Proviral location affects cognate peptide-induced virus production and immune recognition of HIV-1-infected T cell clones.

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Supplementary Materials

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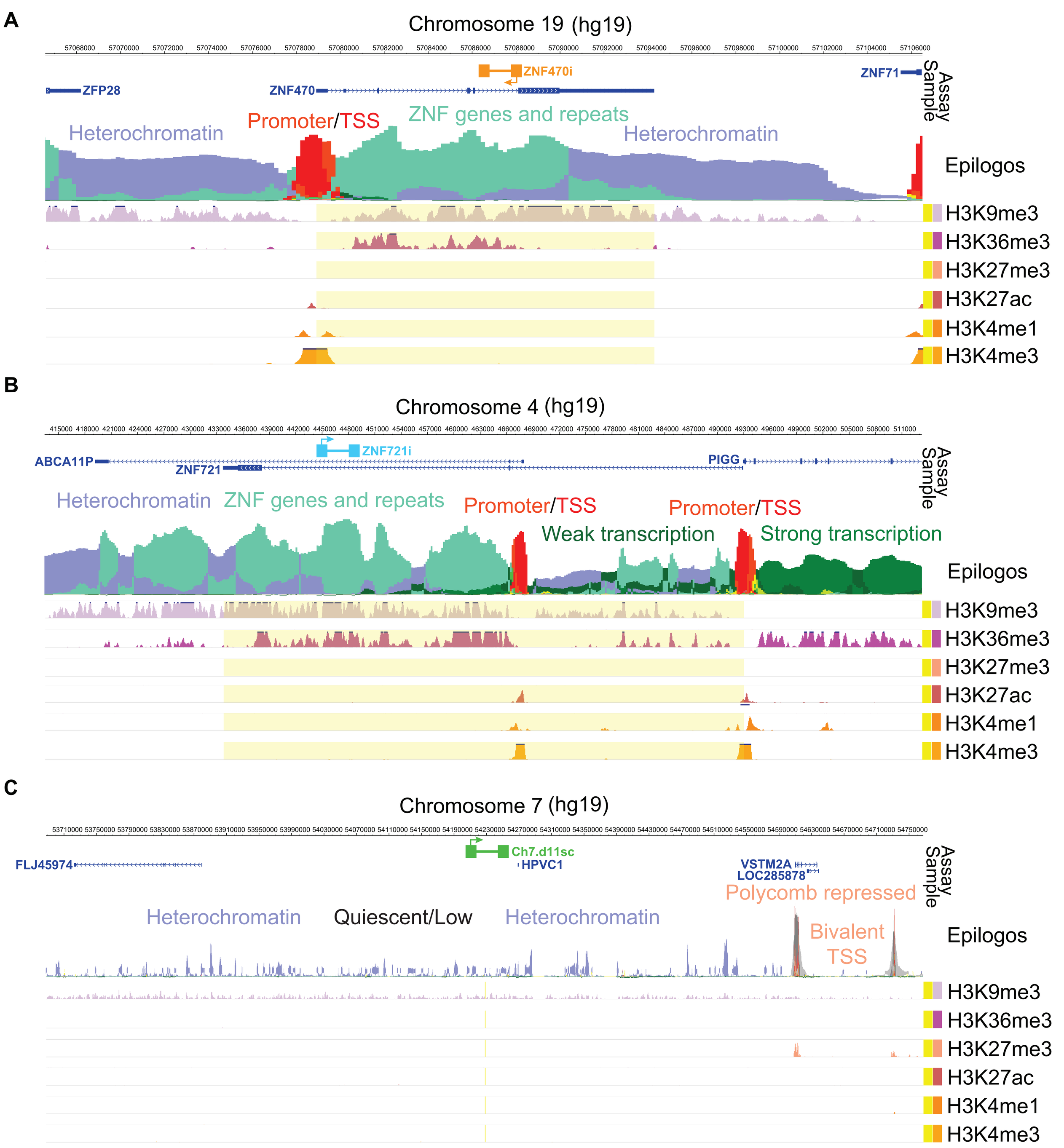
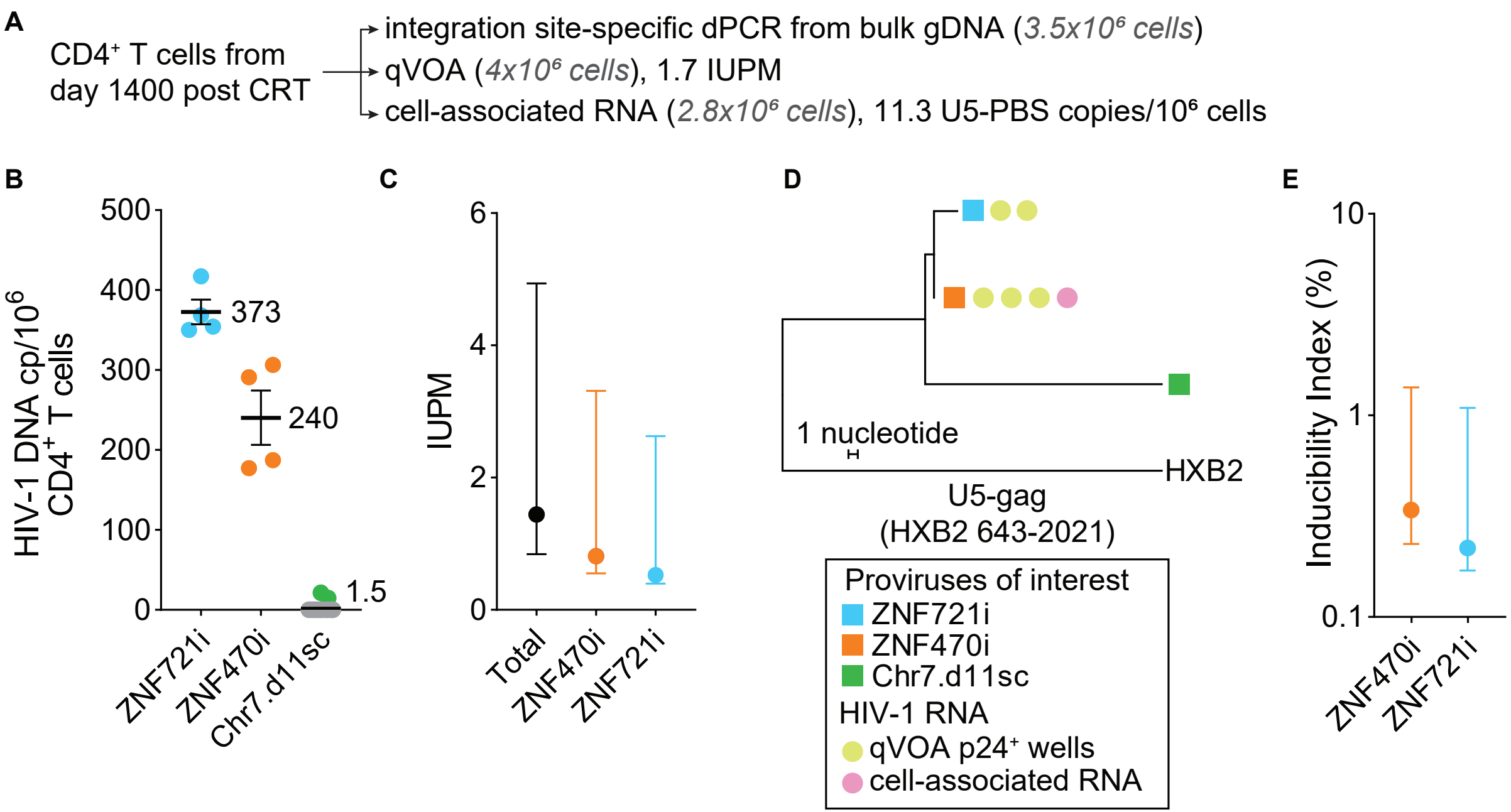
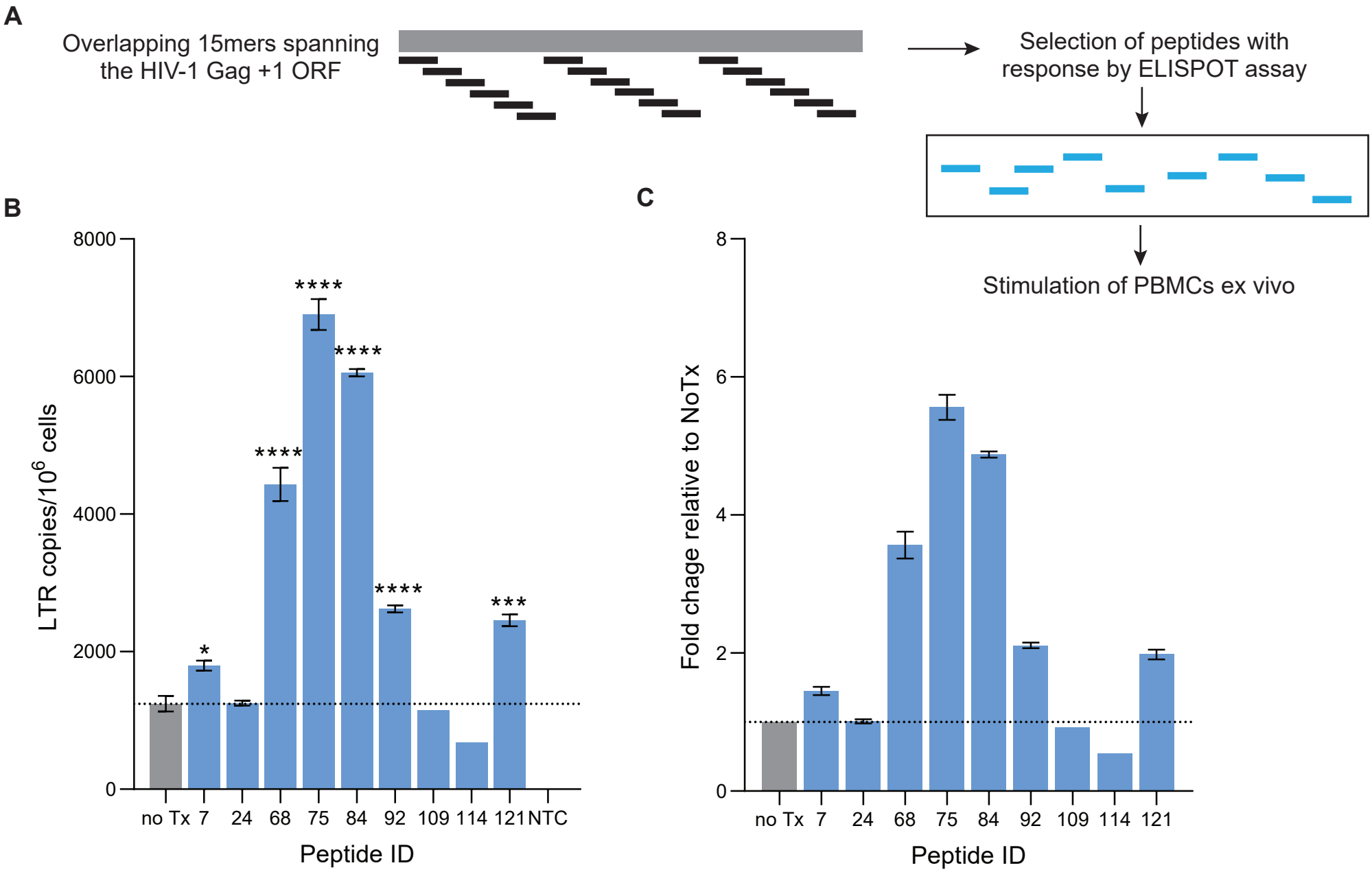


