Supplementary Table 1. T-cell receptor sequences of HERV-K(HML-2)-specific $CD8^+$ T cell clone.

alpha	ATGCTCCTGCTGCTCGTCCCAGTGCTCGAGGTGATTTTTACTCTGGGAGGAACCAGAGCC CAGTCGGTGACCCAGCTTGACAGCCACGTCTCTGTCTCTGAAGGAACCCCGGTGCTGCTG AGGTGCAACTACTCATCTTCTTATTCACCATCTCTCTTCTGGTATGTGCAACACCCCAAC AAAGGACTCCAGCTTCTCCTGAAGTACACATCAGCGGCCACCCTGGTTAAAGGCATCAAC GGTTTTGAGGCTGAATTTAAGAAGAGTGAAACCTCCTTCCACCTGACGAAACCCTCAGCC CATATGAGCGACGCGGCTGAGTACTTCTGTGTTGTGAGTACTCTCAAGATCATCTTTGGA AAAGGGACACGACTTCATATTCTCCCCAATATCCAGAACCCTGACCCTGCCGTGTACCAG CTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGATTCTCAA ACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACTGTGCTAGAC ATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTT GCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCA GAAAGTTCCTGTGATGTCAAGCTGGTCGAGAAAAGCTTTGAAACAGATACGAACCTAAA CTTTCAAAACCTGTCAGTGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAAT CTGCTCATGACGCTGCGGCTGTGGTCCAGCTGA
beta	ATGGGCACCAGCCTCCTCTGCTGGATGGCCCTGTGTCTCCTGGGGGCAGATCACGCAGAT ACTGGAGTCTCCCAGGACCCCAGACACAAGATCACAAAGAGGGGACAGAATGTAACTTT CAGGTGTGATCCAATTTCTGAACACAACCGCCTTTATTGGTACCGACAGACCCTGGGGCA GGGCCCAGAGTTTCTGACTTACTTCCAGAATGAAGCTCAACTAGAAAAATCAAGGCTGCT CAGTGATCGGTTCTCTGCAGAGAGGCCTAAGGGATCTTTCTCCACCTTGGAGATCCAGCG CACAGAGCAGGGGGACTCGGCCATGTATCTCTGTGCCAGCAGCATAGGCCCGTCTGAAG CTTTCTTTGGACAAGGCACCAGACTCACAGTTGTAGAGGACCTGAACAAGGTGTTCCCAC CCGAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCCACACCCAAAAGGCCACA CTGGTGTGCCTGGCCACAGGCTTCTTCCCTGACCACGTGGAGCTGAGCTGGTGGGTGAAT GGGAAGGAGGTGCACAGTGGGGTCAGCACGGACCCGCAGCCCCTCAAGGAGCAGCCCGC CCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTCTGGCA GAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAGAATGACGA GTGGACCCAGGATAGGGCCAAACCCGTCACCCAGATCGTCAGCGCCGAGGCCTGGGGTA GAGCAGACTGTGGCTTTACCTCGGTGTCCTACCAGCAAGGGGTCCTGTCTGCCACCATCC TCTATGAGATCCTGCTAGGGAAGGCCACCCTGTATGCTGTGCTGGTCAGCGCCCTTGTGTT GATG GCCATGGTCAAGAGAAAGGATTTCTGA

Supplementary Table 2. Results summary from IMGT/V-QUEST tool – alpha chain of HERV-K(HML-2)-specific CD8$^+$ T cell clone TCR.

Result summary:	**Productive TRA rearranged sequence** (no stop codon and in-frame junction)		
V-GENE and allele	Homsap TRAV8-2*01 F	score = 1360	identity = **100,00%** (273/273 nt)
J-GENE and allele	Homsap TRAJ30*01 F	score = 209	identity = 91,84% (45/49 nt)
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[26.17.34.11]	[6.8.8]	CVVSTLKIIF

Supplementary Table 3. Results summary from IMGT/V-QUEST tool – beta chain of HERV-K(HML-2)-specific CD8$^+$ T cell clone TCR.

