SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell Culture

PBMCs, HIV-specific CD8+ T cell clones or primary human CD8+ T cells isolated from peripheral blood were cultured in RPMI 1640 (Sigma) supplemented with 10% Fetal Bovine Serum (FBS, Hyclone), 1% Pen/Strep (Sigma), L-glutamine (Sigma) and with IL-2 as indicated. 293T cells used to generate lentivirus particles were maintained in DMEM (Sigma) supplemented with 10% FBS and L-glutamine.

Flow Cytometry and Live Cell-Sorting

Panels for individual experiments were assembled from the following antibodies which were used for cell surface staining: anti-CD3 Alexa 700 (UCHT1, BD Biosciences), anti-CD8 APC-Cy7 (SK1, BD Biosciences), anti-PD-1 PE-Cy7 (EH12.1, BD Biosciences), anti-CD95 PerCP-Cy5.5 (DX2, BD Biosciences), anti-CD45RA BV421 (HI100, Biolegend), anti-CCR7 PE-Cy7 (3D12, BD Biosciences), anti-CD27 BV605 (O323, Biolegend). For dextramer staining, cells were first stained with Live/Dead fixable Violet Dead Cell Stain (L34955; Molecular Probes), followed by incubation with APC-labeled MHC class I–peptide dextramer complexes specific for KK10, KF11, SL9, FL8 or CMV pp65 NV9 (Immunodex) for 30 min at RT. Staining for caspase-8 and cell necrosis was accomplished using the Vybrant FAM caspase-8 assay kit (V35119; Molecular Probes) and Sytox green reagent (S7020; Molecular Probes) according to the manufacturer's instructions. Cells were then washed and fixed in 1%

paraformaldehyde and were acquired on an LSR II flow cytometer (BD Biosciences) equipped with 488, 405, and 633 excitation lines, using FACS Diva software (BD Biosciences). FMOs for caspase-8 staining (FAM group), PD-1 staining, and CD8 activation markers CCR7, CD45RA, and CD27 were collected. Data analysis was performed with the FlowJo software package (TreeStar), using FMOs to determine gating where necessary. Live sorting was performed on a BD FACSAria IIu cell sorter (BD Biosciences) equipped with 488, 405, and 633 excitation lines and a 70um nozzle at 70 psi, housed in a Bioprotect II biosafety cabinet (The Baker Company).

DAPPLE Analysis

DAPPLE (Disease Association Protein-Protein Link Evaluator) analysis was performed as described previously (Rossin et al., 2011) using default inputs http://www.broadinstitute.org/mpg/dapple/dapple.php).

Quantitative RT-PCR

cDNA was generated from mRNA using SuperScript III Reverse Transcriptase kit (Invitrogen) according to the manufacturer's instructions. Gene expression assays for caspase-8 and GAPDH were carried out using Taqman gene expression assays (4331182, 4448484; Life Technologies) on a Roche Lightcycler 480.

Gene Ontology Analysis

Gene ontology analysis was performed using GOrilla (http://cbl-gorilla.cs.technion.ac.il/) to calculate the overrepresented gene ontology terms (http://www.geneontology.org).

Confocal Microscopy

HIV-specific CD8+ T cell clones were incubated for 30 min with immobilized anti-CD3 (OKT3 clone, eBiosciences), anti-CD28 (CD28.2 clone, BD Biosciences), anti-Fas antibody (CH11 clone, EMD Millipore) or mouse IgG1 Isotype control (MOPC-21, BD Biosciences) coated on Poly-L-Lysine coated coverslips. Confocal images were collected on a Zeiss LSM510 Meta Confocal microscope with a plain apochromat 63x oil-immersion objective with a numerical aperture of 1.4. Differential interference contrast images were collected simultaneously with the fluorescence images. Multi-track acquisition mode was used to avoid crosstalk between fluorophores. Images were analyzed with Imaris software (Bitplane).

Imagestream Analysis

Patient samples were analyzed by Imagestream 100 (Amnis) multispectral imaging flow cytometry system equipped with 488, 658, and 405 laser sources with variable laser power and a brightfield laser source. After staining purified CD8+ T cells with FAM-LETD-FMK and APC-labeled dextramers, cells were transferred to 500 μl siliconized microcentrifuge tubes (Sigma). Files of single-

stained control cells (200-500 events) were collected and used to compensate fluorescence spectral overlap between fluorescent channels and determine the optimal laser power to avoid saturation of the camera. Cellular samples were gated for single cell population using the area and aspect ratio features to eliminate debris (low area) and multi-cellular events (large area, high aspect ratio) as described before (Ponomarev et al, 2011). A mask representing the cell membrane was defined by the brightfield image and an internal cytoplasmic mask was defined by eroding the whole cell mask, resulting in the area smaller than the cell membrane (Figure S7B). The gating of positively stained cells was performed based on their pixel intensity. The samples were acquired as files 10,000-20,000 cells size and analyzed for the expression of markers and proteins and mean fluorescent intensity (MFI) by the Image Data Exploration and Analysis Software (IDEAS; Amnis).