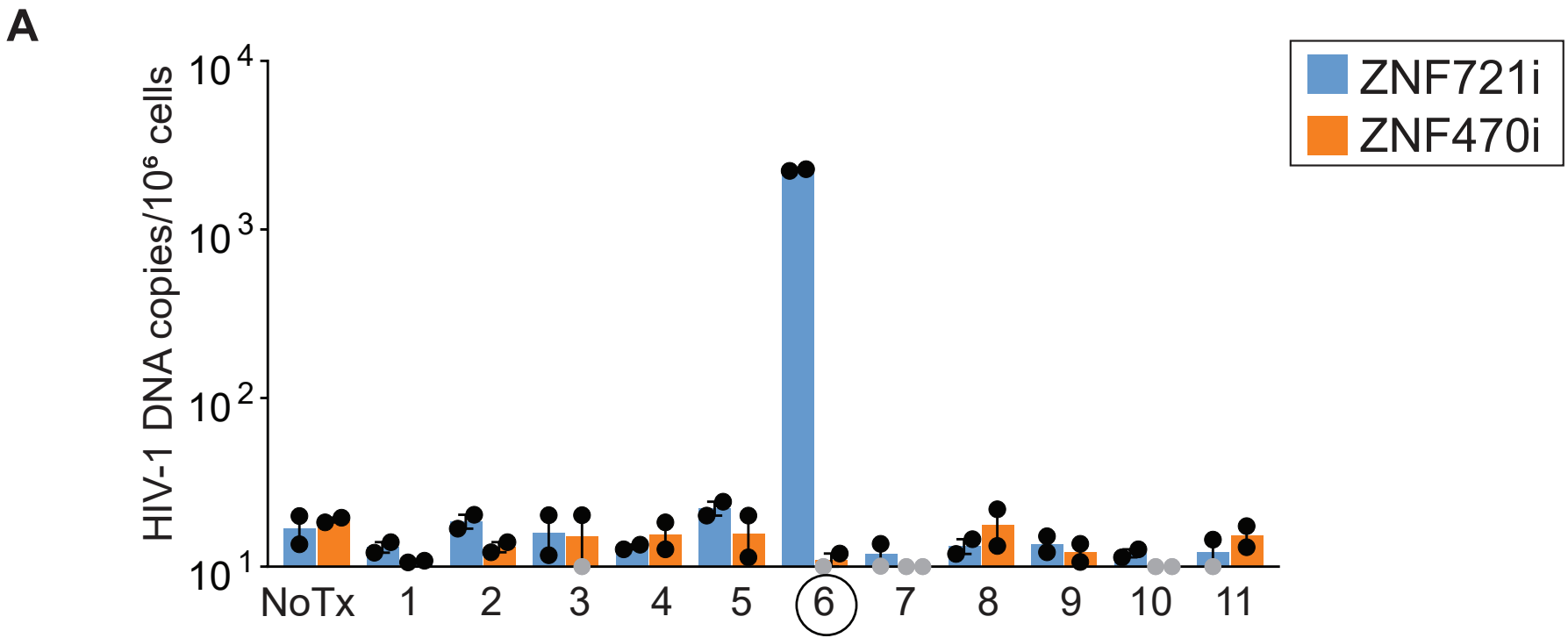
Supplemental Figure S1. Epigenetic signatures surrounding the proviruses of interest. CHIP-seq data from CD4⁺ T cells available through the ROADMAP Epigenomics Program database were visualized with Epigenome broswer and Epilogos (see methods); gene tracks show genes surrounding the proviruses of interest: ZNF470i (**A**), ZNF721i (**B**), and Chr7.d11sc (**C**); Epilogos were generated based on seven datasets from primary CD4⁺ T cells (E037, E038, E039, E040, E041, E042, E043); histone modification tracks from a representative sample (E043 Primary T helper cells from peripheral blood) are displayed below the Epilogos track.