Result summary:	**Productive TRB rearranged sequence** (no stop codon and in-frame junction)		
V-GENE and allele	Homsap TRBV7-9*03 F	score = 1375	identity = **100,00%** (276/276 nt)
J-GENE and allele	Homsap TRBJ1-1*01 F	score = 186	identity = 87,50% (42/48 nt)
D-GENE and allele by IMGT/JunctionAnalysis	Homsap TRBD1*01 F	D-REGION is in reading frame 1	
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[26.17.38.10]	[5.6.10]	CASSIGPSEAFF

Supplementary Table 4. HIV-1 viruses.

Clade/CRF	Catalog #	Reference ID	Full Length Accession	Coreceptor
A	2521	ELI	K03454	X4
A	11245	99KE_KNH1135	AF47605	R5
A	11248	00KE_KNH1209	AF457069	R5
A	11244	99KE_KNH1088	AF457063	R5
A	11247	00KE_KNH1207	AF47068	R5
B	11250	94US_33931N	AY713410	R5
B	7686	91_US1	AY173952	R5
B	7691	90TH_BK132	AY173951	X4
B	510	85US_Ba_L	AY713409	R5
C	11254	02ET_14	AY255825	R5
C	7697	89SM_145	AY713415	R5
C	11258	02_ET288	AY713417	R5
C	11253	98US_MSC5016	AY444801	R5
C	2900	93IN_101	N/A	R5
D	11259	98UG_57128	AF484502	R5
D	11263	00UG_J32228M4	AF484516	R5
D	10047	1165MB	N/A	Not tested
G	3187	G3	NA	R5
CRF02AG	11283	01CM_1475MV	AY371138	R5
CRF02AG	7685	91DJ_263	AF063223	R5

A	3512	HIV-2 7924A	N/A	Not tested
A	3513	HIV-2 60415K	N/A	Not tested
N/A	3060	SIVhu	N/A	Not tested
N/A	253	SIVmac251	N/A	Not tested

Supplementary Table 5. Quantitative PCR primers and probes.

	Forward Primer (5'-3')	Reverse Primer (5'-3')	Probe (5'-3')
HERV-K-HML-2-gag	TCGGGAAACGAGCAAAGG	GAATTGGGAATGCCCCAGTT	6FAM-CTCAGGCCCCACAAC-MGBNFQ
HERV-K-HML-2-pol	GGGAATGCTTAATAGTCCAACTATTTG	TGAAAACTTGTCTCTAACTGGTTGAAG	6FAM-CAGACTTTTGTAGCTCAAG-MGBNFQ
HERV-K-HML-2-env	GGGTACCTGGCCCCATAGA	CATCATCCCTTCTTCCTCAGGTT	6FAM-ATCGCTGCCCTGCC-MGBNFQ
HERV-K-HML-2-rec	GTGACACAAACCCCAGAGAGTATG	CTGCAGACACCATTGATACAATCA	6FAM-TGCTTGCAGCCTTG-MGBNFQ
TBP	AAGTTGGGTTTTCCAGCTAAGTTC	CATCACAGCTCCCCACCATAT	6FAM-TGGACTTCAAGATTCAG-MGBNFQ
PP1A	TCTGCCCACCTTACAGACC	GATCAAATCCGCCACCTCTA	N/A
GAPDH	TTGACCTCAGCTGCACATTC	AGGATGGTCTCGAGTGCTTG	N/A
β-Actin	CACAGGGGAGGTGATAGCAT	CACGAAGGCTCATCATTCAA	N/A

Supplementary Results.

Additional Characterization of HERV-K(HML-2)-Env-specific T cell clone.

Additional Characterization of T-cell Determinant.

