	N	n/a	22	F	n/a	<20	652					X
M18	N	n/a	21	F	n/a	<20	627					X
M19	N	n/a	19	F	n/a	<20	673					X
M20	P	acute	25	F	385	<20	898	X				
M21	P	acute	22	F	120	<20	942	X	X			
M22	P	acute	27	F	1647	<20	1168		X			
M23	P	acute	24	F	926	<20	1033				X	
M24	P	acute	25	F	760	<20	444				X	
M25	P	acute	28	F	3359	21857	1017					X
M26	P	chronic	23	F	85	<20	406	X	X			
M27	P	chronic	19	F	151	2300	708	X				
M28	P	chronic	23	F	17	370	624	X	X		X	

Note: table is continued on the next page

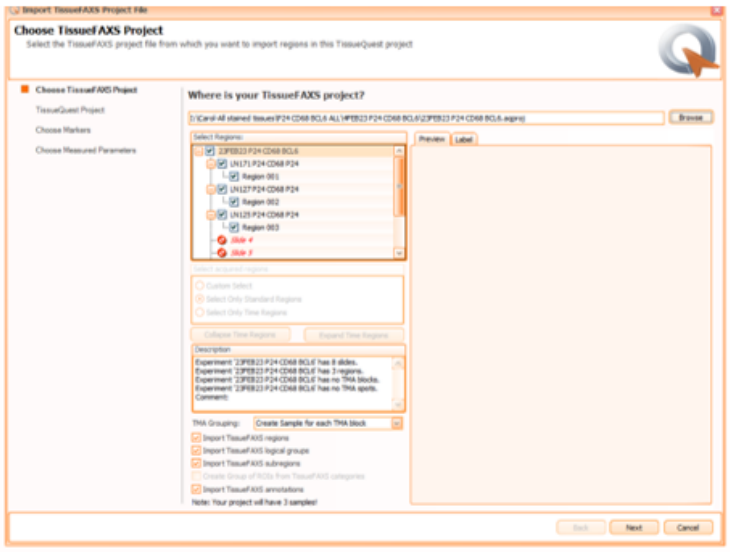
MID	HIV status	Treatment status	Age	Gender	No. of days on treatment before LN excision	Plasma Viral load (cps/ml) nearest to LN excision	CD4 count (cells/ul) closest to LN excision	Figure 1 and Supplentary Fig. S1&S3	Figure 2 and Supplementary Fig. S4-S6	Figure 3 and Supplementary Fig.S7	Figure 4 and Supplementary Fig. 8	Figure 5 and Supplementary Fig. 8
M29	P	chronic	21	F	35	59	1369	X	X			
M30	P	chronic	20	M	174	<20	599	X			X	
M31	P	chronic	23	F	10	140	733	X				
M32	P	chronic	23	F	104	11000	639		X			
M33	P	chronic	22	M	115	<20	834	X	X			
M34	P	chronic	22	F	9	180	472	X				
M35	P	chronic	36	F	3810	5478	409		X	X		
M36	P	chronic	28	F	2454	<20	656			X		
M37	P	chronic	21	F	747	<20	323				X	
M38[a]	P	chronic	30	F	1907	6000	555	X	X		X	
M38[b]	P	chronic	33	F	3130	2359	897		X			
M39	P	chronic	23	F	39	3000	554	X	X		X	
M40	P	chronic	26	M	1014	<20	406	X				
M41	P	chronic	28	F	143	<20	856	X				
M42	P	chronic	28	F	1002	<20	847	X				
M43	P	chronic	21	F	186	<20	225	X			X	
M44[a]	P	chronic	32	F	3001	<20	751					X
M44[b]	P	chronic	33	F	3256	1363	423			X		
M45	P	chronic	33	F	3297	<20	618			X		
M46	P	chronic	20	F	37	290	521				X	
M47	P	chronic	33	F	2807	<30	846					X
M48[a]	P	chronic	26	F	2170	580	785					X
M48[b]	P	chronic	28	F	2775	<20	857		X	X	X	
M49	P	chronic	33	F	3479	<30	969		X			X
M50	P	chronic	19	F	79	<20	467		X			
M51	P	chronic	40	F	2496	<20	751			X		
M52	P	chronic	28	F	n/a	<20	851			X		
M53	P	UTx	45	F	n/a	2488	45			X		
M54	P	UTx	29	M	n/a	400000	251		X			
M55	P	UN	22	F	n/a	<20	883	X				
M56	P	UN	38	F	n/a	<20	741	X				
M57	P	UN	61	F	n/a	<20	375	X				
M58	P	UN	44	F	n/a	n/a	n/a	X				

[a,b] Repeat biopsies for the same donor at different timepoints and/or clinical states (only three study donors) are indicated by “a” or “b”



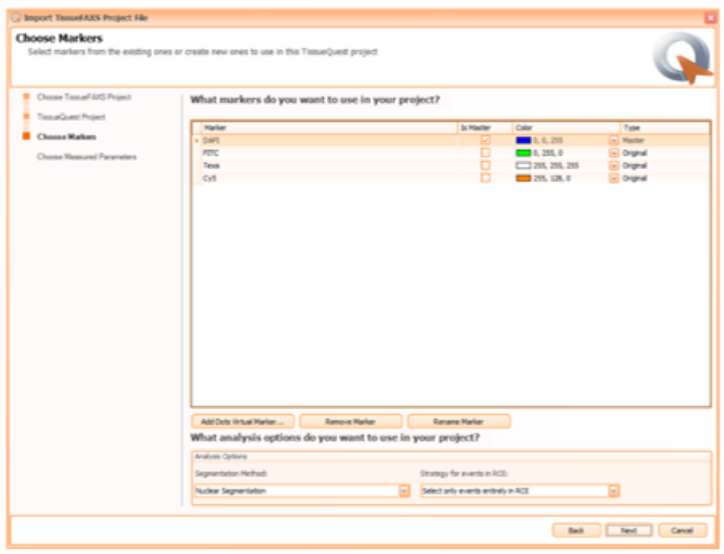
Supplementary Figure 1. Macrophage phenotyping and localization. (A) The localization of $CD68^+$ (white) macrophages relative to the LN GC indicated by BCL-6 (green) positivity from n=3 LN tissue donors. The white dashed oval defines the GC. **(B)** Representative multicolor IF images from n=3 LN tissue donors of $CD206^+$ (red) macrophages along LN lymphatic and blood vessels (white arrows). **(C)** Representative multicolor IF images from n=5 PLWH and n=5 PLWoH of $CD68^+$ (white) and $CD206^+$ (red) macrophages. Images acquired at 40x and all cell nuclei were stained with DAPI (blue). Scale bars= 100µm and 200µm.

A.



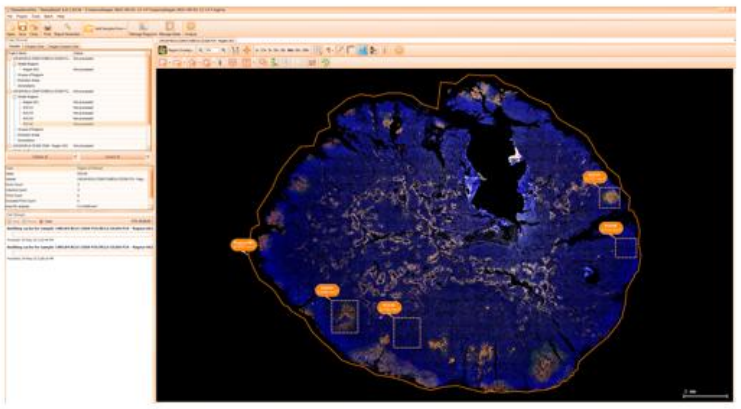
Files of stained tissue sections are uploaded to TissueQuest software

B.