shRNA Lentiviral Vectors and Transduction

Isolation of CD8+ T cells was performed using magnetic anti-CD8+ beads and the MACS cell separation system (Miltenyi Biotec) according to the manufacturer's instructions. All cell enrichment procedures were conducted by positive selection. Lentiviral vectors encoding shRNA hairpin sequences targeting RIPK3 or a scrambled sequence control, as well as a puromycin resistance cassette in the pLKO.1 backbone, were obtained from The RNAi Consortium (TRC, http://broadinstitute.org/rnai/trc) of the Broad Institute (Cambridge, MA). Lentivirus-containing supernatant was generated as described

previously (Pertel et al., 2011) via a lipid-based delivery system using Lipofectamine2000 (Invitrogen) and 293T cells.

For lentiviral transduction of primary human CD8+ T cells, cells were placed at a concentration of 2 x 10^5 cells/well in 96 well plates previously coated with 2 μg/ml of antibodies against CD3 (OKT3 clone, eBiosciences) and CD28 (CD28.2 clone, BD Biosciences) antibodies in media comprised of RPMI 1640 supplemented with 10% FBS, 1% of pen/strep, L-glutamine and HEPES in the presence of 500 U/mL of recombinant human IL-2 (Roche Diagnostics). Following 2d of stimulation, cells were counted and placed in wells of 6 well plate at a concentration of 2 x 10^6 cells/well with lentivirus-containing supernatant at a multiplicity of infection (MOI) of 1. Lentivirus-containing supernatant was removed and replaced with fresh media supplemented with 500 U/mL of IL-2 after 2-3d. Following transduction, puromycin (5 μg/mL) containing media was added for selection, which was allowed to occur for a total of 5 days. All subsequent experiments with lentivirus-transduced T cells were conducted following a 2d rest period in the absence of IL-2.

SDS-PAGE

Protein samples were prepared by diluting with 4X NuPAGE LDS sample buffer (Invitrogen) containing 2X NuPAGE sample reducing buffer (Invitrogen) and boiling at 95^{o}C for 5 min. NuPAGE 4-12% Bis-Tris gels (Invitrogen) were run for 70 min at 150 V in 1X NuPAGE MES SDS running buffer (Invitrogen) in the

NuPAGE gel apparatus. Protein bands were visualized by staining using Simply Blue SafeStain for 30 min and destaining in water.

Western Blotting

Unstained gels were soaked in western semi-dry transfer buffer (0.039 M glycine, 0.048 M Tris, 0.0375% (w/v) SDS) for 10 min. Immobilon-P PVDF transfer membranes (Millipore) were briefly soaked in methanol for 30s, followed by 15 min in semi-dry transfer buffer. The gel and membrane were assembled in semi-dry blotting apparatus (Bio-Rad) and electrophoretic transfer of proteins was carried out at a constant current of 1.0 mA/cm^2 for 16 h at room temperature (RT). After transfer, the membrane was blocked by overnight incubation with 5% milk in PBST (PBS with 0.05% (v/v) Tween 20) at 4^oC. After washing membranes three times with 10 mL PBST for 5 min, antibodies at their appropriate dilution were incubated for 2h at room RT. The membrane was washed and then incubated for 1 h at RT with secondary antibody conjugated with horseradish peroxidase. Unbound antibody was washed away and antibody-antigen complexes were detected using ECL reagents (Amersham Biosciences). The specific antibodies utilized in this study included a rabbit polyclonal anti-RIPK3 antibody (Imgenex; IMG-5846A) and a rabbit monoclonal anti-GAPDH antibody (Cell Signaling Technologies; D16H11).

