

Supplementary files

**Single-Cell TCR Sequencing Reveals Phenotypically
Diverse Clonally Expanded Cells Harboring Inducible
HIV Proviruses During ART**

Gantner et al.

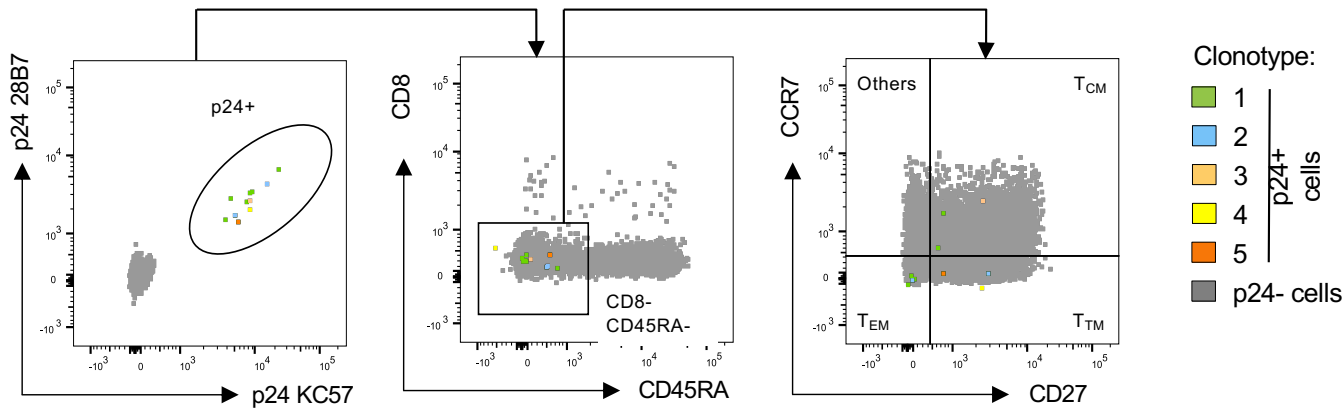
a

Well ID#	TRBV	CDR3 sequence (amino-acids)	TRBJ
A1	V6-5*01	CASRRARWGALSNSPLHF	J1-6*01
A2	V6-5*01	CASRRARWGALSNSPLHF	J1-6*01
A3	V4-1*01	CASSLTEAYGYTF	J1-2*01
A4	V20-1*01	CSAKDRVETQFF	J2-5*01
A5	V29-1*01	CSVKDSYNEQFF	J2-1*01
A6	V6-5*01	CASRRARWGALSNSPLHF	J1-6*01
A7	V10-3*01	CGVRDPFYPPHF	J1-5*01
A8	V6-5*01	CASRRARWGALSNSPLHF	J1-6*01
A9	V6-5*01	CASRRARWGALSNSPLHF	J1-6*01
A10	V6-5*01	CASRRARWGALSNSPLHF	J1-6*01
A11	V6-5*01	CASRRARWGALSNSPLHF	J1-6*01
A12	V4-1*01	CASSLTEAYGYTF	J1-2*01
B1	V6-5*01	CASRRARWGALSNSPLHF	J1-6*01

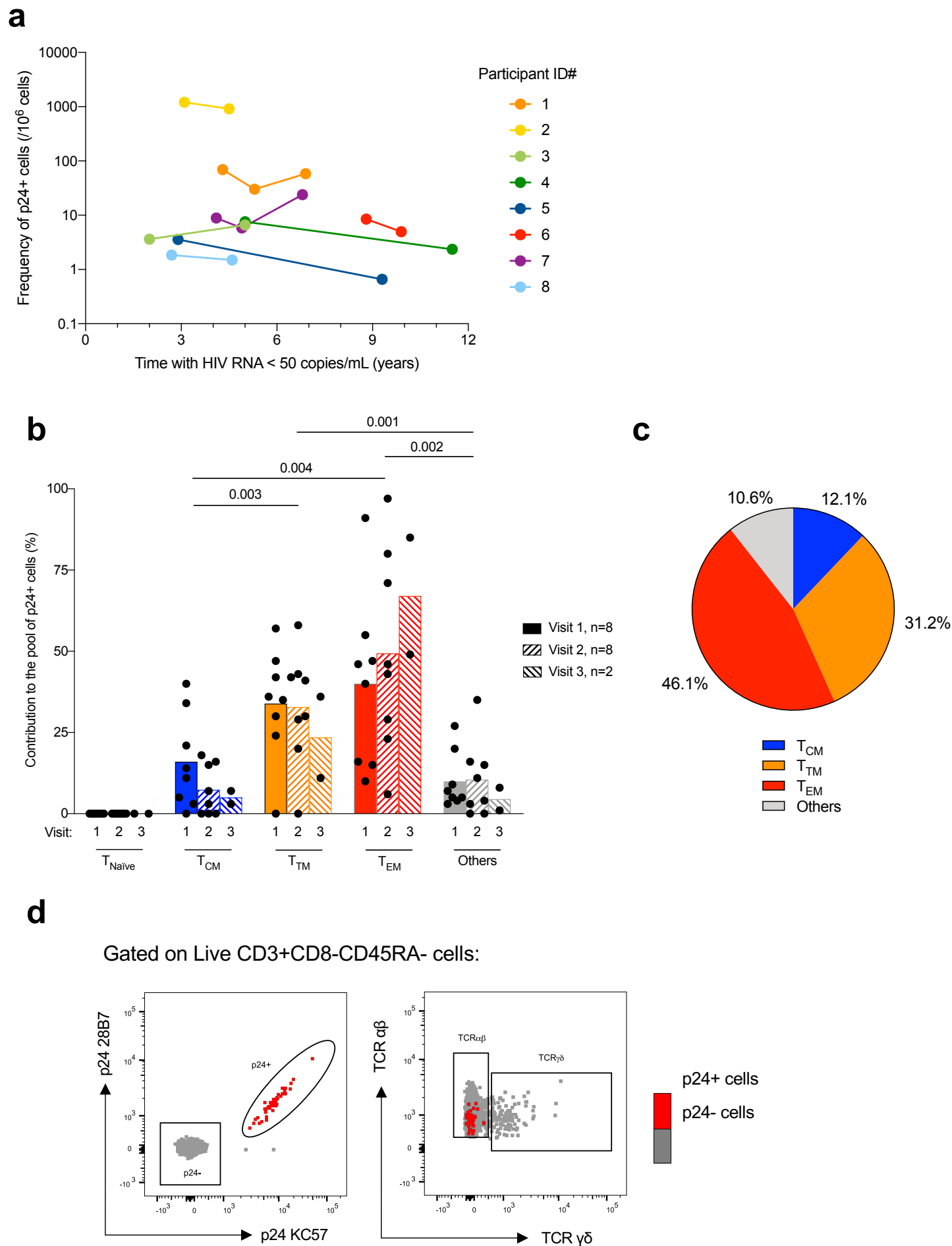
Clonotype list:

- 1
- 2
- 3
- 4
- 5

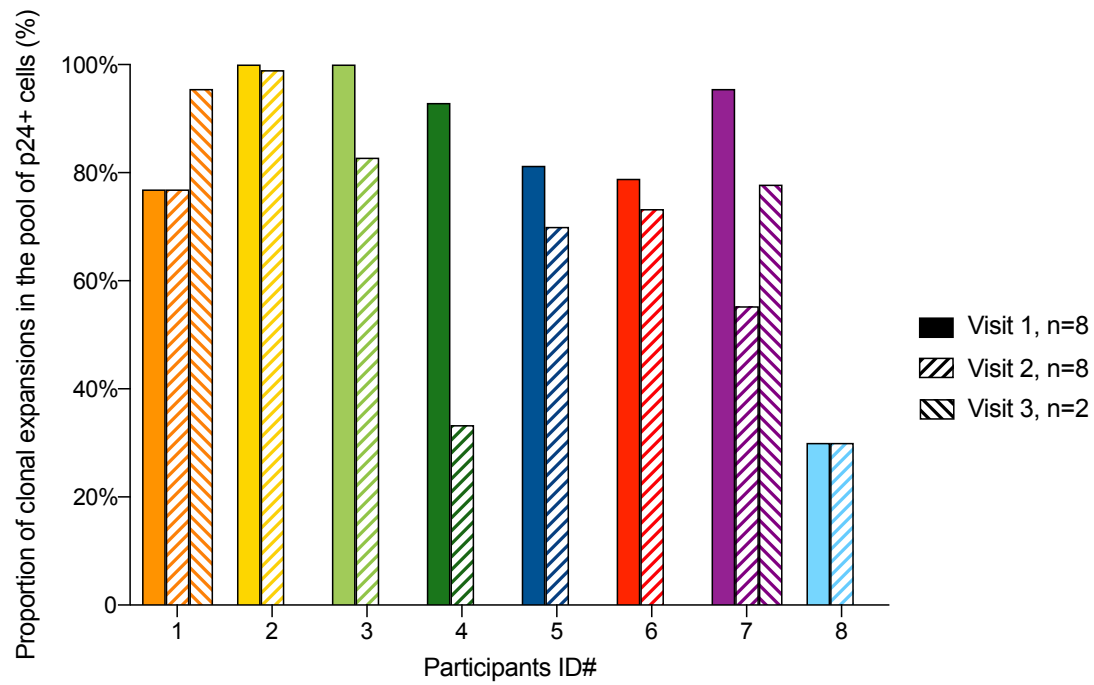
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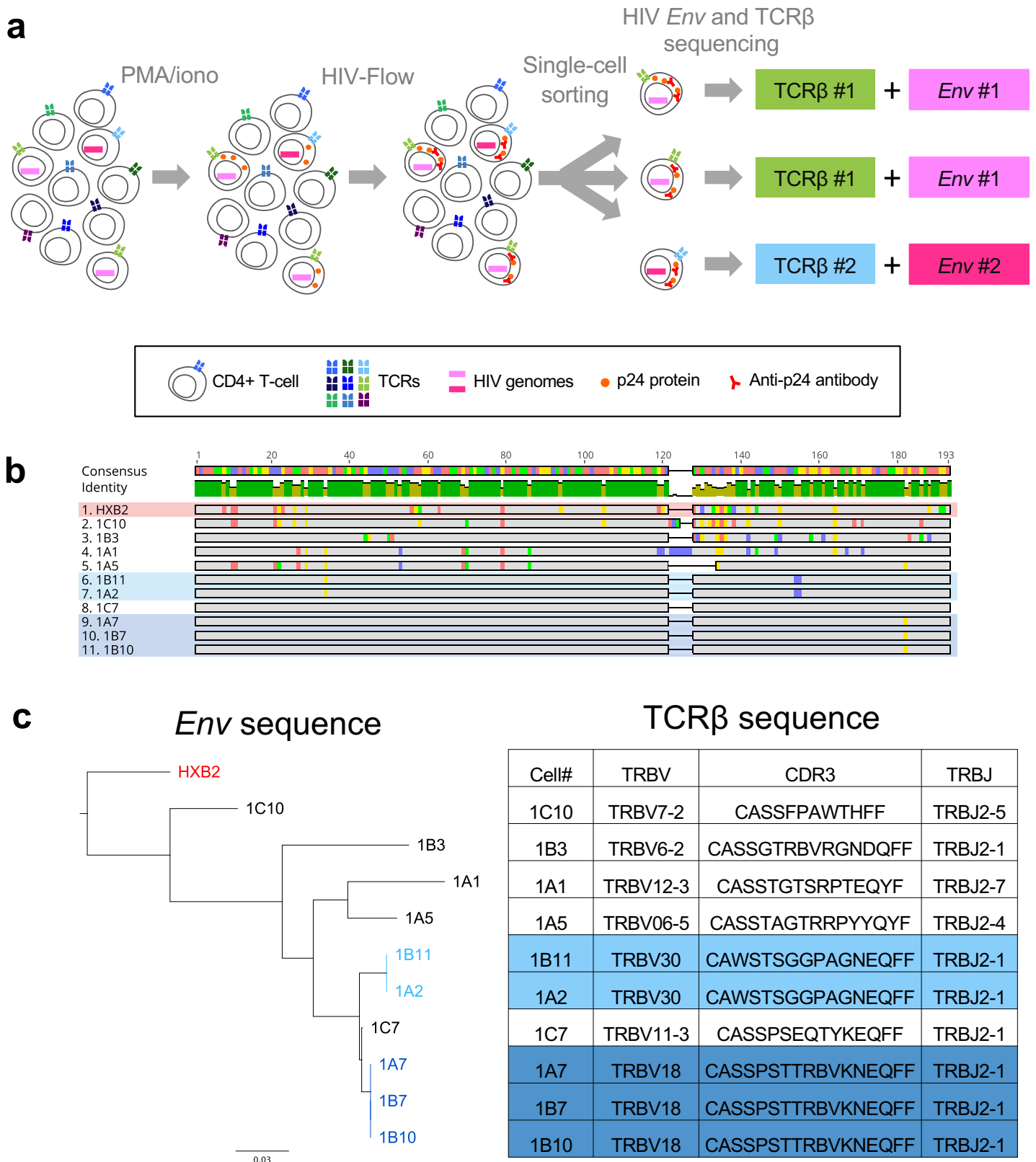
Supplementary Fig. 1. Phenotype and TCR sequences of individual p24+ cells from a representative participant. **a.** TRBV, TRBJ and CDR3 sequences from single sorted p24+ cells (n=13) from participant #1, visit 1. Sequences from two expanded clonotypes are shown in green and blue. **b.** Dot plots showing the level of expression of CD45RA, CCR7 and CD27 in individual p24+ cells (colored dots), overlaid onto total memory CD4+ T cells (in grey). The colors of individual p24+ cells correspond to the clonotypes shown in **a**.