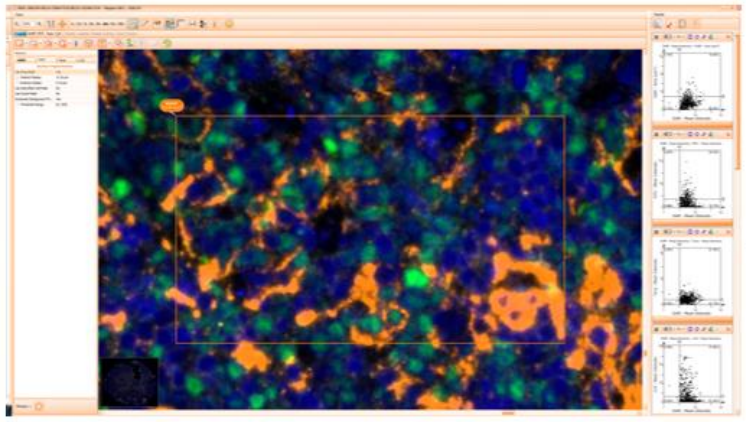
The nuclear segmentation method is selected and DAPI is used as the master channel. The parameters for the other channels including FITC, Texas Red and Cy5 are set.

C.



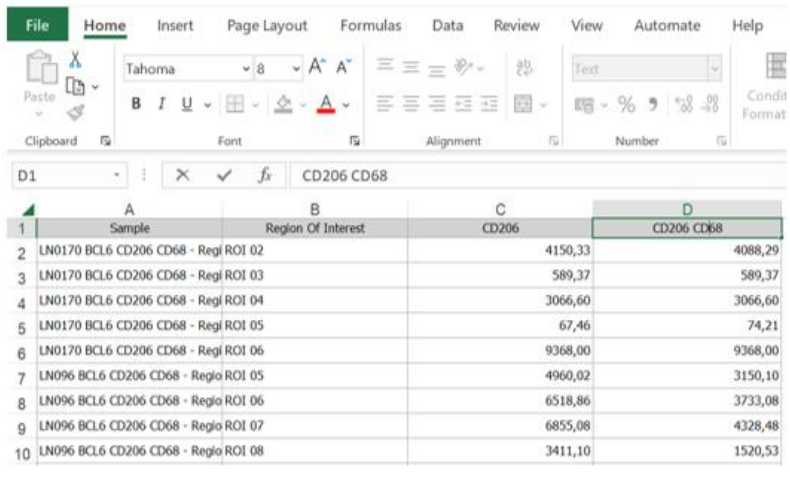
Different regions of interest (ROIs) are selected using a free-hand drawing tool

D.



Selected ROIs are analyzed and single cell data is generated for all channels used

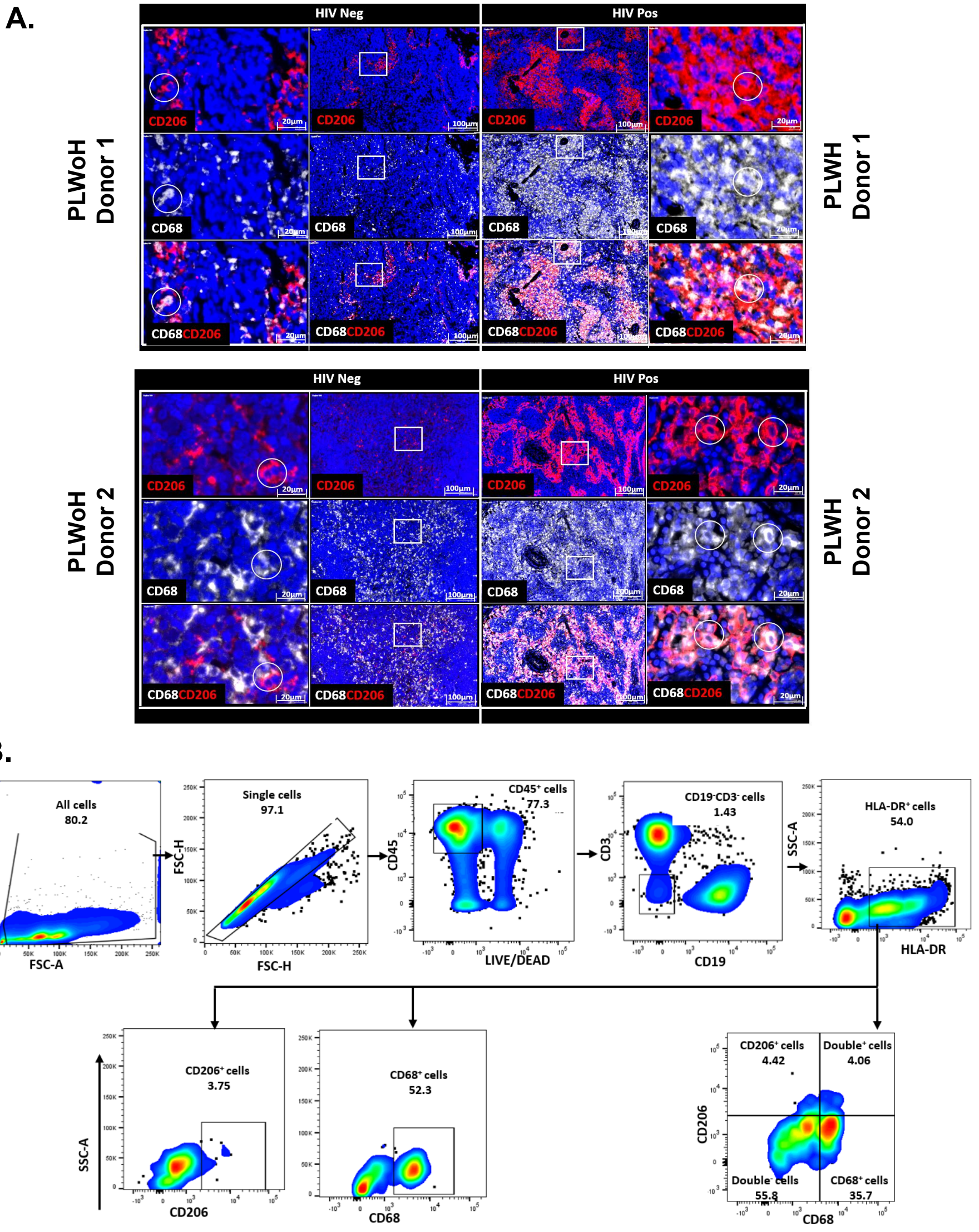
E.