The EC_{50} of the HERV-K(HML-2)-Env-specific T cell clone to the optimal epitope, at approximately 4μM, is considerably higher than that typically observed with virus-specific T cell responses. One possible explanation for this is that this T cell clone is of relatively low avidity. However it is also possible that this response was primarily raised against a distinct HERV-K-Env sequence containing mismatches with the consensus HERV-K(HML-2)-Env CIDSTFNWHQRI sequence. We assembled an alignment of HERV-K-Env sequences and selected the following variants of this sequence: CIDLTFNWQHRI, CIDSTFDWQHHI, CIDSTFDWQHYI, CIDSTSDWQHHI. Peptides corresponding to these sequences were manufactured, but

each of these completely failed to stimulate the clone (data not shown). This does not represent an exhaustive list of HERV-K variant sequences, however, and we feel that it may be important for future studies to identify precisely which subset of HERV-K insertions are induced upon HIV-1 infection.

A third possible explanation for the high EC_{50} of the clone recognizes is that it may recognize only a modified version of the determinant that is present at low abundance in our synthetic peptide. It is important to note that we can strictly rule out the possibility that the clone could be recognizing a minor foreign contaminating peptide by virtue of the consistent recognition of 6 different batches of the CIDSTFNWQHRI peptide (from two different manfacturers), by the logical fine-mapping of the epitope where any peptide containing the minimal epitope is recognized while shorter peptides are not, and by the very similar titration curves observed with different peptide preparations (ex. CIDSTFNWQHRILLV vs CIDSTFNWQHRI, Figure 3D). One possibility is that the clone recognizes the native peptide sequence but that this sequence is present at low abundance in the synthetic peptide. It has previously been demonstrated that sulfhydryl modification of cysteine is responsible for reducing the antigenicity of the influenza virus nuclear protein determinants NP_{39-47} and $NP_{218-226}$ and that replacing cysteine residues in these determinants with either serine or alanine resulted in substantial increases in antigenicity (1). Similarly, substitution of the cysteine residue in the lympocytic choriomeningitis virus T cell determinant KAVYNFATC with methionine, to give KAVYNFATM, results in a decrease in the EC_{50} from >5μM to 21nM by preventing the formation of cysteine dimers (2). We tested the effect of replacing the N-terminal cysteine in CIDSTFNWQHRI with either alanine, serine methionine or tyrosine

(AIDSTFNWQHRI, SIDSTFNWQHRI, MIDSTFNWQHRI and YIDSTFNWQHRI) on the antigenicity of the peptide. Substitution with either alanine, serine, or threonine completely abolished recognition by the HERV-K(HML-2)-Env-specific T cell clone, while the methionine-substituted peptide was recognized at a reduced level (data not shown). We further tested the response of the T cell clone to reduced (TCEP treated) CIDSTFNWQHRI peptide as well as oxidized dimeric peptide and observed lesser antigenicity of both of these modified peptides as compared to the untreated peptide (data not shown). Taken together, these data suggest that a modification of the cysteine residue in this peptide may be required for optimal immunogenicity – but that this is not simply related to redox state.

Next, we tested the possibility that a minor species of the CIDSTFNWQHRILLV peptide bearing an unknown modification is the primary T cell determinant recognized by the HERV-K(HML-2)-Env specific T cell clone, 50mg of crude peptide was divided into 60 fractions by C18 reversed-phase HPLC (JPT Peptide Technologies) and each fraction was tested for its ability to stimulate the T cell clone in IFN-γ ELISPOT assays. The T cell clone responded maximally to fraction 8 with lesser responses to fractions 9-11 and no response observed to later fraction. This overlapped only partially with the elution profile of the native CIDSTFNWQHRILLV peptide (identified by a 923 mw/z peak) that was the primary product in fractions 8 – 18 (data not shown). The elution of CIDSTFNWQHRILLV peaked at fractions 12 and 13, while the clone did not respond to these fractions (data not shown). These data indicate that the T cell determinant of the HERV-K(HML-2)-Env specific T cell clone is a modified version of CIDSTFNWQHRILLV, which is slightly less hydrophobic than the native peptide (elutes