Supplementary Fig. 2. Frequencies and phenotype of p24+ cells. **a.** Frequencies of p24+ cells in CD4+ T cells measured by HIV-Flow in longitudinal samples from all participants. **b.** Contribution of each memory subset to the pool of p24+ cells. Central memory (T_{CM} , blue); transitional memory (T_{TM} , orange); effector memory (T_{EM} , red); others (grey). Data are plotted for all study visits. The contribution to the pool of p24+ cells was higher for the most differentiated subsets (n=18 samples analyzed, two-sided Wilcoxon for paired comparisons). **c.** The mean contribution of each CD4+ T cell subset (T_{CM} , T_{TM} , T_{EM} and others) to the pool of p24+ cells is represented as a pie chart (all participants and all visits). **d.** Representative dot plots showing the expression of TCR $\alpha\beta$ and TCR $\gamma\delta$ in p24+ cells (in red), overlaid onto total memory CD4+ T cells (in grey).

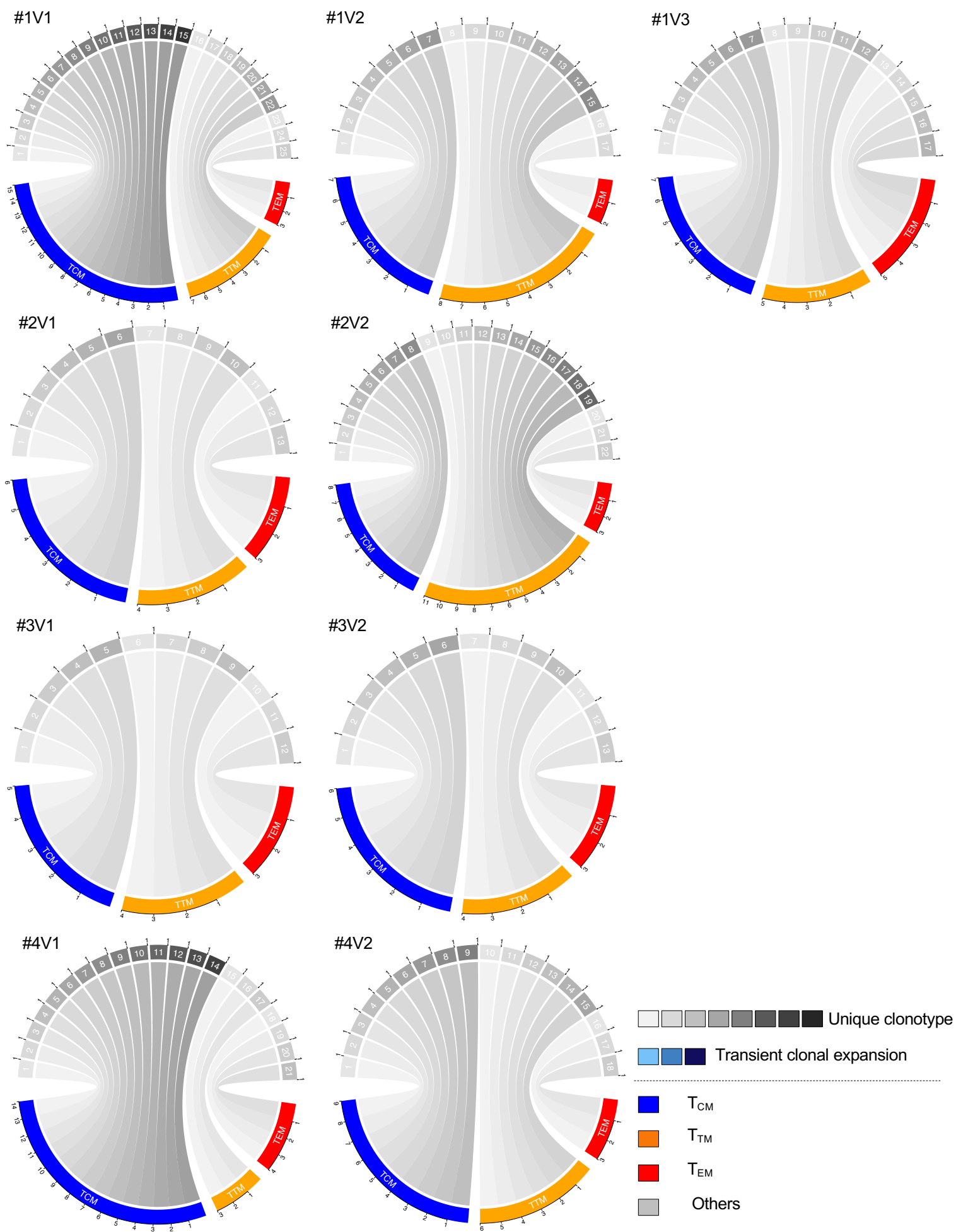


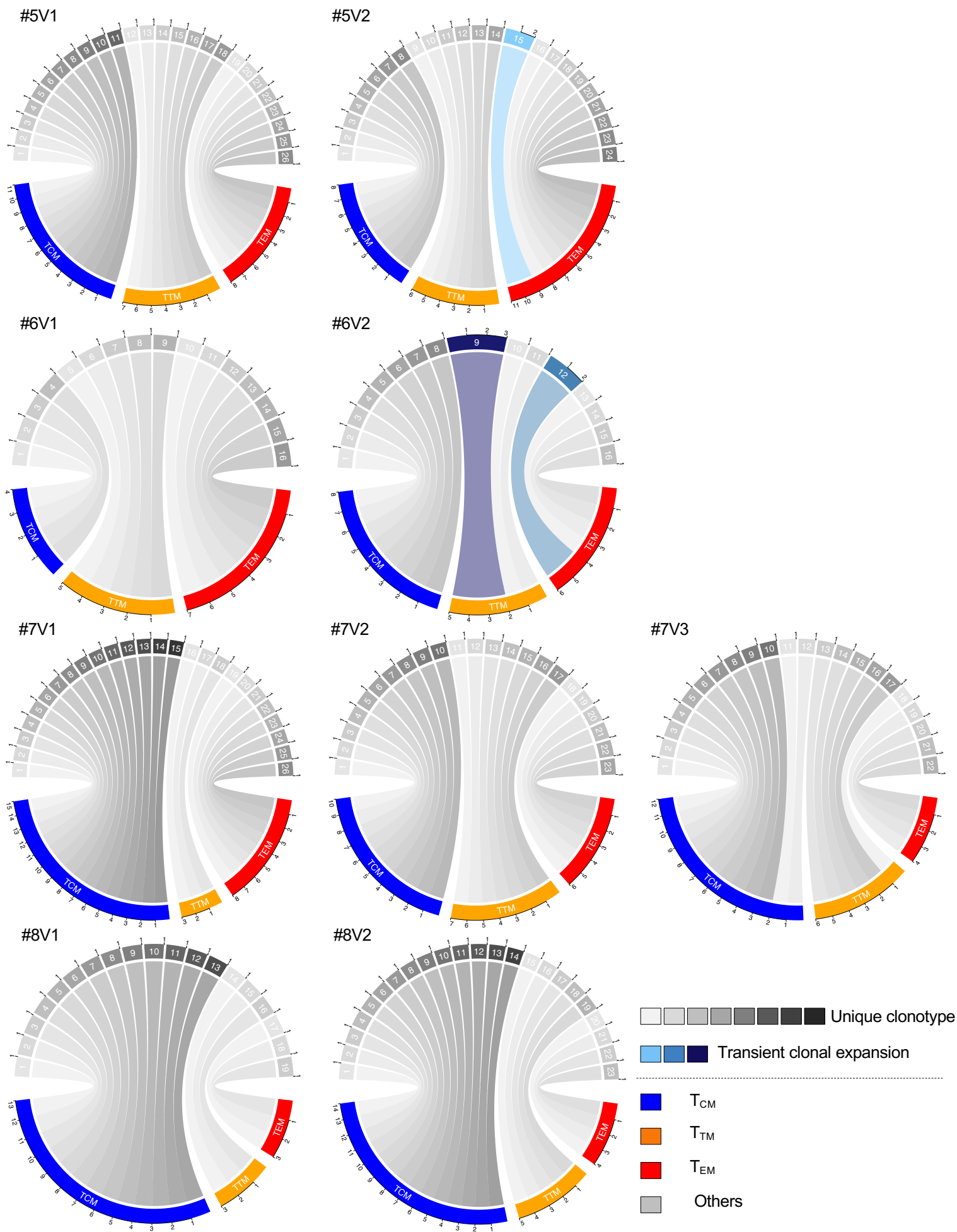
Supplementary Fig. 3. High proportion of clonal expansions in the translation-competent reservoir. Proportion of clonally expanded cells in the pool of p24+ cells for all participants and all study visits.