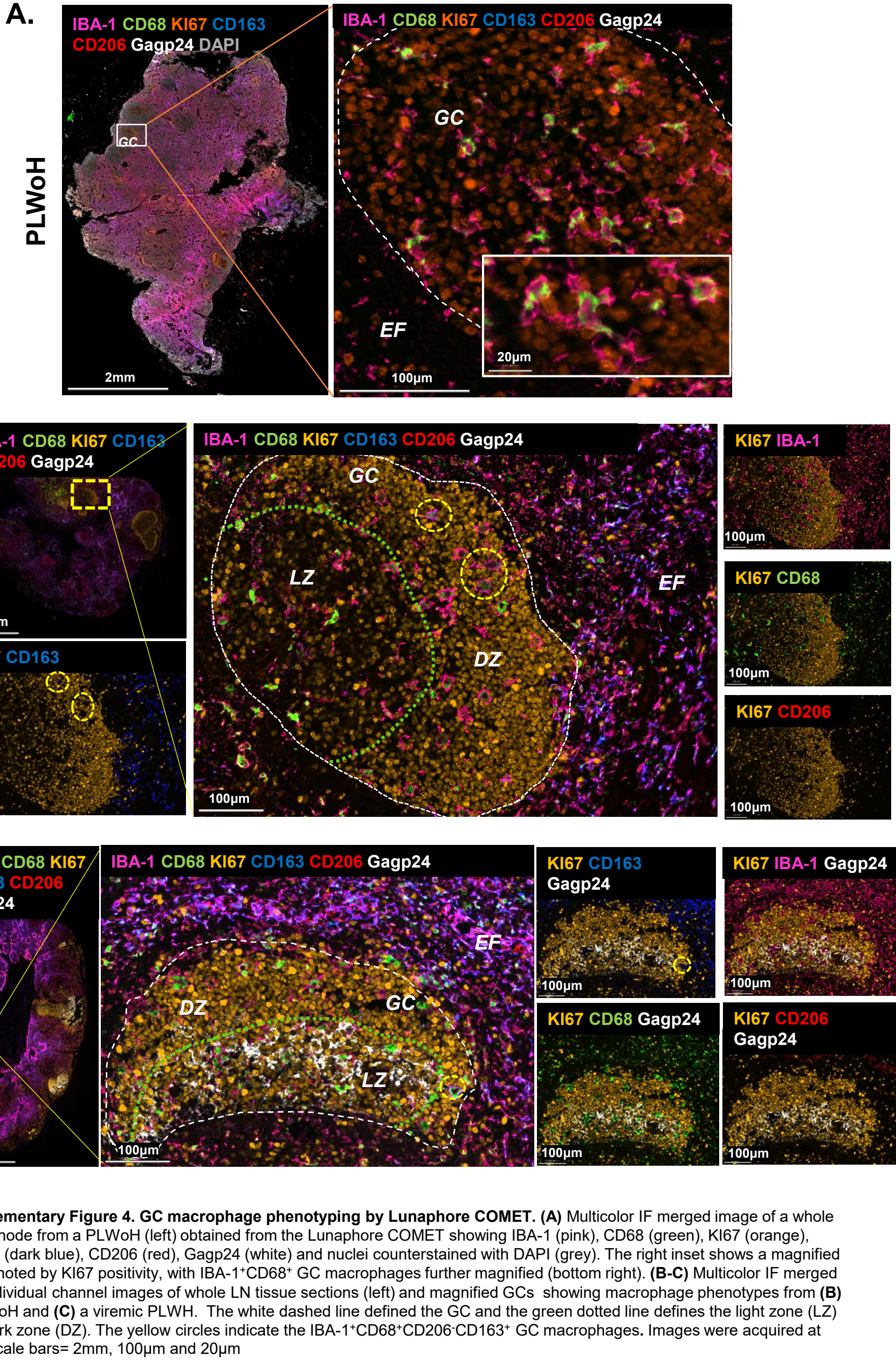
	A	B	C	D
1	Sample	Region Of Interest	CD206	CD206 CD68
2	LN0170 BCL6 CD206 CD68 - Regi	ROI 02	4150,33	4088,29
3	LN0170 BCL6 CD206 CD68 - Regi	ROI 03	589,37	589,37
4	LN0170 BCL6 CD206 CD68 - Regi	ROI 04	3066,60	3066,60
5	LN0170 BCL6 CD206 CD68 - Regi	ROI 05	67,46	74,21
6	LN0170 BCL6 CD206 CD68 - Regi	ROI 06	9368,00	9368,00
7	LN096 BCL6 CD206 CD68 - Regio	ROI 05	4960,02	3150,10
8	LN096 BCL6 CD206 CD68 - Regio	ROI 06	6518,86	3733,08
9	LN096 BCL6 CD206 CD68 - Regio	ROI 07	6855,08	4328,48
10	LN096 BCL6 CD206 CD68 - Regio	ROI 08	3411,10	1520,53

Generated results are exported as excel files and analyzed using GraphPad prism

Supplementary Figure 2. TissueQuest image analysis summary. Summary of quantitative image analysis by TissueQuest.