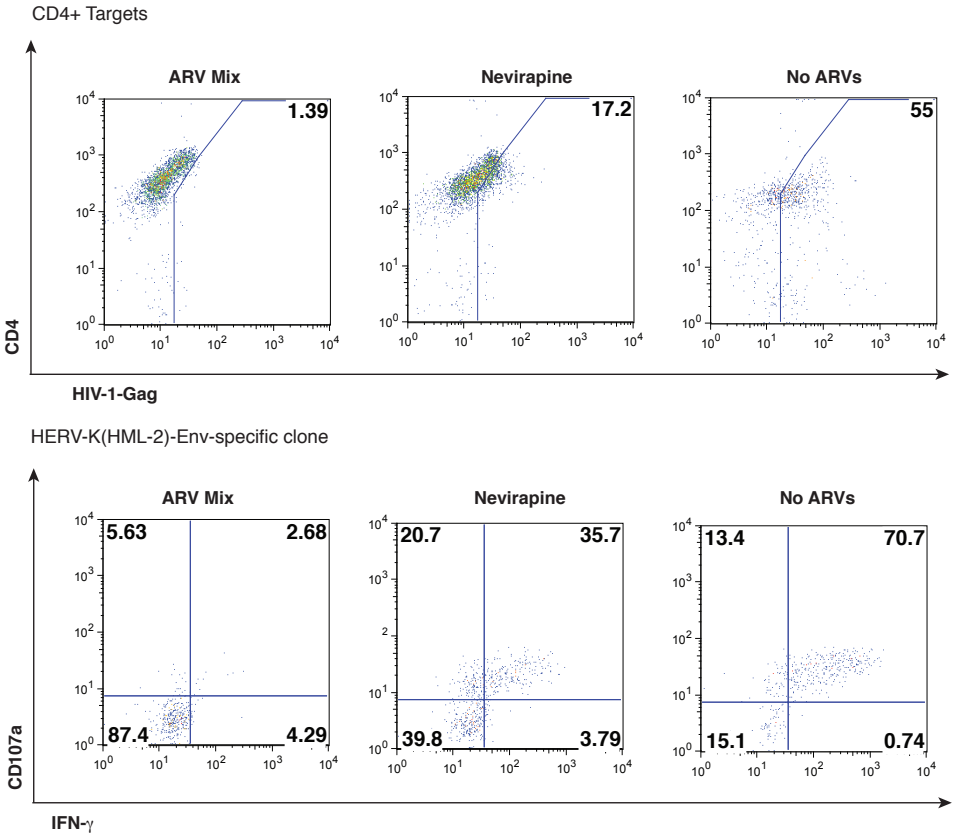
earlier). Peptide titration experiments performed on fraction 8 demonstrated an EC_{50} of 2μM, only slightly lower than the 4μM of the previously tested >98% pure CIDSTFNWQHRILLV. This corresponds with the observation that fraction 8 does not represent an isolated modified species but rather is still comprised primarily of native peptide. In fact, no unique peak could be observed in fractions 8-11 on the corresponding mass spectra, suggesting that the T-cell agonist is still present as only a minor component in these fractions. This has prevented us from using these data to identify the peptide modification needed for antigenicity. Deamidation of asparagine or glutamine residues represents one common peptide modification that has been shown to alter antigenicity(3). We therefore tested whether demamidation of the asparagine (N) residue in CIDSTFNWQHRI to asparatate or iso-aspartate (CIDSTFDWHQRI or CIDSTF-iso-Asp-WQHRI) resulted in a more antigenic peptide. Both of these modified peptides completely failed to stimulate the HERV-K(HML-2)-Env-specific T-cell clone (data not shown).