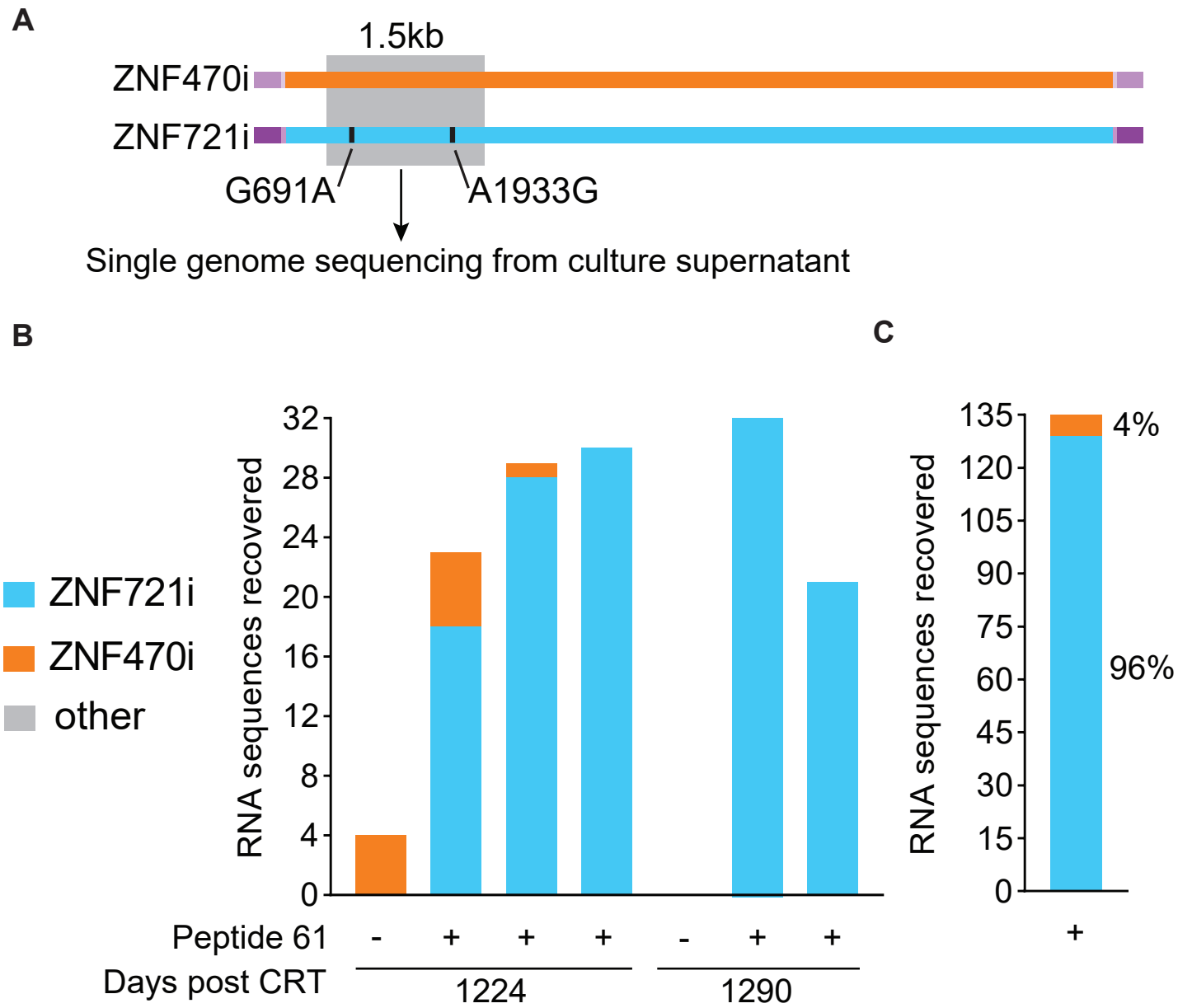
Supplemental Figure S2. Additional reservoir characterization of CD4⁺ T cells post chemoradiation. (**A**) Schematic of experimental design; CD4⁺ T cells were isolated from PBMCs collected from day 1400 post CRT; cells were used for quantitative viral outgrowth assay seeding 20 wells with 200,000 cells each; the remaining cells were used for the quantification of proviruses from gDNA, and isolation of cell-associated RNA from aliquots at limiting dilution for HIV-1 RNA positive cells; aliquots were screened for U5-PBS RNA and used as input for single genome sequencing; cells assayed in each method are indicated in grey. (**B**) Frequency of proviruses of interest measured by dPCR assays targeting the human-LTR junction; each symbol represents a replicate; error bars indicate mean and standard deviation; mean values are indicated in black; grey symbols indicate negative values. (**C**) Infectious units per million estimated from the qVOA; p24⁺ wells were sequenced, and IUPM were calculated for each variant matching proviruses of interest as in **D**; error bars indicate the 95% confidence intervals. (**D**) U5-gag maximum likelihood tree showing variants recovered from qVOA and cell-associated RNA, together with reference sequences of proviruses of interest. (**E**) Estimate of inducibility in vitro calculated by dividing the number of qVOA wells positive for a specific variant by the total number of its corresponding proviruses seeded in the qVOA experiment (based on their frequency as in **B**).