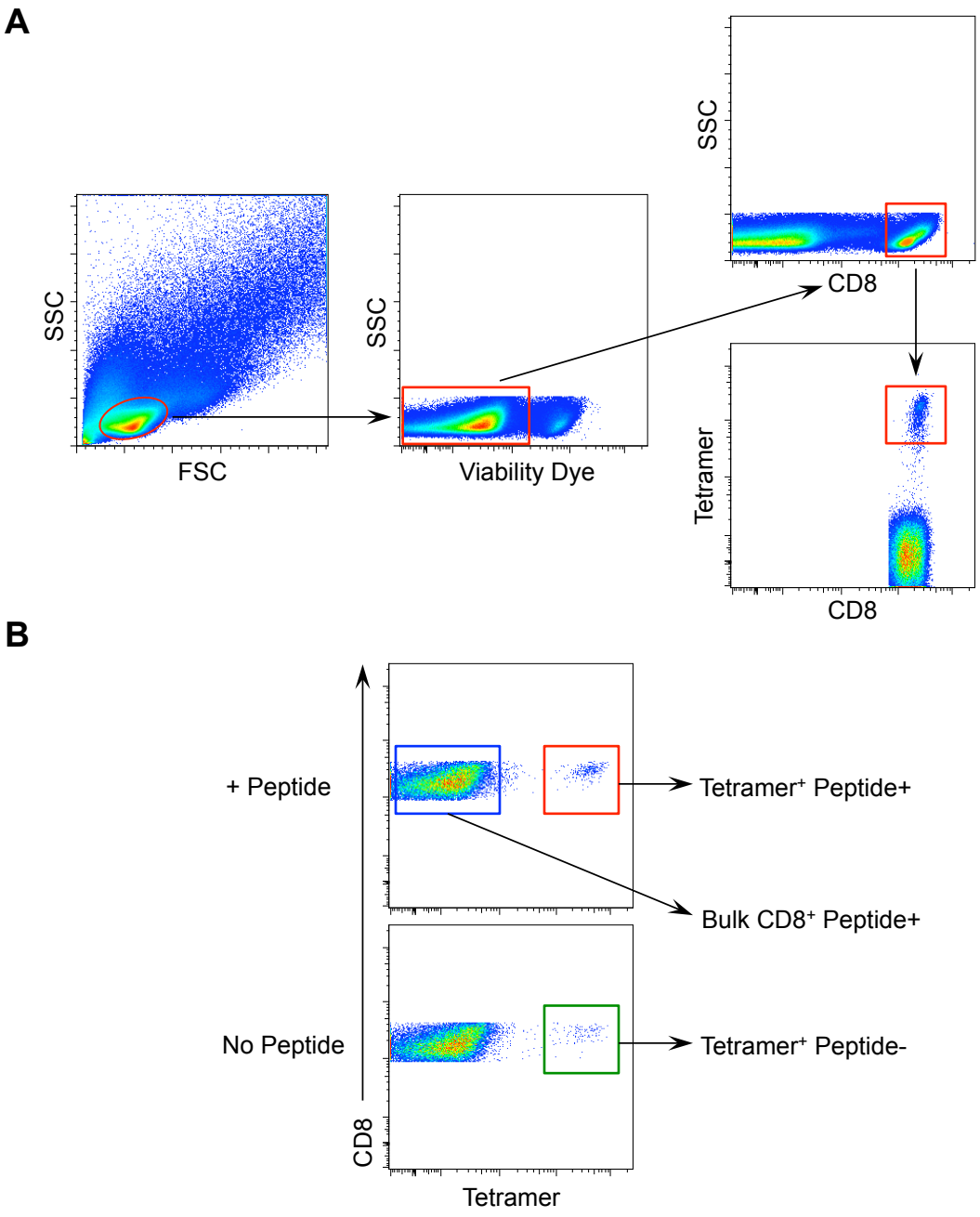

Figure S1, related to Figure 1 and Experimental Procedures: Flow cytometry gating strategy and sorting strategy of HIV-specific CD8⁺ T cells. (A) Whole PBMCs were stained with HIV tetramer, a viability dye and anti-CD8 antibody. Frequency of tetramer⁺ CD8⁺ T cells was determined by gating on viable CD8⁺ T cells. (B) PBMCs were incubated in the presence or absence of KK10 peptide (10 ng/mL) for 6 days. Cells were stained with B*2705 KK10-tetramer and anti-CD8 antibody and three cell subsets (Tetramer+ Peptide-Stimulated, Tetramer+ Non-Peptide Stimulated, and Bulk CD8 T cells) were isolated for the four B*2705 controller and four B*2705 progressors presented in Figure 1A. Total RNA was harvested for each cell subset (24 total) and analyzed by transcriptional profiling.

	HIV Controllers	Chronic Progressors
Number of Subjects	4	4
Gender Male (%)	3 (75)	4 (100)
Female (%)	1 (25)	0 (0)
Plasma HIV RNA, copies/mL Mean (Range)	Below Detection (<49)	39,042 (3,874-67,600)
CD4+ cell count, cells/mm^3 Mean (Range)	863 (328-1,238)	370 (153-565)
Duration of HIV diagnosis **Years**, Mean (Range)	10.75 (8-15)	8.5 (2-17)
KK10 Tetramer+ CD8+ T cells **%**, Mean ± SEM	1.73 ± 0.95	2.29 ± 0.67
KK10 viral sequence WT	3	4
Mutant	1 (L6M)	0

Table S1, related to Figure 1: Patient characteristics of HLA-B*2705$^+$ elite controllers and chronic progressors selected for cell sorting and transcriptional profiling. Clinical and molecular features of the patients utilized for our transcriptional profiling studies.