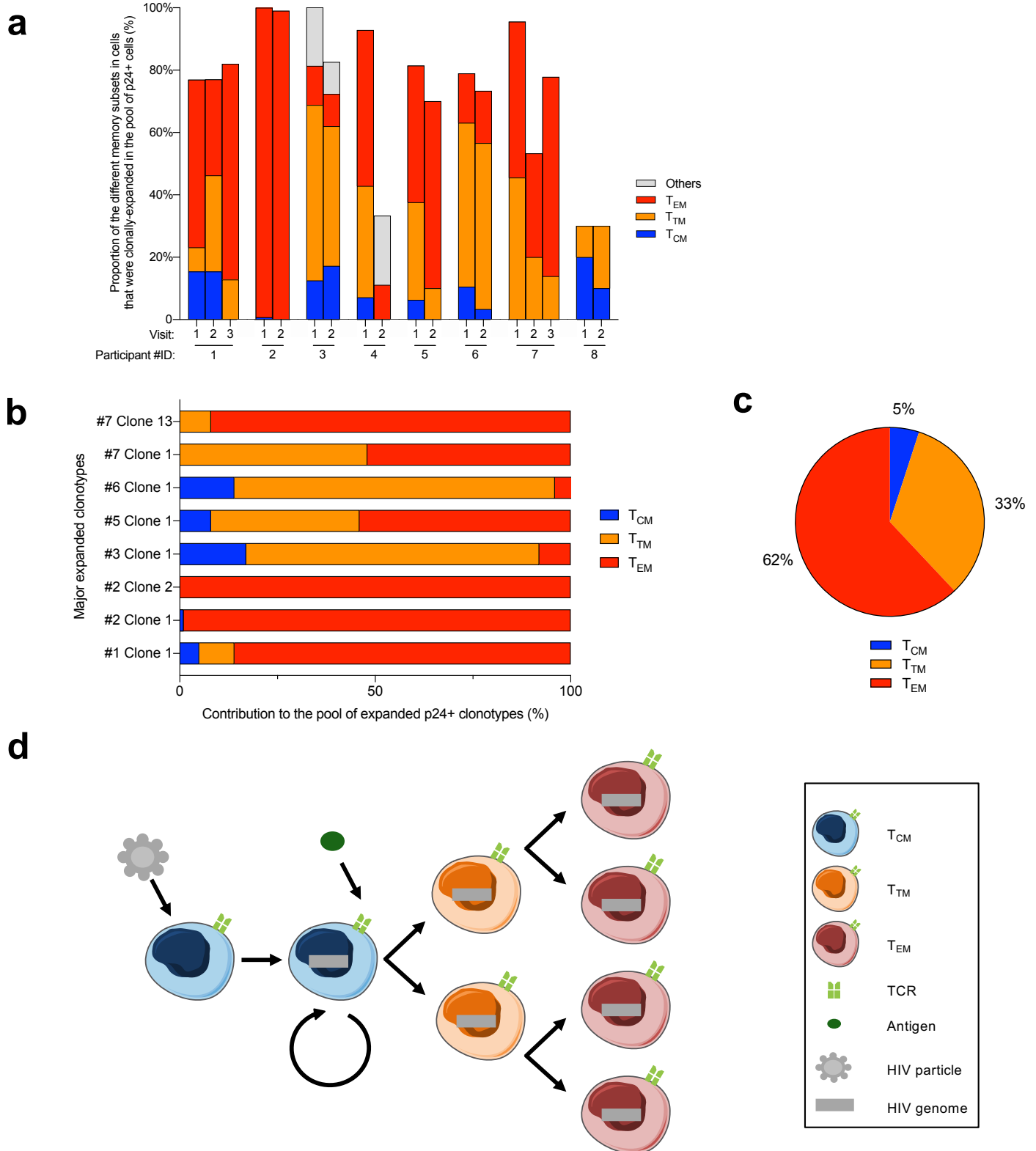
Supplementary Fig. 4. Combined analyses of HIV *Env* and TCR sequences in single sorted p24+ cells. **a.** To obtain both TCR and HIV sequences from individual cells, we adapted our assay (described in Fig. 1a) to co-amplify TCR β and HIV *Env* C3-V5 in a multiplex PCR. **b.** Alignment of *Env* sequences obtained from single-sorted p24+ cells (n=10). Two clusters of identical *Env* sequences (n=2 and n=3) were identified. All other sequences differed by at least 2 nucleotides. **c.** When pairing *Env* and TCR sequences, we observed that cells sharing the same *Env* sequence also shared the same TCR sequence (highlighted in blue).