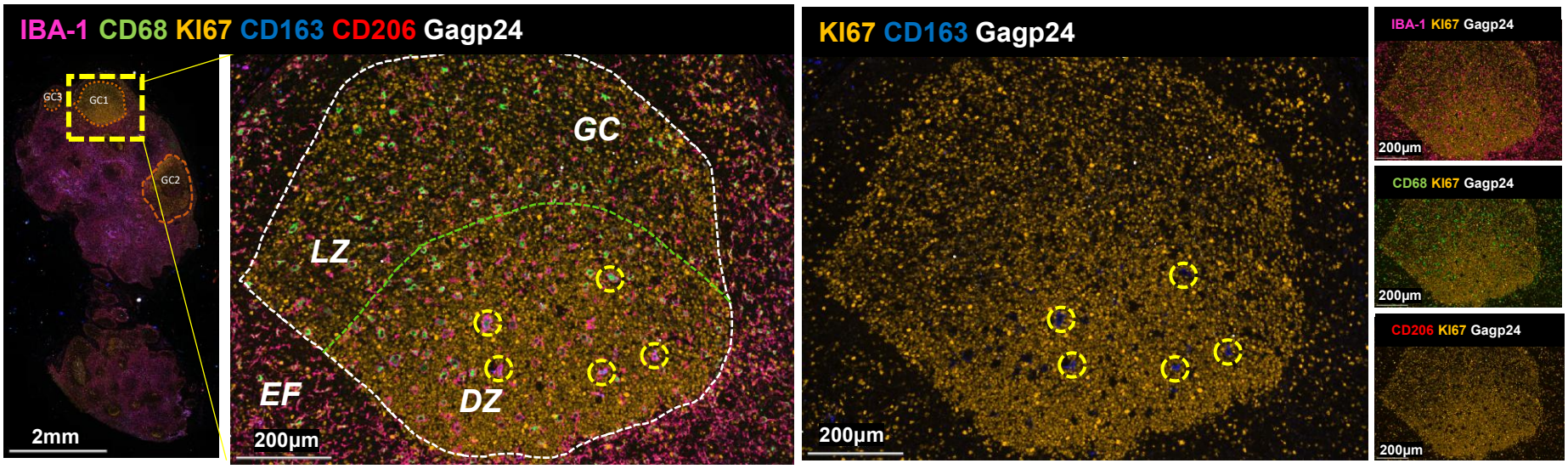


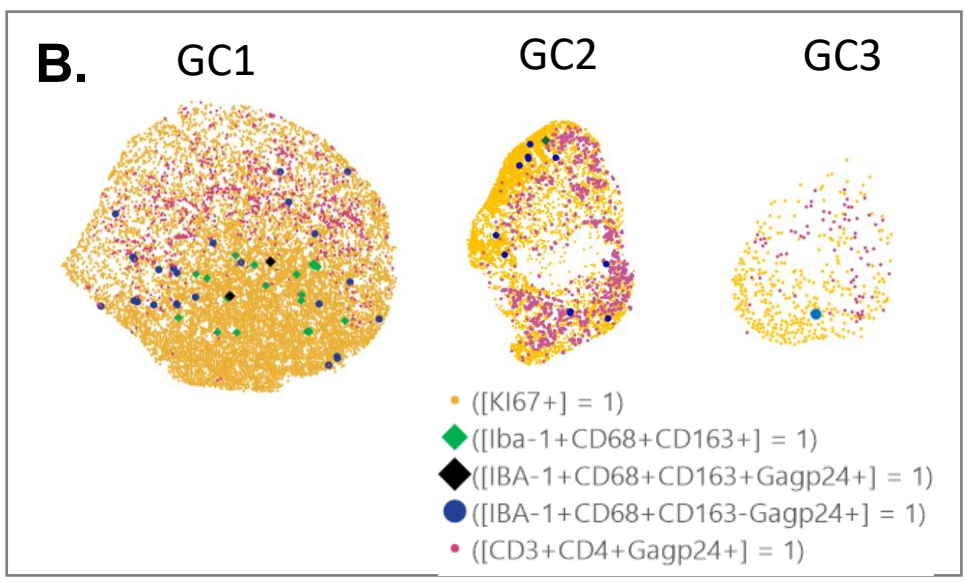
Supplementary Figure 3. HIV modulates macrophage frequency. (A) Multicolor IF images of the individual channels of CD206$^+$ macrophages (red), CD68$^+$ macrophages (white), and the composite image of CD68$^+$CD206$^+$ macrophages and nuclei counterstained with DAPI (blue) in LN tissue sections from n=2 representative PLWoH and n=2 representative PLWH. The white rectangles from the inner panels indicate regions of interest which are further magnified on the outer panels. The white circles indicate double positive CD68$^+$CD206$^+$ macrophages. Images were acquired at 40x. Scale bars= 100µm and 20µm. **(B)** Flow cytometry gating strategy of CD206$^+$, CD68$^+$ and CD68$^+$CD206$^+$ macrophages from LNMCs.

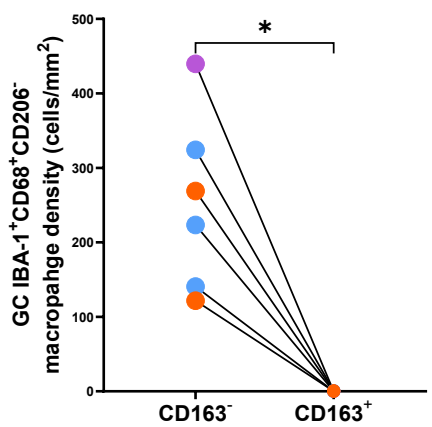


Supplementary Figure 4. GC macrophage phenotyping by Lunaphore COMET. (A) Multicolor IF merged image of a whole lymph node from a PLWoH (left) obtained from the Lunaphore COMET showing IBA-1 (pink), CD68 (green), KI67 (orange), CD163 (dark blue), CD206 (red), Gagp24 (white) and nuclei counterstained with DAPI (grey). The right inset shows a magnified GC denoted by KI67 positivity, with IBA-1^{+}CD68^{+} GC macrophages further magnified (bottom right). **(B-C)** Multicolor IF merged and individual channel images of whole LN tissue sections (left) and magnified GCs showing macrophage phenotypes from **(B)** a PLWoH and **(C)** a viremic PLWH. The white dashed line defined the GC and the green dotted line defines the light zone (LZ) and dark zone (DZ). The yellow circles indicate the IBA-1^{+}CD68^{+}CD206^{-}CD163^{+} GC macrophages**.** Images were acquired at 20x. Scale bars= 2mm, 100µm and 20µm

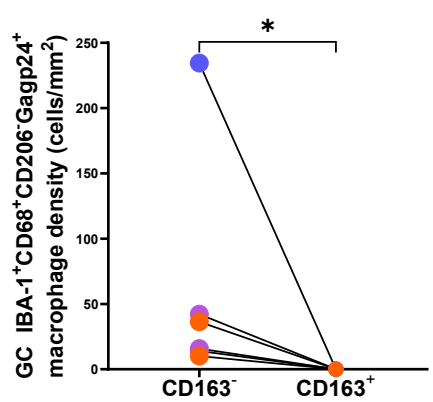
A. Virally suppressed donor





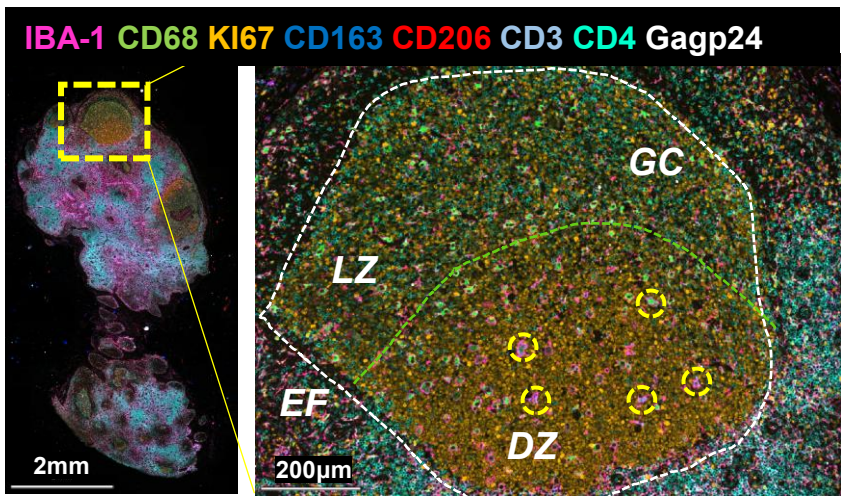


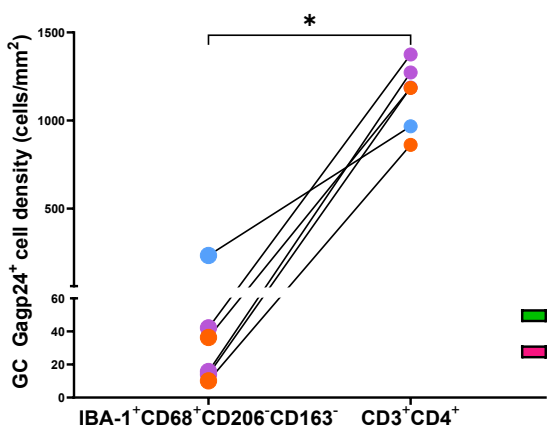
	$CD163^-$	$CD163^+$
GCs	n=6	n=6
Median	246.2	0.045
IQR	217.2	0.652



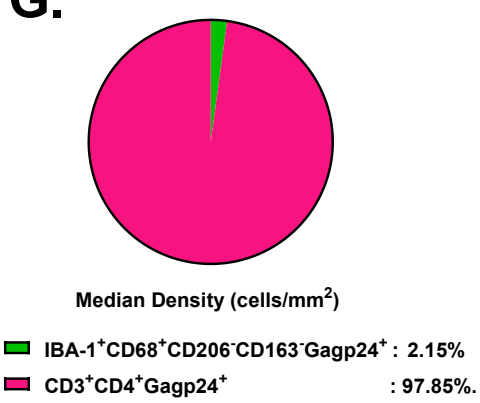
	$CD163^-$	$CD163^+$
GCs	n=6	n=6
Median	26.06	0.000
IQR	77.24	0.0192

E.