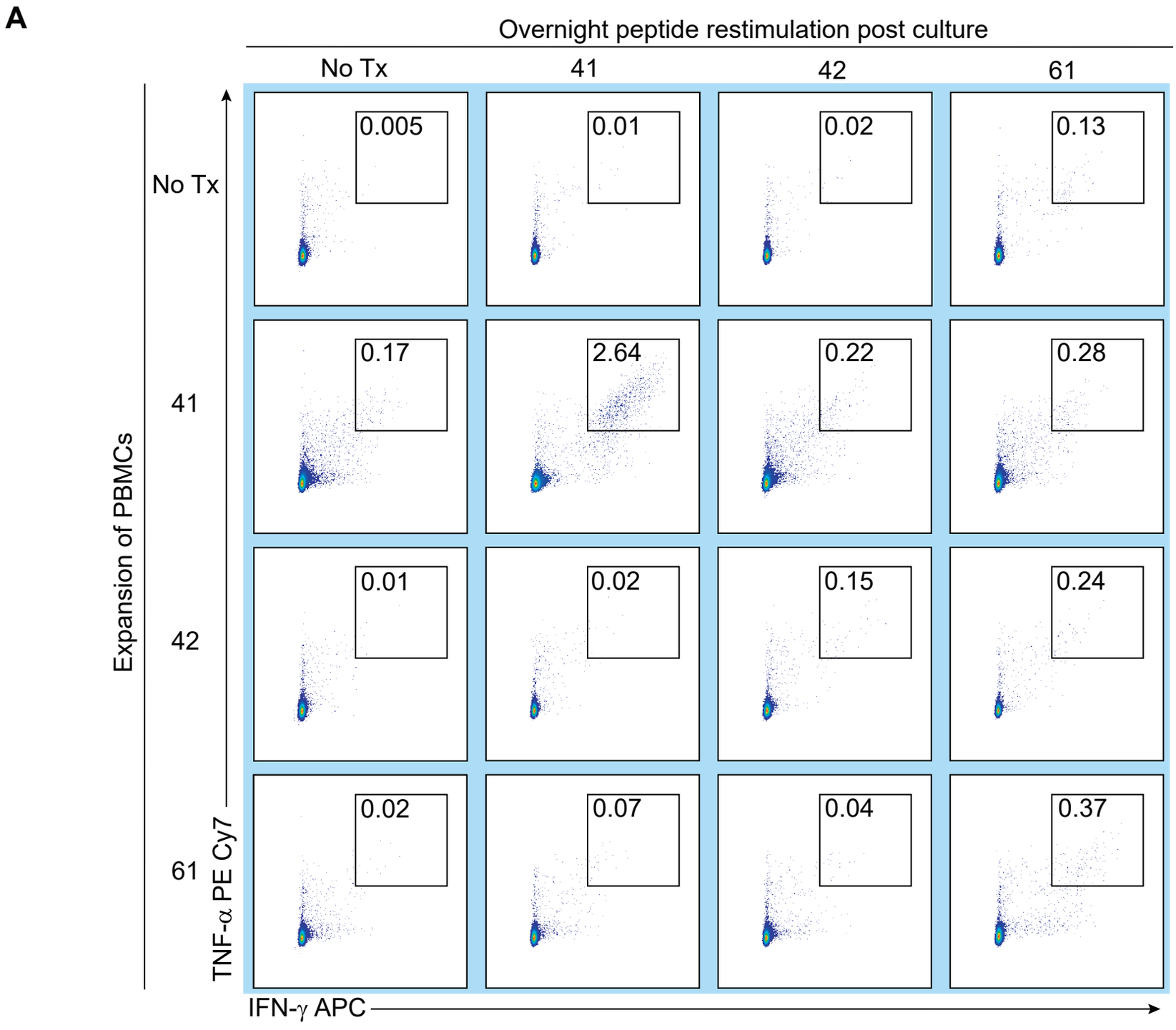
Supplemental Figure S3. Proliferation of HIV-1-infected cells upon stimulation with selected Gag peptides. (**A**) Schematic of experimental design; overlapping Gag peptides were selected based on response to elispot assay. (**B**) CD8-depleted PBMCs were stimulated in presence of individual peptides for 9 days; genomic DNA from total cells was used to quantify total HIV-1 LTR copies; no Tx indicate cells left untreated; NTC indicates water dPCR water control; dashed line indicates baseline HIV-1 DNA level in unstimulated cells; statistical significance was tested by one-way ANOVA; * $p<0.05$, ** $p<0.01$, *** $p<0.001$ **** $p<0.0001$. (**C**) Results expressed as fold-change relative to no treatment.