Taken together, these data indicate that the HERV-K(HML-2)-Env-specific T-cell clone is remarkably specific for its cognate determinant, as a number of very subtle changes in the peptide sequence abolished recognition. The peptide fractionation experiment clearly demonstrates that the unmodified CIDSTFNWQHRILLV peptide is not the determinant recognized by the clone – however, we have been unable to identify the precise identity of the modification required to render this peptide antigenic. The fact that the exact chemical composition of determinants recognized by T-cells is generally unknown has been highlighted by others (4)

Recognition of low-level HIV-1 infections at 16 hours post-infection by HERV-K(HML-2)-Env-specific T-cells.

The majority of recognition assays presented in this manuscript tested the ability of HERV-K(HML-2)-Env-specific T-cells to recognize high-levels of HIV-1 infection. One of the side observations from the ARV suppression recognition assay presented in Figure 6 was that even the small amount of HIV-1 infection that occurred in nevirapine-treated cells was sufficient to induce measurable recognition by the T-cell clone, although this was reduced as compared to cells infected to high-levels in the absence of ARVs. We repeated this experiment and again observed that even a low-level HIV-1 infection – defined by both a low frequency of Gag^+ cells and low-levels of Gag expression on a per-cell basis – was sufficient to trigger robust recognition by the HERV-K(HML-2)-Env-specific T-cell clone. Also of note, the co-culture between clone and target was initiated at 16 hours post-infection. The addition of brefledin A at the time of co-culture would have blocked additional MHC-I antigen presentation over the course of this co-culture. Thus, low levels of HIV-1 infection are detectable by HERV-K(HML-2)-Env-specific T-cell clones within at least 16 hours of infection.