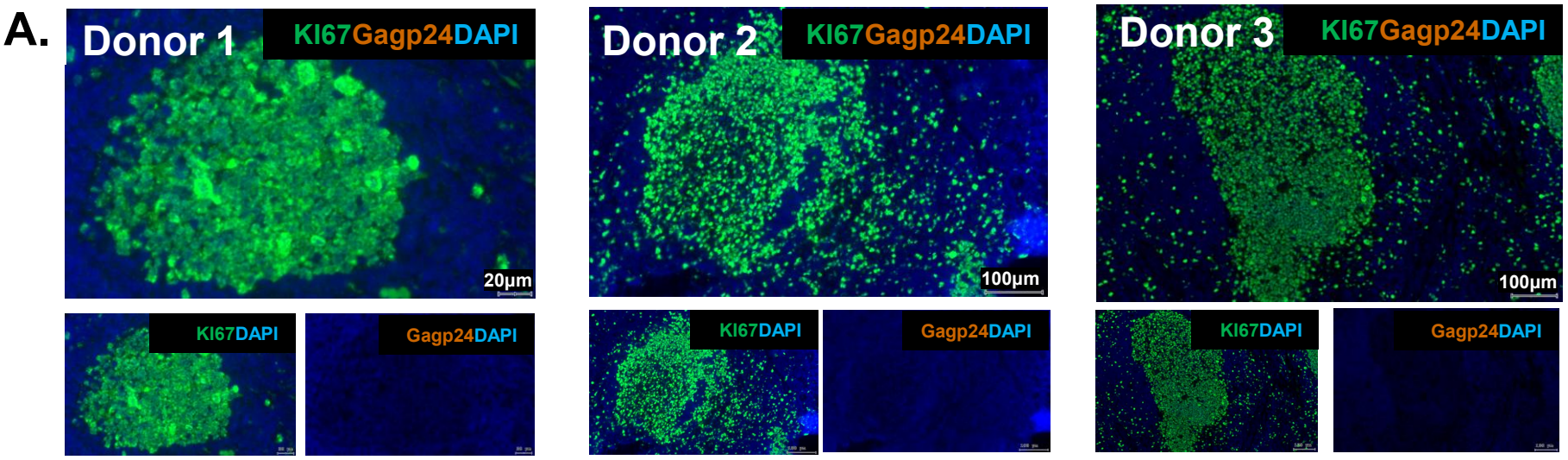


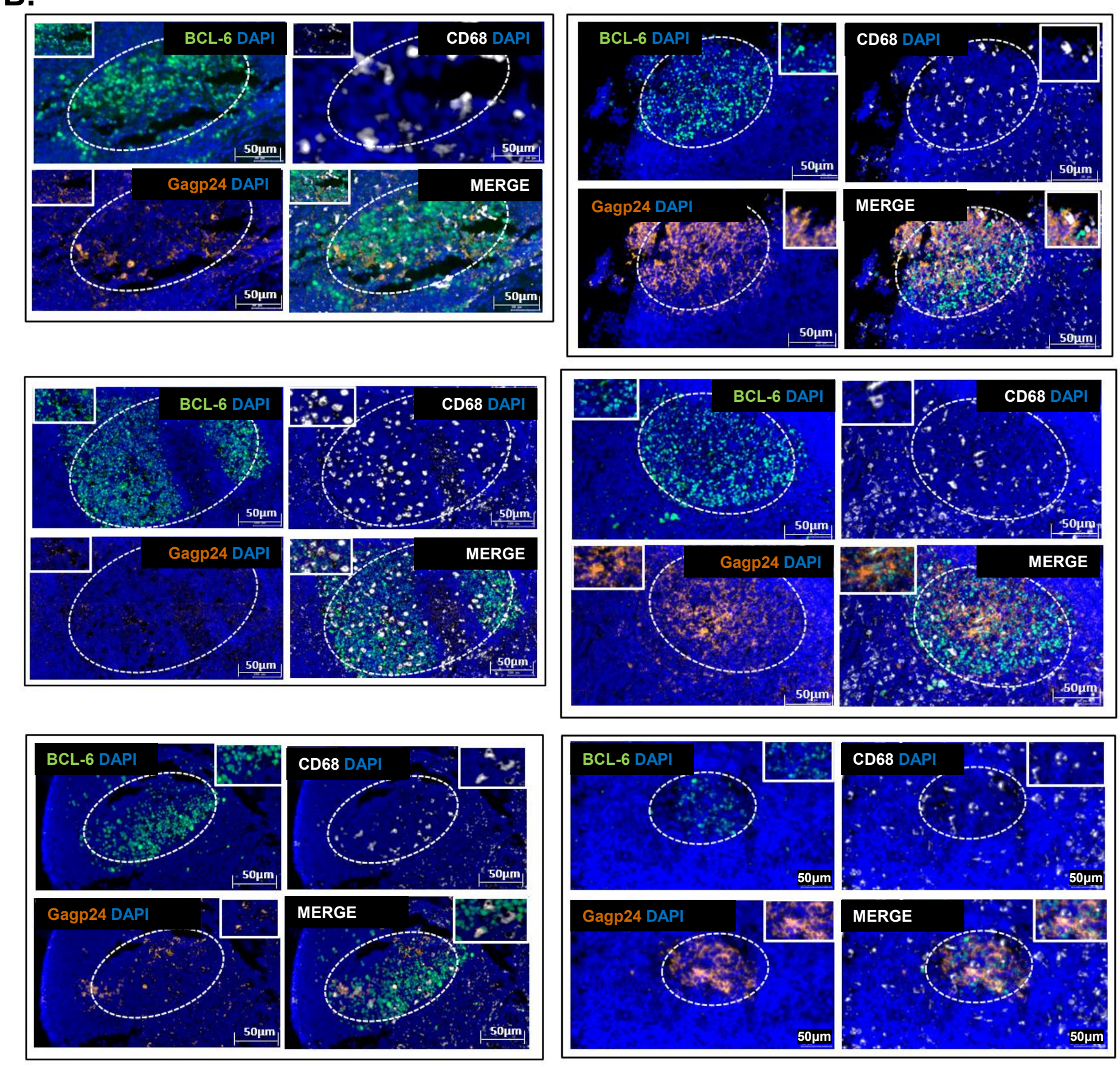


	$IBA\text{-}1^+CD68^+CD206^-CD163^-$	$CD3^+CD4^+$
GCs	n=6	n=6
Median	26.06	1186
IQR	77.24	356.9