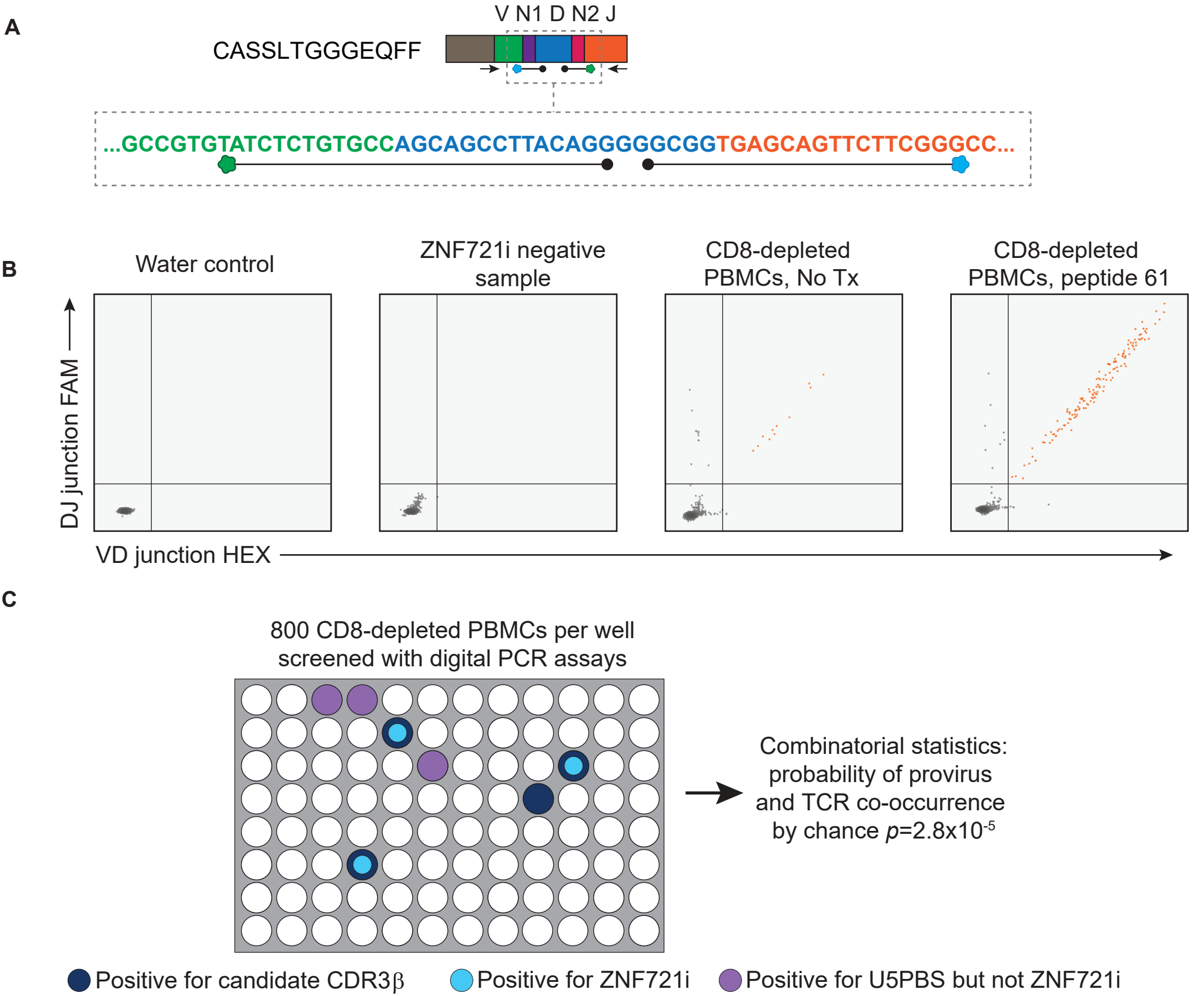
Supplemental Figure S4. Cells carrying ZNF470i are not reactive to Gag peptides. (**A**) CD8-depleted PBMCs were cultured for 9 days without treatment (no Tx) or upon addition of mini-pools of Gag peptides, each containing 10-11 overlapping peptides; genomic DNA was extracted from total cells at the end of culture to quantify copies of proviruses of interest harnessing the site of HIV-1 integration; each circle represents a dPCR technical replicate; grey circles indicate values below the limit of detection. Mini-pool number 6 is circled to indicate it led to a significant increase in ZNF721i copies.