Supplementary Fig. 5

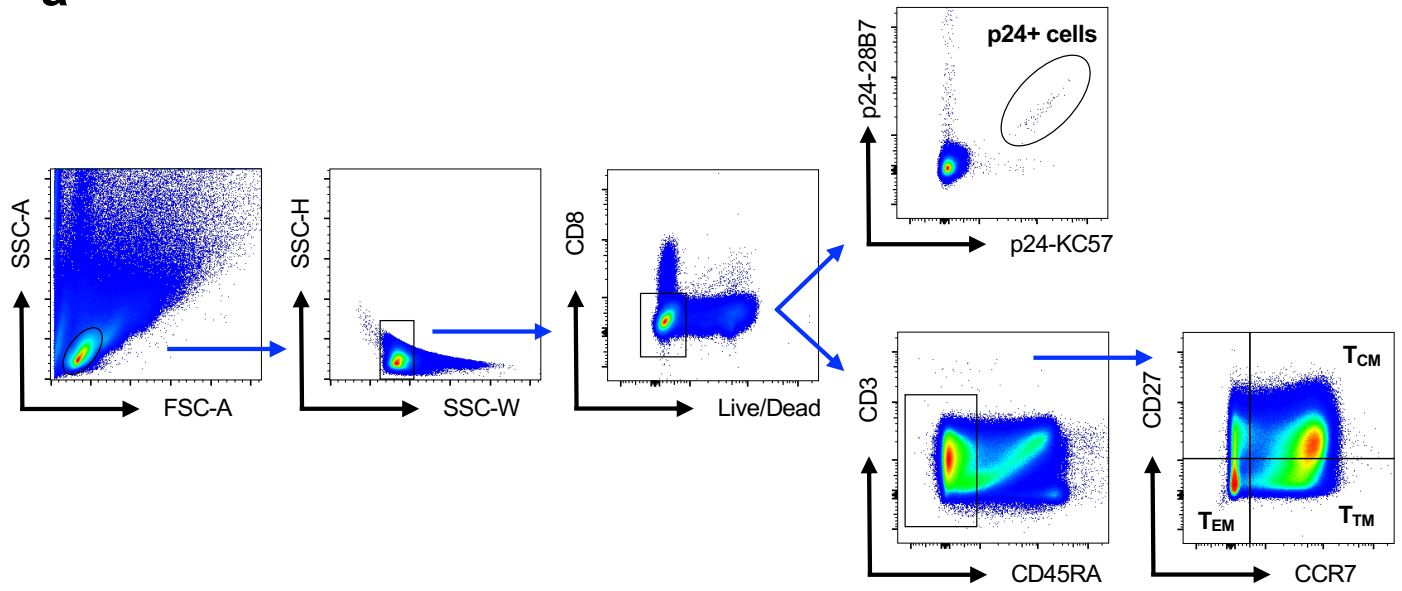
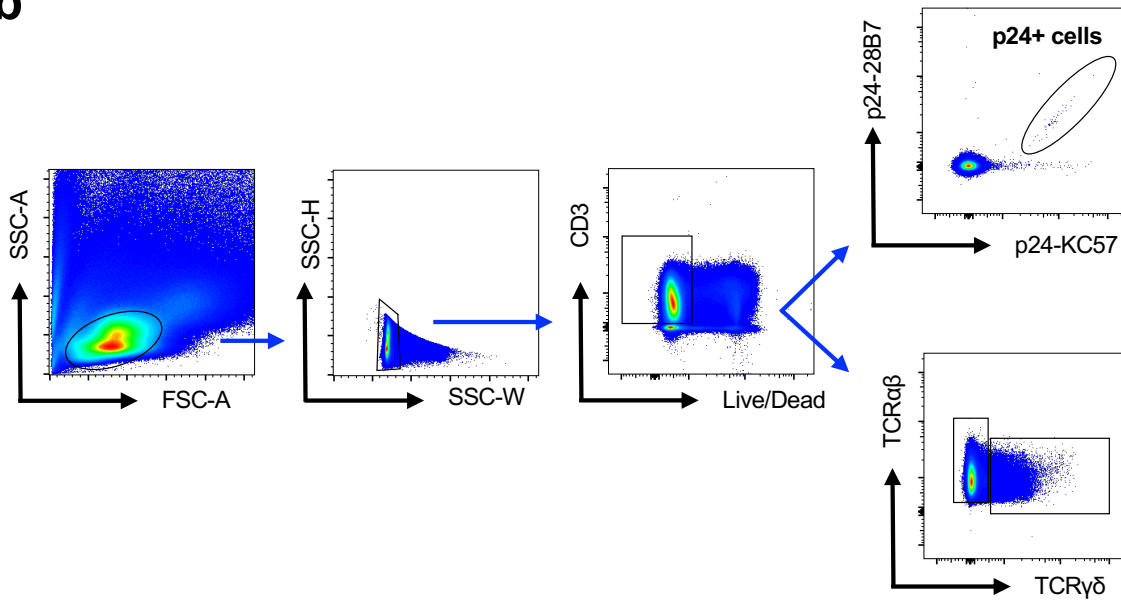