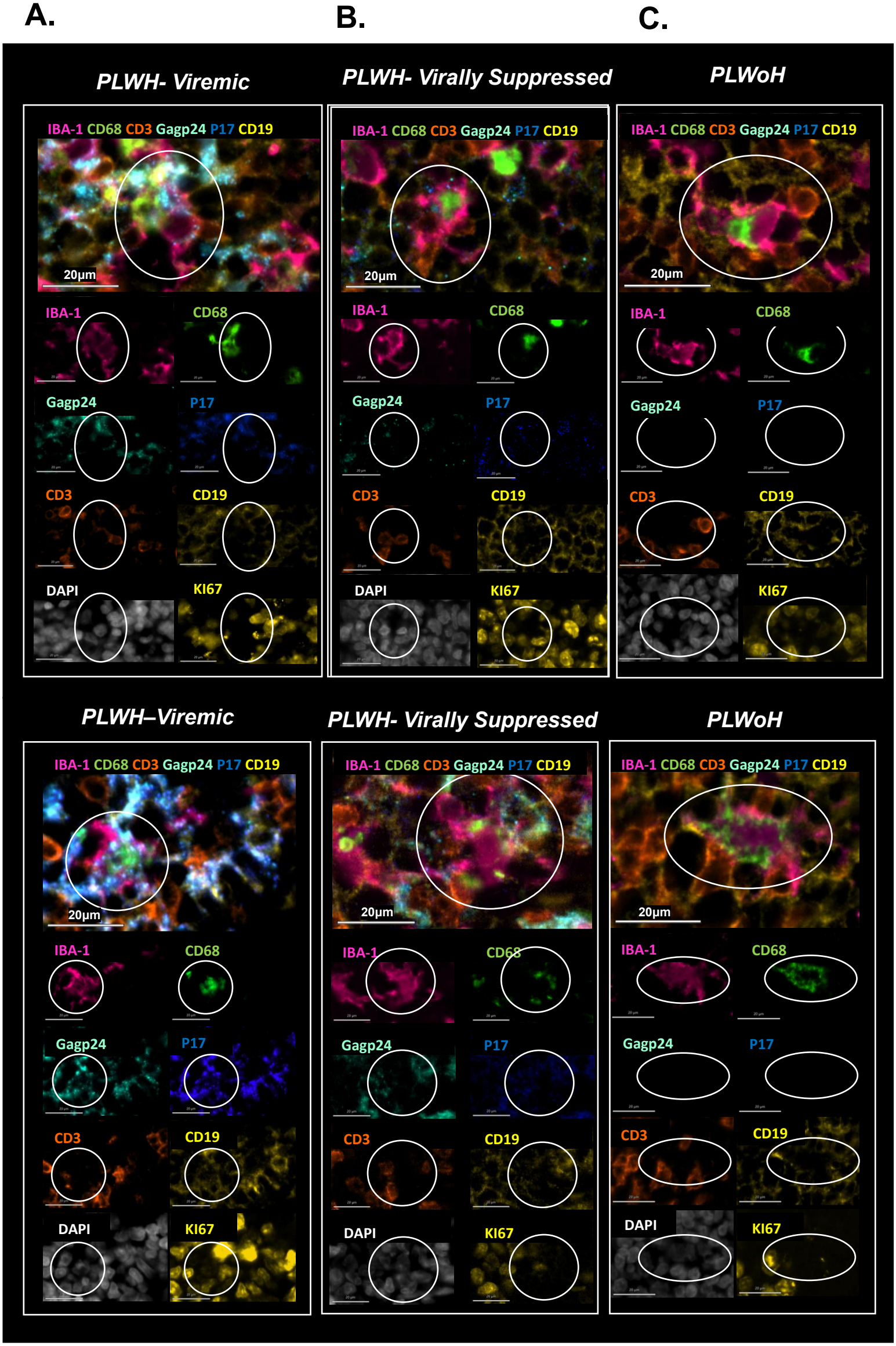


Supplementary Figure 5. GC macrophage phenotyping in virally suppressed individuals by Lunaphore COMET and HALO image analysis. (A) Multicolor IF merged image of a whole LN from a virally suppressed individual (left) obtained from the Lunaphore COMET showing IBA-1 (pink), CD68 (green), KI67 (orange), CD163 (dark blue), CD206 (red) and Gagp24 (white). The right inset shows the merged image of a magnified GC denoted by KI67 positivity, and individual channels for macrophage markers on the side. The white dashed line defined the GC and the green dotted line defines the light zone (LZ) and dark zone (DZ). Yellow circles indicate the IBA-$1^+CD68^+CD206^-CD163^+$ GC macrophages. **(B)** HALO spatial plots of n=3 GCs as denoted in panel A as GC1, GC2 and GC3 showing spatial distributions of $Gagp24^+$ GC macrophage phenotypes and the observation of IBA-$1^+CD68^+CD163^+$ macrophages, and $CD3^+CD4^+$ $Gagp24^+$ T cells as per the key. **(C)** HALO FISH IF quantitative analysis of GC macrophage density (cells/mm2) between IBA-$1^+CD68^+CD206^-CD163^-$ and IBA-$1^+CD68^+CD206^-CD163^+$ phenotypes and **(D)** Gagp24 positivity between these macrophage phenotypes. **(E)** Multicolor IF merged image of a whole LN with the CD3 (light blue) and CD4 (mint) **(F)** HALO FISH IF quantitative analysis of GC cell density (cells/mm^2) between IBA-$1^+CD68^+CD206^-CD163^-Gagp24^+$ macrophages and $CD3^+CD4^+Gagp24^+$ T cells, with **(G)** the proportion of the median densities represented in the pie chart. Comparisons made using the Wilcoxon sum-ranked test where each colour represents an individual donor and each circle represents a GC. Images were acquired at 20x. Scale bars= 2mm and 200µm. The level of significance as follows $p<0.05$*.



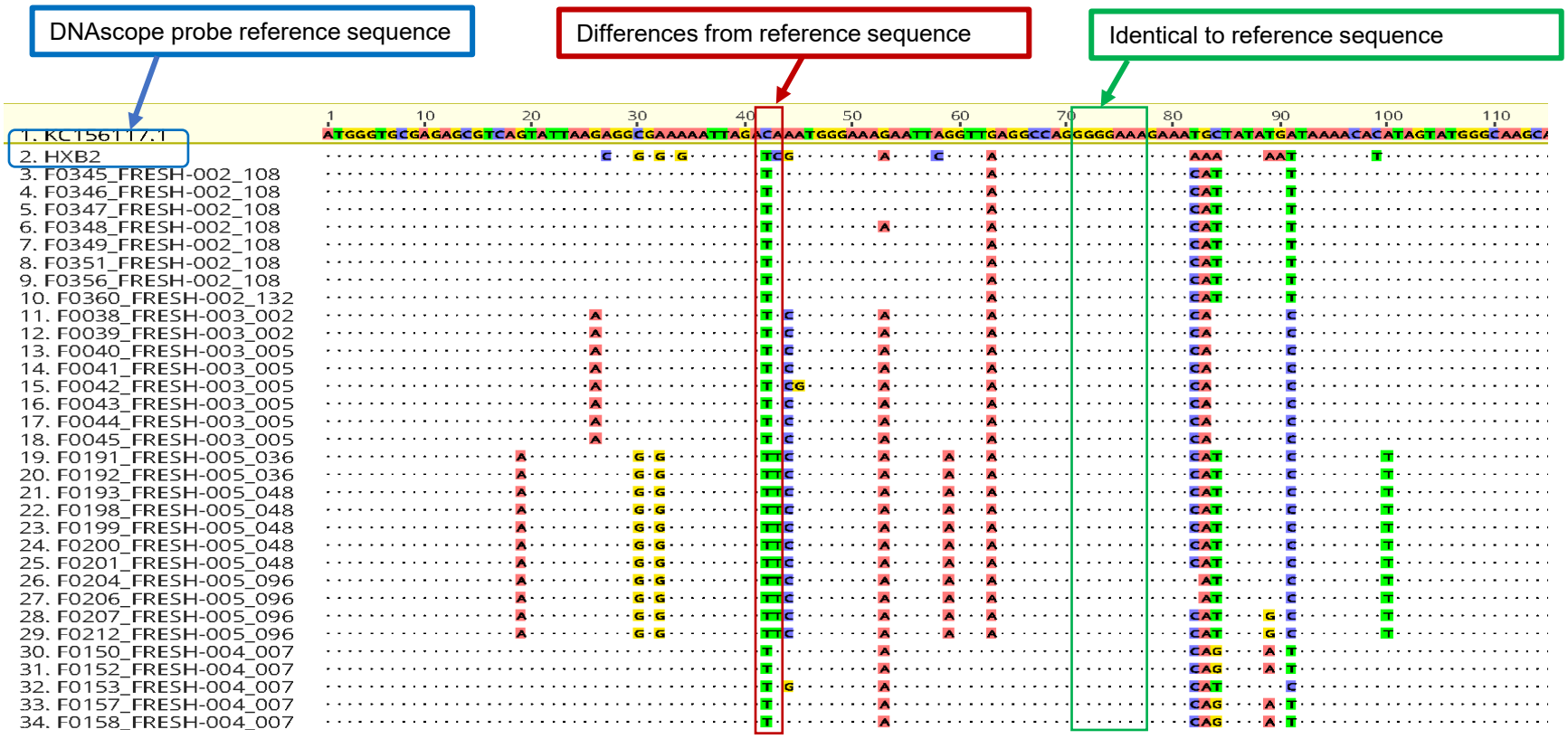


Supplementary Figure 6. HIV Gagp24 antibody controls and additional representative images from donors
(A) Representative Gagp24 negative control multicolor IF images from n=3 PLWoH of HIV Gagp24 antigen (brown) and KI67 (green) to define GCs. **(B)** Representative multicolor IF images from n=6 PLWH of GCs (white dashed ovals) indicated by BCL-6 (green) positivity and their respective expressions of $CD68^{+}$ (white) macrophages and HIV Gagp24 antigen (brown). Nuclei are stained with DAPI (blue). Images acquired at 40x. Scale bars= 20µm, 50µm and 100µm. .