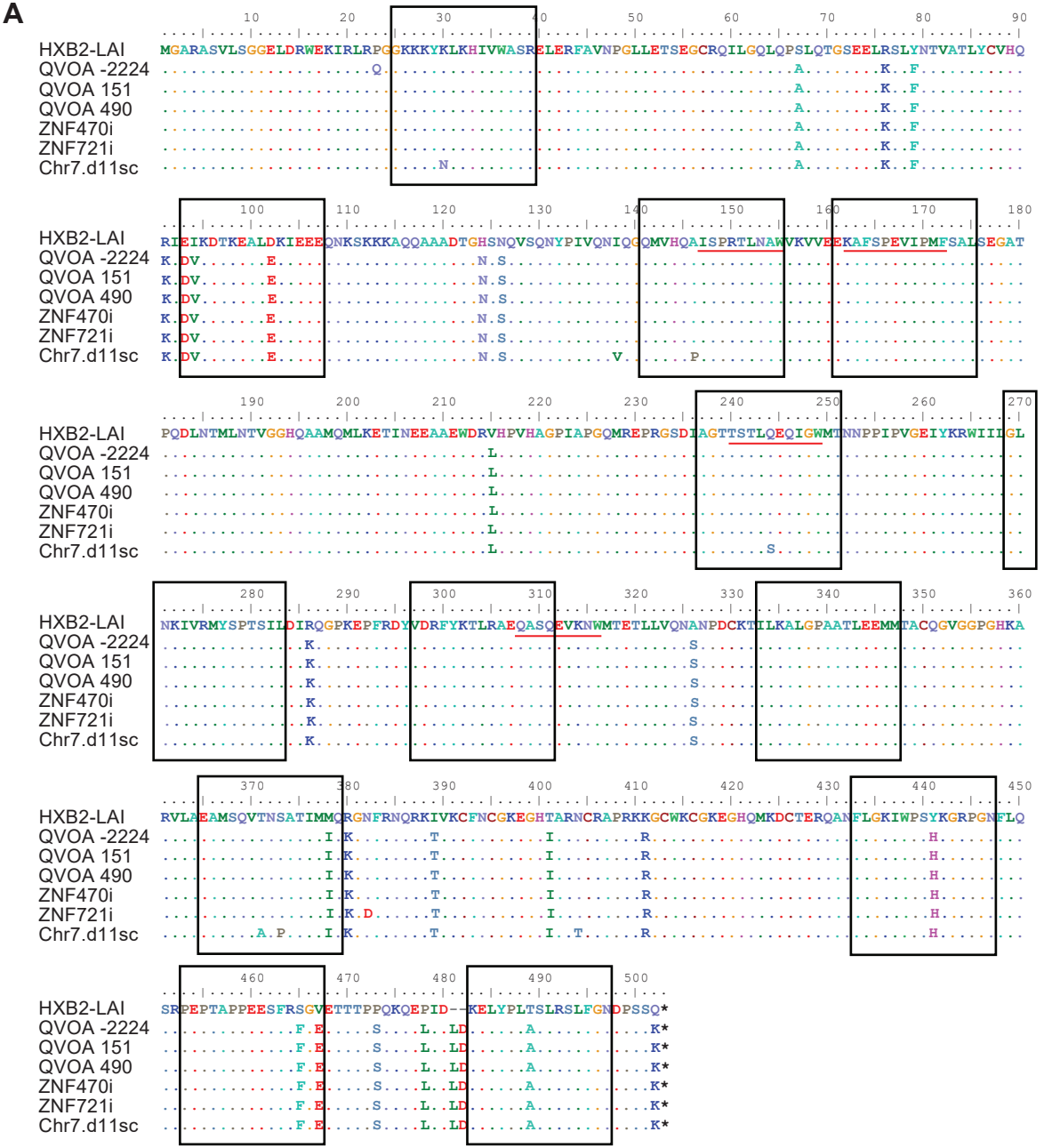
Supplemental Figure S5. Analysis of U5-gag sequence from culture supernatant, related to Figures 4 and 5. (**A**) Schematic of the sequencing approach to profile HIV-1 variants produced in response to CD8-depleted PBMC stimulation with peptide 61; the gray shaded area indicated the amplicon spanning between the 5'-leader and gag, which contains the two mutations that distinguish ZNF470i from ZNF721i. (**B**) Distribution of variants of interest in two control samples wihtout treatment (No Tx) and in 5 smaples treated with peptide 61. (**C**) Cumulative data from all 5 samples stimulated with peptide 61.



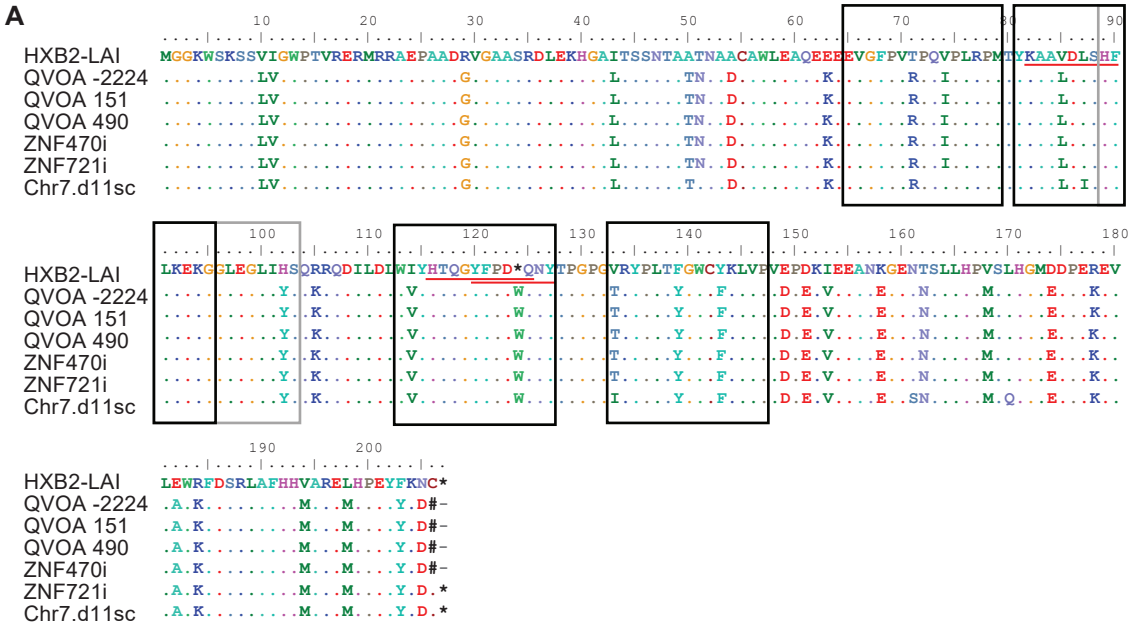
Supplemental Figure S6. Intracellular cytokine staining of CD8⁺ T cells stimulated with Gag peptides 41, 42, and 61. (**A**) Intracellular cytokine staining of CD8⁺ T cells re-stimulated with Gag peptides of interest after PBMC expansion; numbers within gates indicate the percentage of cells positive for both TNFα and IFNγ.



Supplemental Figure S7. Quantification of the CASSLTGGGEQFF clonotype by duplex VDJ-specific ddPCR and analysis of provirus-TCR co-occurrence. (**A**) Design of duplex strategy to specifically amplify the VDJ rearrangement of the CASSLTGGGEQFF CDR3β; probes were designed to anneal across the VDJ junction to exploit V, D, and J chain diversity, as well as junctional diversity (N1 and N2 nucleotides). (**B**) Representative 2D plots of droplet digital PCR reactions with water negative control, whole genome amplified DNA not containing the ZNF721i provirus, and DNA from $CD4^+$ T cells cultured for 10 days with no treatment, or stimulation with the cognate Gag peptide 61. (**C**) Co-occurrence analysis and conbinatorial statistics to determine whether ZNF721i and CASSLTGGGEQFF belong to the same cell clonotype.



Supplemental Figure S8. Gag protein sequence analysis of proviruses of interest. (**A**) Gag protein sequences were codon aligned to the HXB2 reference; black boxes indicate targeted epitopes; red lines indicate HLA-B*57–restricted epitopes; numbers next to QVOA sequences indicate days from the start of chemoradiation.