Supplementary Fig. 5. Phenotypic and clonotypic analysis of p24- cells. The distribution of TCR β clonotypes of p24- cells among memory subsets is represented as a chord diagram for participants #1 to #8 and according to the study visit (V1, V2 and V3). The circular representation shows the link between a specific clonotype (top half of the circle) and its memory phenotype (bottom half of the circle). The circular axis represents the number of p24- cells in each clonotype/subset. Expanded clonotypes detected at a single visit are depicted in shades of blue; unique clonotypes are depicted in shades of grey. The memory subset color code is as follows: central memory (T_{CM}) in blue; transitional memory (T_{TM}) in orange; and effector memory (T_{EM}) in red.



Supplementary Fig. 6. Major expanded p24+ clonotypes display multiple memory phenotypes. **a.** Proportion of the different memory phenotypes among cells that were clonally-expanded cells in the pool of p24+ cells for all participants and all study visits. **b.** Proportion of each memory subset in major clonally-expanded p24+ clonotypes ($n \geq 10$ p24+ cells). Major expanded infected clonotypes were systematically overrepresented in the most differentiated subsets (i.e. T_{TM} and T_{EM}) and displayed at least two different memory phenotypes. Rare T_{CM} cells were commonly observed within major expanded clonotypes. **c.** Pie chart depicting the mean contribution of each memory subset to the major expanded clones shown in **b.** **d.** The pool of HIV infected cells is maintained by cell proliferation. Whereas T_{TM} and T_{EM} cells represent the main contributors to the pool of infected cells, TCR analysis reveals that these cells are the progeny of infected T_{CM} cells that have long survival and self-renewal capacities. Our results suggest a model in which T_{CM} cells represent a long-lived source of reservoir cells that can expand upon antigen stimulation and replenish the pool of infected cells.

a**b**

Supplementary Fig. 7. Gating strategies. **a.** Gating strategy used for index cell sorting of p24+ cells with recording of memory subsets. **b.** Gating strategy used to determine the TCR lineage of HIV p24+ cells.

Supplementary Table 1. Participants' characteristics

#ID	Sex	Ethnicity	HLA	Route of HIV acquisition	Nadir CD4 (cells/mm ³)	Visit	Age (years)	CD4 (cells/mm ³)	CD8 (cells/mm ³)	HIV RNA (copies/mL)	Infection duration (years)	ART duration (years)	Undetectability duration (years)	Current ART	Total HIV DNA (cop./10 ⁶ CD4 T cells)	Integrated HIV DNA (cop./10 ⁶ CD4 T cells)	HIV-Flow (cells/10 ⁶ CD4 T cells)
1	M	Hispanic	DRB1*01:01 DRB1*08:04	IVDU	384	1	57	587	945	<48	24.7	16.7	4.3	FPV/r+ABC+3TC	1601	1340	69.10
						2	58	612	834	<20	25.7	17.7	5.3	FPV/r+ABC+3TC	1311	1292	30.10
						3	60	677	788	<20	27.3	19.3	6.9	DRV/r+ABC/3TC	1249	1417	58.30
2	M	African American	DRB1*07:01 DRB1*13:04	HTS	21	1	39	267	518	<20	13.6	7.8	3.1	EFV/TDF/FTC	2749	3851	1208.00
						2	40	285	504	<20	15.0	9.2	4.5	EFV/TDF/FTC	3153	6059	914.60
3	M	Caucasian	DRB1*03:01 DRB1*08:02	MSM	186	1	43	1065	1408	<48	15.8	11.3	2.0	EFV/TDF/FTC	681	22	3.62
						2	46	887	1134	<20	18.8	14.3	5.0	EFV/TDF/FTC	640	12	6.61
4	M	Caucasian	DRB1*10:01 DRB1*13:02	MSM	70	1	48	268	673	<20	23.0	5.3	5.0	RAL+ETR+MVC	427	310	7.58
						2	55	316	733	<20	29.5	11.8	11.5	RAL+ETR+MVC	596	444	2.36
5	M	African American	DRB1*13:01 DRB1*15:03	MSM	181	1	32	248	378	<20	3.7	3.2	2.9	EFV/TDF/FTC	1945	1267	3.58
						2	39	420	568	<20	10.0	9.5	9.3	EVG/c/TAF/FTC	921	639	0.66
6	M	Caucasian	DRB1*04:04 DRB1*15:01	MSM	386	1	66	601	819	<40	22.2	15.7	8.8	DRV/r	2704	1424	8.49
						2	67	602	880	<40	23.3	16.8	9.9	DRV/r	2142	658	4.97
7	M	Caucasian	DRB1*01:02 DRB1*15:01	HTS	377	1	36	471	322	<40	10.5	4.2	4.1	RPV/TDF/FTC	221	175	8.87
						2	37	377	222	<40	11.3	5.1	4.9	RPV/TDF/FTC	262	229	5.83
						3	39	445	227	<40	13.2	7.0	6.8	RPV/TDF/FTC	354	106	23.90
8	F	African American	DRB1*03:02 DRB1*09:01	HTS	29	1	44	460	1879	<20	5.5	5.5	2.7	FPV/r+RAL+FTC/TDF	3300	2937	1.85
						2	46	541	1729	<20	7.4	7.4	4.6	DTG+TDF/FTC	3300	3239	1.49