Supplementary Figure 1

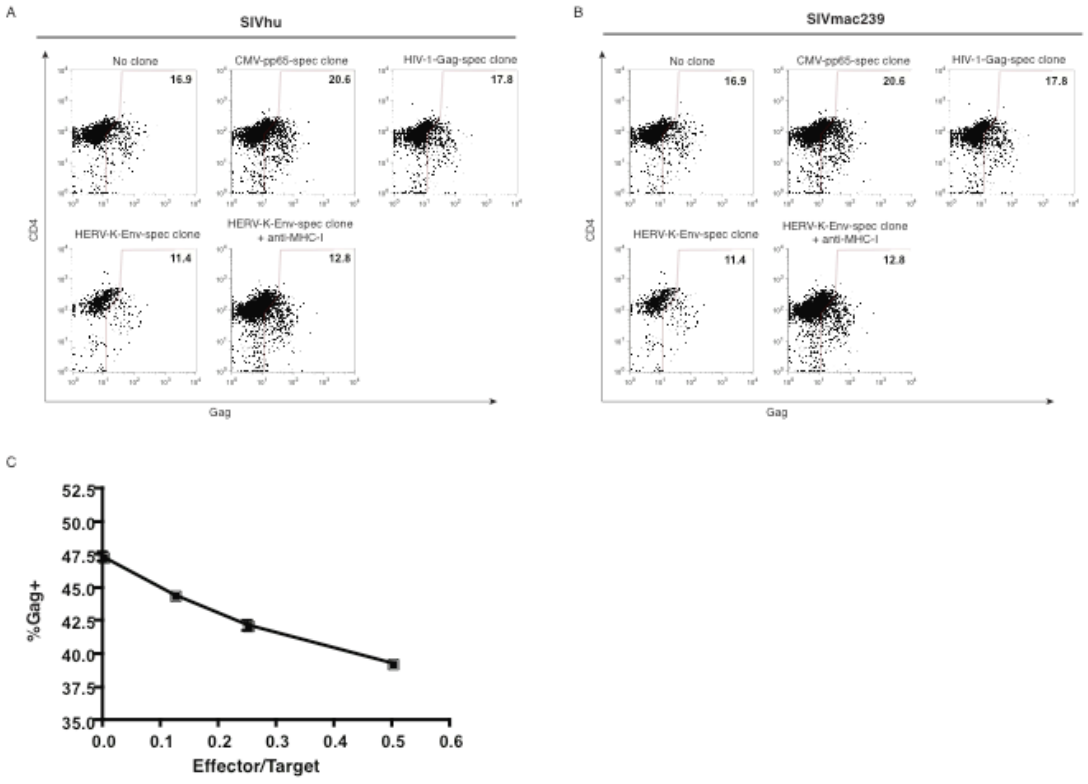


Supplementary Figure 1. HERV-K(HML-2)-Env-specific T-cells recognize low-levels of HIV-1 infection within 16 hours of infection. Primary $CD4^+$ T-cells autologous to the HERV-K(HML-2)-Env-specific T-cell clone were treated with either nevirapine, a mix of ARVs (see Figure 6 legend), or maintained as no ARV controls. These cells were subsequently infected with HIV-1. At 16 hours post-infection, these infected targets were co-cultured with the HERV-K(HML-2)-Env-specific T-cell clone in the presence of brefeldin A for 12 hours. Shown are flow cytometry plots gated on $CD8^+$ cells and depicting CD107a by IFN-γ.

Elimination of SIV-infected cells by HERV-K(HML-2)-Env-specific T-cells.

Here we present additional data supporting that the HERV-K(HML-2)-Env-specific T-cell clone specifically eliminates SIV-infected cells. The percentage of SIV-infected primary $CD4^+$ T-cells was substantially reduced upon co-culture with HERV-K(HML-2)-specific, but not with CMV-pp65- or HIV-1-Gag-specific $CD8^+$ T-cell clones (Supplementary Figure 2A, B). Pretreatment of these target cells with the anti-MHC-I antibody DX17 reduced this effect. We also observed dose-dependent elimination of SIVmac251-infected targets upon the co-culture with different clone (effector) to target ratios of HERV-K(HML-2)-Env-specific T-cell clone (Supplementary Figure 2C).

Supplementary Figure 2



Supplementary Figure 2. HERV-K(HML-2)-Env-specific T-cells eliminate SIV-infected cells. Primary CD4+ T-cells from subject OM9 were infected with the indicated SIV viruses. Infections were allowed to proceed for 72 hours and then either CMV-pp65-, HIV-1-Gag, or HERV-K(HML-2)-Env-specific T-cell clones were added at a ratio of 1 clone:10 targets (**A, B**) or at the indicated effector (clone) to target ratios and cultured for 24 hours. Where indicated, these target cells were pre-incubated with the anti-MHC-I antibody DX17 at 10μg/ml prior to the addition of T-cell clone. (**C**) SIVmac239-infected target cells were co-cultured with the HERV-K(HML-2)-Env-specific T-cell clone at the indicated effector (clone) to target ratios. Levels of infection were measured by flow cytometry staining for CD4 and intracellular HIV-1-Gag.

Supplementary References.

1. Chen, W., Yewdell, J.W., Levine, R.L., and Bennink, J.R. 1999. Modification of cysteine residues in vitro and in vivo affects the immunogenicity and antigenicity of major histocompatibility complex class I-restricted viral determinants. *J Exp Med* 189:1757-1764.
2. Puglielli, M.T., Zajac, A.J., van der Most, R.G., Dzuris, J.L., Sette, A., Altman, J.D., and Ahmed, R. 2001. In vivo selection of a lymphocytic choriomeningitis virus variant that affects recognition of the GP33-43 epitope by H-2Db but not H-2Kb. *J Virol* 75:5099-5107.
3. Arentz-Hansen, H., Korner, R., Molberg, O., Quarsten, H., Vader, W., Kooy, Y.M., Lundin, K.E., Koning, F., Roepstorff, P., Sollid, L.M., et al. 2000. The intestinal T cell response to alpha-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. *J Exp Med* 191:603-612.
4. Yewdell, J.W. 2005. The seven dirty little secrets of major histocompatibility complex class I antigen processing. *Immunol Rev* 207:8-18.