Supplemental Figure S9. Nef protein sequence analysis of proviruses of interest. (**A**) Nef protein sequences were codon aligned to the HXB2 reference; black boxes indicate targeted epitopes; grey box indicate overlapping epitope at positions 83-103; red lines indicate HLA-B*57–restricted epitopes; numbers next to QVOA sequences indicate days from the start of chemoradiation.

Supplemental Table S1. Characteristics of previously published proviruses integrated into ZNF721/ABCA11P.

Study		Provirus information							Participant characteristics							
Reference	Pubmed_id	Retrovirus Integration Database_id	Chromosome	Insert position*	Provirus orientation	*ZNF721* gene orientation	**Assays used**	**Evidence of intactness**	**Evidence of inducibility**	Participant id	Clinical notes	Age (years)	Sex	Race ethnicity	On ART	Years on ART	Plasma HIV RNA
This study			chr4	447418	+	-	NFL proviral sequencing, LM-PCR/ISA, QVOA	Yes	induced by cognate antigen via PBMC stimulation	ES24	EC on ART	60	M	African American	yes	3	suppressed
Guo 2022, Halvas 2020	36044447	rid36044447_23888	chr4	443311	+	-	NFL proviral sequencing, LM-PCR/ISA, QVOA	Yes	inducible by QVOA, source of NSV	F07	NSV	59	M	African American	yes	19	NSV
Guo 2022	36044447	rid36044447_23886	chr4	439930	+	-	LM-PCR/ISA		unknown								
		rid36044447_23887	chr4	440816	+	-											
		rid36044447_23890	chr4	446377	+	-											
Guo 2022, Halvas 2020	36044447	rid36044447_35276	chr4	425766	+	-	NFL proviral sequencing, LM-PCR/ISA, QVOA	Yes	inducible by QVOA	R09	NSV	73	M	Caucasian	yes	10	NSV
Guo 2022	36044447	rid36044447_35281	chr4	434674	-	-	LM-PCR/ISA		unknown								
		rid36044447_35282	chr4	441824	+	-											
		rid36044447_35284	chr4	441923	+	-											
		rid36044447_35286	chr4	462538	+	-											
		rid36044447_35294	chr4	488671	+	-											
		rid36044447_10322	chr4	425632	+	-				C03	NSV	43	M	Caucasian	yes	9	NSV
		rid36044447_10323	chr4	439826	+	-											
		rid36044447_10324	chr4	443536	+	-											
		rid36044447_10328	chr4	449724	+	-											
		rid36044447_10329	chr4	465001	+	-											
		rid36044447_10330	chr4	466440	+	-											
Eiunkauf 2019	30688658	rid30688658_36	chr4	442619	+	-	MIP-seq	Yes	unknown	P1		59	M		yes	11.5	suppressed
Jiang 2020, Lian 2021	32848246		chr4	444791	+	-	MIP-seq	Yes	unknown	P9/EC9	EC						suppressed
Lian 2021, Sun 2023	34910552		chr4	448830	+	-	MIP-seq, PHEP-seq	Yes	unknown	27/P5		62	M		yes	14	suppressed
Huang 2021	34636876	rid34636876_9	chr4	450716	+	-	Q4PCR, MIP-seq	Yes	unknown	5203		59	M	Caucasian	yes	21	suppressed
Coffin 2019	31217357	rid31217357_2486	chr4	443254	+	-	LM-PCR/ISA			CH62-1	Fiebig IV/V				yes	3.7	suppressed
Coffin 2019	31217357	rid31217357_3040	chr4	457831	+	-	LM-PCR/ISA			CH68-5	Fiebig IV/V				yes	3.6	suppressed
Bale 2021	33832973	rid33832973_8584	chr4	449365	+	-	LM-PCR/ISA		unknown	ZA011_preA	MTCT	0.8	F		no	0	viremic
Bale 2021	33832973	rid33832973_10090	chr4	439658	+	-	LM-PCR/ISA			ZA012_preA	MTCT	0.4	M		no	0	viremic
Bale 2021	33832973	rid33832973_10091	chr4	440131	+	-	LM-PCR/ISA										

Supplemental Table S2. Oligonucleotides.

Primers and probes used for specific and total virus quantification

Assay	Primer Name	PCR Reaction	Primer Type	Sequence (5'- 3')	Coordinates on HXB2	Reference
ZNF721i Quantification	721_IS_Fw	Digital PCR	Forward	TGTACTTTCCAAGATACCAGAA	52-71	This work
	721_IS_Rev	Digital PCR	Reverse	GTAGCCTTGTGTGTGGTAGA		
	721_IS_Probe	Digital PCR	Probe	5FAM/AGTATCTCT/ZEN/ATAGATACTGGAAGGGCT/3IABkFQ		
ZNF470i Quantification	470_IS_Fw	Digital PCR	Forward	5HEX/CTTATTTAT/ZEN/TAATTGGAAGGGCTAATTTACTC/3IABkFQ	52-71	This work
	470_IS_Rev	Digital PCR	Reverse	ATAACTTATCTGGGCTTTCCT		
	470_IS_Probe	Digital PCR	Probe	GTAGCCTTGTGTGTGGTAGA		
Chr7.d11sc competition assay	U5PBS_delvar_Fw	Digital PCR	Forward	CTAGAGATCCCTCAGACCCTTT	588-609	This work
	U5PBS_delvar_Rev	Digital PCR	Reverse	ATTYGGCGTACTCACCAGTC	739-758	
	delvar_PSP	Digital PCR	Probe	5HEX/CGAACAGGG/ZEN/ACTTGAAAACCAGAGGAGC/3IABkFQ	643-681	
	U5PBS_probe	Digital PCR	Probe	5FAM/AAAGGGAAA/ZEN/CCAGAGGAGCTCTCTCGACAC/3IABkFQ	663-692	
Chr7.d11sc Quantification	Chr7.d11sc_IS_Fw	Digital PCR	Forward	ATCTGAGACAAGGCTCAATCAA	58-89	This work
	Chr7.d11sc_IS_Rev	Digital PCR	Reverse	GTTCTGCCAATCAGGGAAGTA		
	Chr7.d11sc_IS_Probe	Digital PCR	Probe	5FAM/CAGGAGGTC/ZEN/CTAATTGGAAGGGCTAATT/3IABkFQ		
U5-R	RU5-F	Digital PCR	Forward	CTTAAGCCTCAATAAAGCTTGCC	517-539; 9601-9623	Anderson et al. (30253074)
	RU5-R	Digital PCR	Reverse	GGATCTCTAGTTACCAGAGTC	577-597; 9661-9681	
	RU5-probe	Digital PCR	Probe	5FAM/AGTAGTGTG/ZEN/TGCCCGTCTG/3IABkFQ	552-570; 9636-9654	