M, male; F, female; IVDU, intravenous drug user; MSM, men who have sex with men; HTS, heterosexual; cop., copies; Und., Undetectability; ART, antiretroviral therapy; ABC, abacavir; FPV, fosamprenavir; DRV, darunavir; DTG, dolutegravir; EFV, efavirenz; ETR, etravirine; EVG, elvitegravir; FTC, emtricitabine; MVC, maraviroc; /r, ritonavir; RAL, raltegravir; RPV, rilpivirine; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; 3TC, lamivudine.

Supplementary Table 2. Primers used for amplification and sequencing of TCR β

Name	Sequence 5'-3'
PCR1 : Forward primers (tagged with M13F)	
VB2	GTAAACGACGGCCAGTATACTTCTATTGGTACAGACAAATCTTGG
VB3	GTAAACGACGGCCAGTCTATGTATTGGTATAACAGGACTCTAAG
VB4	GTAAACGACGGCCAGTCAYARSGCTATGTATTGGTACAAGC
VB5/9	GTAAACGACGGCCAGTCACTGTGTCCTGGTACCAACAG
VB6	GTAAACGACGGCCAGTTACATGTACTGGTATCGACAAGACC
VB7	GTAAACGACGGCCAGTTACCCTTTATTGGTACCGACAGAGCCTGG
VB11	GTAAACGACGGCCAGTCTTTACTGGTACCGGCAGAWCYTGG
VB12	GTAAACGACGGCCAGTTTTTCTGGTACAGACAGACCATGATG
VB13	GTAAACGACGGCCAGTCACTGTCTACTGGTACCAGCAGG
VB14	GTAAACGACGGCCAGTTGGACATGATAATCTTTATTGGTATCGAC
VB15	GTAAACGACGGCCAGTCATGTACTGGTACCAGCAGAAGTC
VB16	GTAAACGACGGCCAGTGTTATGTTTTTGGTACCAACAGGTCC
VB17	GTAAACGACGGCCAGTCATGTTTGTTCACTGGTACCGACAGAATC
VB18	GTAAACGACGGCCAGTAGTCATGTTTACTGGTATCGGCAGC
VB19	GTAAACGACGGCCAGTTGCCATGTACTGGTACCGACAG
VB20	GTAAACGACGGCCAGTCACAACATGTTTTGGTATCGTCAG
VB21	GTAAACGACGGCCAGTTAGTTATGTTTACTGGTATCATAAGACGC
VB23	GTAAACGACGGCCAGTATACTTTTTGTTTATTGGTATCAACAGAATCAG
VB24	GTAAACGACGGCCAGTATGTACTGGTATCGACAAGACCC
VB25	GTAAACGACGGCCAGTTGACAAAATGTACTGGTATCAACAAGATC
VB29	GTAAACGACGGCCAGTTGATGTTCTGGTACCGTCAGCAAC
VB30	GTAAACGACGGCCAGTCAACCTATACTGGTACCGACAGG
PCR1 : Reverse primers (tagged with M13R)	
JB1-1	CAGGAAACAGCTATGACCAACTGTGAGTCTGGTGCCTTGCCAAAG
JB1-2	CAGGAAACAGCTATGACAACCTGGTCCCCGAACCGAAGG
JB1-3	CAGGAAACAGCTATGACAACAGTGAGCCAACCTCCCTCTCCAAAATA
JB1-4	CAGGAAACAGCTATGACCAGAGAGCTGGGTCCACTGCCAAAAAACA
JB1-5	CAGGAAACAGCTATGACAGAGTCGAGTCCCATCACCAAAATGC
JB1-6	CAGGAAACAGCTATGACCTGGTCCCATTCCCAAAGTGGAGG
JB2-1	CAGGAAACAGCTATGACAGCCGTGTCCCTGGCCCCGAAGAAC
JB2-2	CAGGAAACAGCTATGACCGTTTTTTGGAGAAGGCTCTAGGCTGACC
JB2-3	CAGGAAACAGCTATGACCAGCCGGTGCCTGAGCCAAAATAC
JB2-4	CAGGAAACAGCTATGACCGGGTCACGGCGCCGAAGTAC
JB2-5	CAGGAAACAGCTATGACAGCCGCGTCCTGGCCCCGAAG
JB2-6	CAGGAAACAGCTATGACCTGCCGGCCCCGAAAGTCAGG
JB2-7	CAGGAAACAGCTATGACCCTGGTGCCCGACCCGAAG
PCR2 and Sequencing: Forward and Reverse primers	
M13F	GTAAACGACGGCCAGT
M13R	CAGGAAACAGCTATGAC
PCR1 : HIV Env C3-V5	
OutC3F	CCTCAGGAGGGGACCCAGAA
OutV5R	TGGGTGCTACTCCTAATGGTTCA
PCR2 and Sequencing : HIV Env C3-V5	
InC3F	AGGGGAATTTTCTACTGTAA
InV5R	ACTTCTCCAATTGTCCCTCA