Supplementary Figure 7. Bonafide GC macrophages co-localize with dual HIV protein makers. Representative merged and individual channel Lunaphore COMET images showing. (A) Representative Lunaphore COMET merged and individual channel images from n=2 (A) viremic PLWH and **(B)** virally suppressed PLWH of bona fide GC macrophages denoted by positivity for IBA-1 (pink), CD68 (green) and negativity for CD3 (orange) and CD19 (yellow), co-localizing with HIV Gagp24 (mint) and P17 (dark blue). A control representative image from a PLWoH is shown **(C)**. Individual channels for KI67 (yellow) to denote GC localization and nuclei counterstained with DAPI (grey) are shown. Images were acquired at 20x magnification and cell nuclei were detected by DAPI (grey). Scale bars equal to 20µm.

A.



	KC156117.1	HXB2	F0345_FRE...	F0346_FRE...	F0347_FRE...	F0348_FRE...	F0349_FRE..
KC156117.1		88.948%	92.357%	92.242%	92.242%	92.173%	92.196%
HXB2	88.948%		88.119%	87.981%	87.981%	87.958%	88.027%
F0345_FRESH-002_108	92.357%	88.119%		99.537%	99.583%	99.374%	99.652%
F0346_FRESH-002_108	92.242%	87.981%	99.537%		99.537%	99.467%	99.560%
F0347_FRESH-002_108	92.242%	87.981%	99.583%	99.537%		99.467%	99.652%
F0348_FRESH-002_108	92.173%	87.958%	99.374%	99.467%	99.467%		99.444%
F0349_FRESH-002_108	92.196%	88.027%	99.652%	99.560%	99.652%	99.444%	
F0351_FRESH-002_108	92.311%	88.096%	99.606%	99.699%	99.652%	99.490%	99.676%
F0356_FRESH-002_108	92.127%	88.004%	99.490%	99.398%	99.444%	99.328%	99.652%
F0360_FRESH-002_132	92.334%	88.096%	99.977%	99.513%	99.560%	99.351%	99.629%
F0038_FRESH-003_002	91.342%	87.454%	92.907%	92.837%	92.907%	92.791%	92.884%
F0039_FRESH-003_002	91.388%	87.500%	92.953%	92.884%	92.953%	92.837%	92.930%
F0040_FRESH-003_005	91.365%	87.523%	92.930%	92.860%	92.930%	92.814%	92.907%
F0041_FRESH-003_005	91.388%	87.500%	92.953%	92.884%	92.953%	92.837%	92.930%
F0042_FRESH-003_005	91.365%	87.477%	92.930%	92.860%	92.930%	92.814%	92.907%
F0043_FRESH-003_005	91.388%	87.500%	92.953%	92.884%	92.953%	92.837%	92.930%

% identity to reference sequence

Nucleotide Statistics:

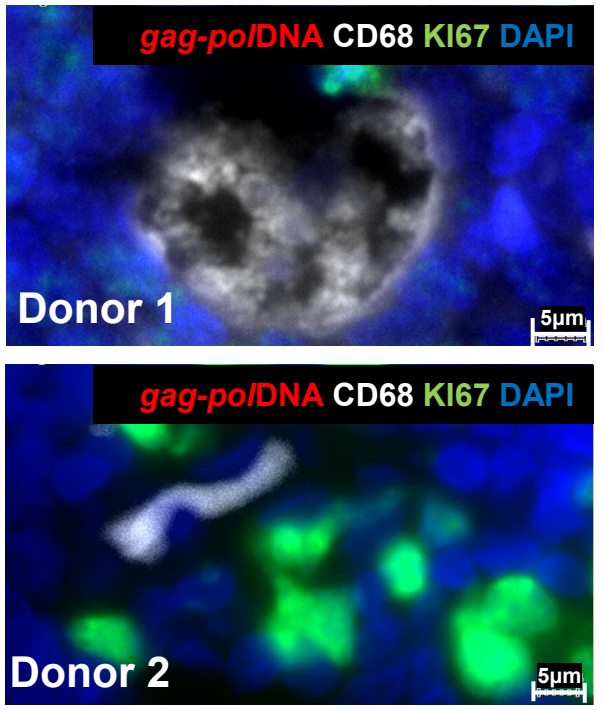
Length (mean): 4,297 bp

Sequences: 241

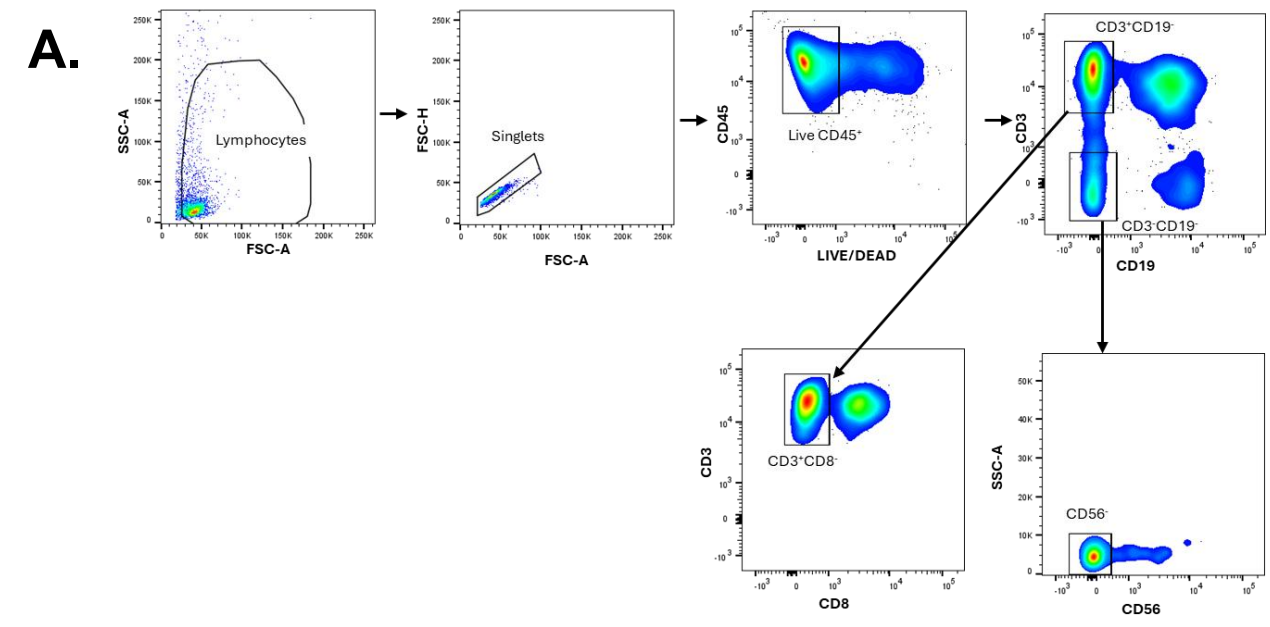
Identical Sites: 2,572 (58.0%)