Primers and probes used for specific TCR quantification

Assay	Primer Name	PCR Reaction	Primer Type	Sequence (5'- 3')	
ZNF721i TCR Quantification	721TCR_Fw	Digital PCR	Forward	GCACACAGCAGGAGGAC	This work
	721TCR_Rev	Digital PCR	Reverse	GCTCACCCTCTCTCCCA	
	721TCR_Probe1	Digital PCR	Probe	5FAM/CCCGAAGAA/ZEN/CTGCTCACCGC/3IABkFQ	
	721TCR_Probe2	Digital PCR	Probe	5HEX/ATCTCTGTG/ZEN/CCAGCAGCCTTACAGG/3IABkFQ	

Primers used for cDNA synthesis, single genome sequencing (SGS) and sanger sequencing

Assay	Primer Name	PCR Reaction	Primer Type	Sequence (5'- 3')	Coordinates on HXB2	Reference
cDNA synthesis/SGS	ES24_gag RO	outer	Reverse	TTAGCCTGTCTCTCAGTACAATC	2062-2084	This work
SGS/sanger sequencing	ES24_gag RN	nested/sanger	Reverse	TCATTTGGTGTCCTTCCTTTCC	2038-2059	
SGS	Delvar_4187_FO	outer	Forward	TGGGTACCAGCGCACAAAG	4152-4170	
SGS/sanger sequencing	Delvar_4210_FN	nested/sanger	Forward	GAATTGGAGGAAATGAACAAGTAG	4171-4194	
SGS	Delvar_5716_RO	outer	Reverse	TAGATATGTTGCCCTAAGCTATG	5676-5698	
SGS/sanger sequencing	Delvar_5687_RN	nested/sanger	Reverse	TAGCCTAGGAAAATGTCTAACAGC	5646-5669	
SGS	U5_gag_FO	outer	Forward	GTARCTAGAGATCCCTCAGAC	583-603	
SGS/sanger sequencing	U5_gag_FN	nested/sanger	Forward	AAATCTCTAGCAGTGGCGCC	621-640	
SGS	env_FO	outer	Forward	GCCAGTAGTRTCAACYGAA	6979-6997	Simonetti et al. (33301425)
SGS/sanger sequencing	env_FN	nested/sanger	Forward	CTGCTAAATGGCAGTCTAGC	7001-7020	
SGS	env_RO	outer	Reverse	GCARATGAGTTTTCYAGAGCA	8015-8035	
SGS/sanger sequencing	env_RN	nested/sanger	Reverse	TTGCCTGGAGCTGYTTRATGC	7938-7958	
Sanger sequencing	INT3A2	sanger	Reverse	AGCTTCCTCATTGATGGTCTCTTT	1393-1416	
SGS	5CP1	outer	Forward	GAAGGGCACACAGCCAGAAATTGCAGGG	1981-2008	
SGS/sanger sequencing	2.5	nested/sanger	Forward	CCTAGGAAAAAGGGCTGTTGGAAATGTGG	2011-2039	
SGS	RT3.1	outer	Reverse	GCTCCTACTATGGGTTCTTTCTCTAACTGG	3830-3859	
SGS/sanger sequencing	RT3798R	nested/sanger	Reverse	CAAACTCCCACTCAGGAATCCA	3777-3798	
SGS	RT3597mixF	outer	Forward	AAAACAGGAAARTATGCAA	3597-3615	
SGS/sanger sequencing	RT3626F	nested/sanger	Forward	TGCCCACACTAATGATGTAA	3626-3645	Einkauf et al. (30688658)
SGS	SC05R	outer	Reverse	AGCTCTTCGTCGCTGTCTCCGCTT	5980-6003	
SGS/sanger sequencing	SC02R	nested/sanger	Reverse	CTTCCTGCCATAGGAGATGCCTA	5957-5979	
SGS	VP5450F	outer	Forward	CAGGACATAACAAGGTAGGATC	5450-5471	
SGS/sanger sequencing	VP5549F	nested/sanger	Forward	AGAGGATAGATGGAACAAGCCCCAG	5550-5574	
SGS	CO602	outer	Reverse	GCCCATAGTGCTTCCTGCTGCTCCCAAGAACC	7785-7816	
SGS/sanger sequencing	V3CR	nested/sanger	Reverse	TGCTCTTTTTTCTCTCTSCACCACT	7735-7759	

Primers used to confirm integration site

Assay	Primer Name	PCR Reaction	Primer Type	Sequence (5'- 3')	Coordinates on HXB2	Reference
ZNF470i	ZNF470_FO	outer	Forward	CAGTAGCGCTACTGTTTCTAATTC		This work
	ZNF470_FN	nested/sanger	Forward	CTACATATAACTTATCTGGGCTTTCC		
	ZNF470_RO	outer	Reverse	GTTACCCAGGCTGGAGTTGAA		
	ZNF470_RN	nested/sanger	Reverse	CACACCTGGCCCTACTTTCAGT		
ZNF721i	ZNF721_FO	outer	Forward	AATTTTATGTACTTTCCAAGATACCAG		This work
	ZNF721_FN	nested/sanger	Forward	TACCAGAAAAAAGTATCTCTATAGA		
	ZNF721_RO	outer	Reverse	TTTGTACTGATACACCTTTACTTCT		
	ZNF721_RN	nested/sanger	Reverse	GGCGTATCTGCTAGAGATTTTC		
Chr7.d11sc	Delvar_FO	outer	Forward	CCATGCCTGTTAGAGAGTGTG		This work
	Delvar_FN	nested/sanger	Forward	GAGACAAGGCTCAATCAATTTAG		
primers annealing to HIV	ES24_gag RO	outer	Reverse	TTAGCCTGTCTCTCAGTACAATC	2062-2084	Simonetti et al. (33301425)
	ES24_gag RN	nested/sanger	Reverse	TCATTTGGTGTCCTTCCTTTCC	2038-2059	
	env_FO	outer	Forward	GCCAGTAGTRTCAACYGAA	6979-6997	
	env_FN	nested/sanger	Forward	CTGCTAAATGGCAGTCTAGC	7001-7020	