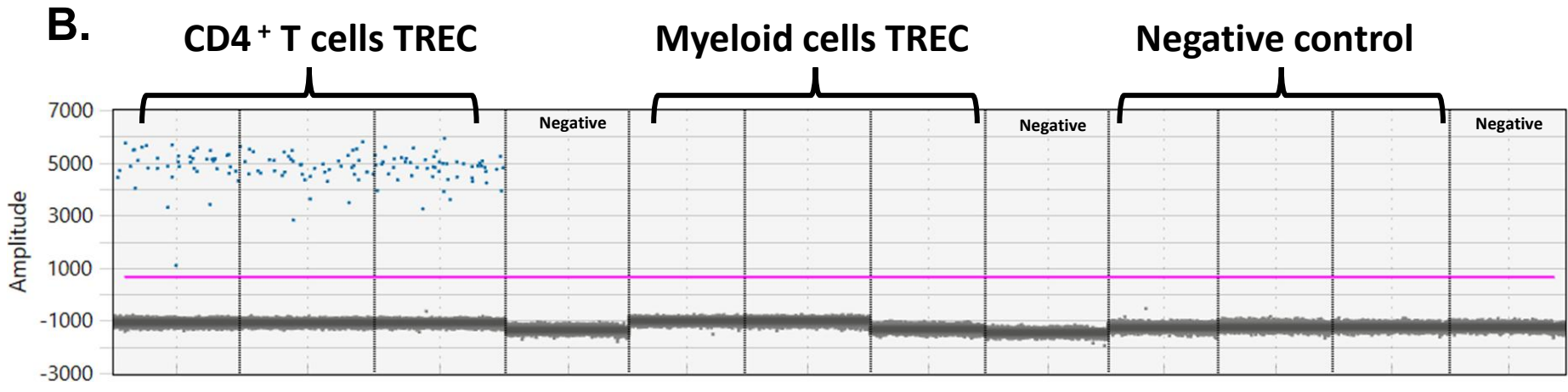
Pairwise Identity: 92.8%

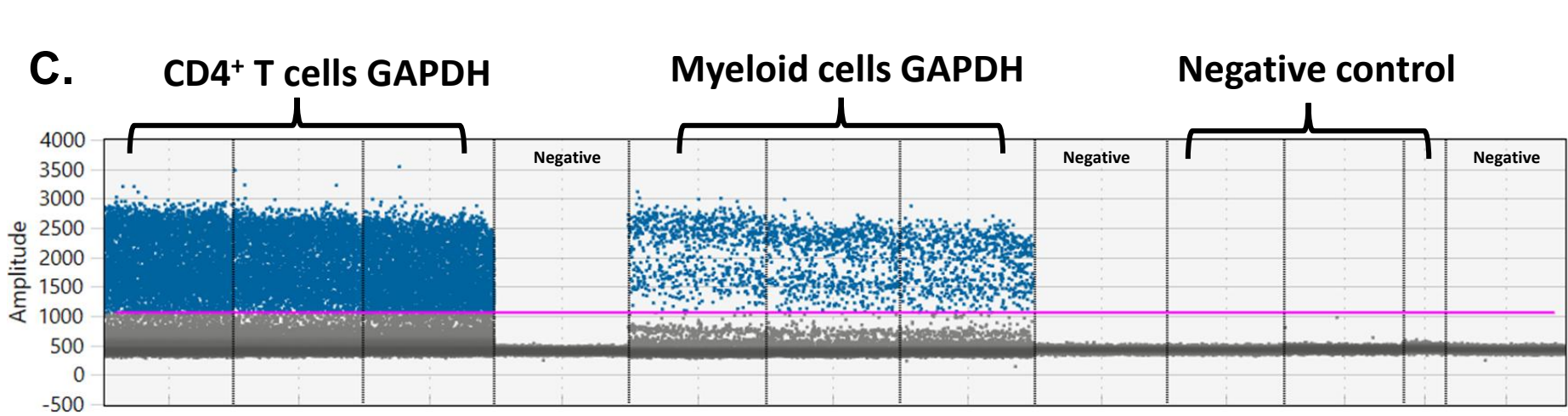
B.

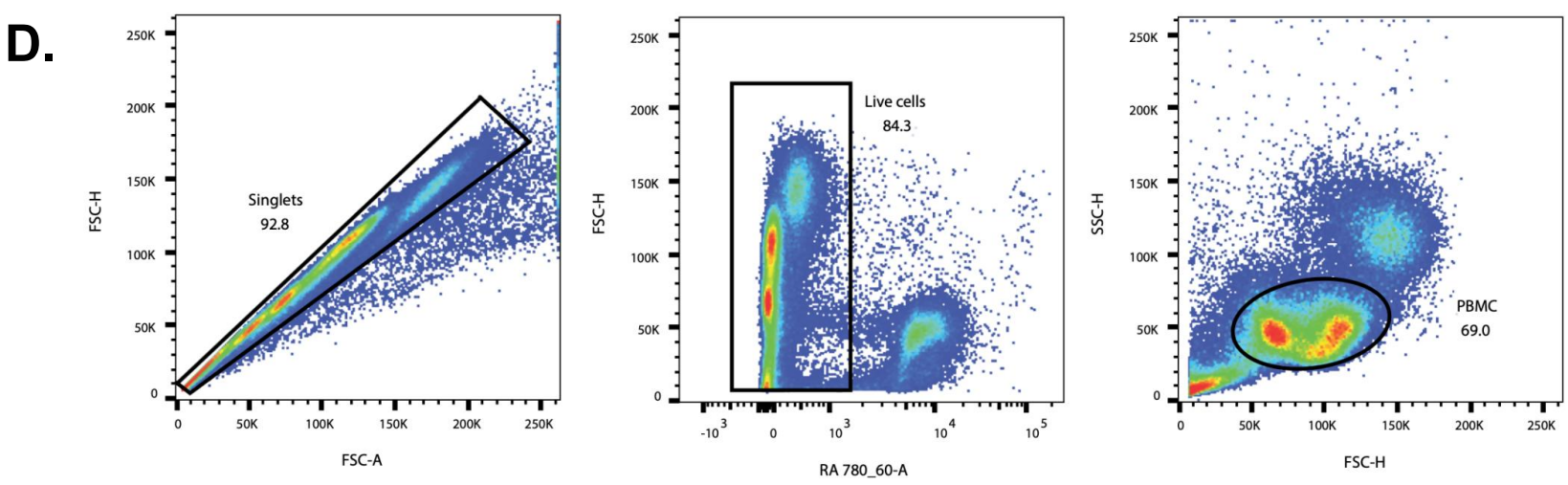


Supplementary Figure 8. DNAscope validation. (A) Alignment of DNAscope probe reference sequence to the sequences of our study cohort, validated with a 92.8% pairwise identity. **(B)** Representative DNAscope negative control images from n=2 PLWoH of *gag-pol* HIV DNA (red), CD68 (white) andKI67 (green). Cell nuclei were counterstained with DAPI (blue). Images acquired at 40x. Scale bars= 5µm.









Supplementary Figure 9. Isolation of myeloid and CD4⁺ T cells from LNMCs and purity of myeloid cells. (A) Flow cytometry gating strategy for CD4⁺ T cells and myeloid cells from LNMCs for ddPCR. **(B)** TREC-2D Plot indicating no T cell contamination in the myeloid population. **(C)** House-keeping gene GAPDH expression in CD4⁺ T cell and myeloid populations.**(D)** Flow cytometry gating of live PBMCs for the SQuHIVLa assay