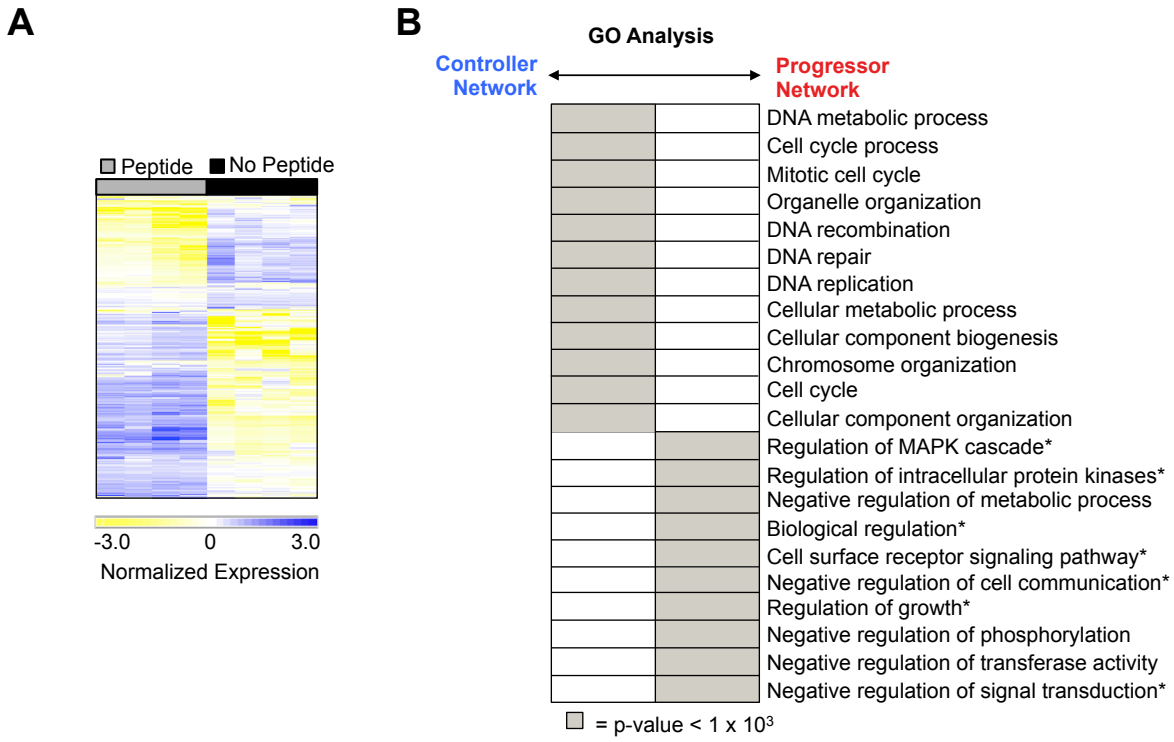

Figure S2, related to Figure 1: Differentially expressed genes and gene ontology analysis following peptide stimulation in HIV-specific CD8⁺ T cells from elite controllers and chronic progressors (A) The top 200 differentially expressed genes in peptide-stimulated and non-peptide stimulated KK10-specific CD8⁺ T cells in B*2705 controllers. Each column represents an individual sample and each row an individual gene, colored to indicate normalized expression (blue = increased expression, yellow = decreased expression). (B) Controller and progressor networks reveal a functional distinction between patient groups. We used GOrilla (http://cbl-gorilla.cs.technion.ac.il/) to calculate the overrepresented gene ontology terms (http://www.geneontology.org) within the controller and progressor differentially expressed and induced gene networks. A selected list of terms with p-value < 1 x 10-3 was visualized. The asterisk (*) denotes those GO terms within the progressor network in which caspase-8 was involved.

GENE	FUNCTION
CONTROLLER NETWORK	
SPC25	Chromosome segregation and spindle checkpoint activity
MLF1IP	Centromere Protein U
BUB1B	Enzyme that controls spindle checkpoint function
RAD51	Assists in double strand break repair
RAD51AP1	Accessory protein to RAD51 involved in DSBR
C1orf59	Chromosome 1 ORF, numerous transcription sites
BRCA1	T lymphocyte lineage development
RPA3	DNA recombination, repair and replication
PRIM1	DNA primase that synthesizes RNA primers during DNA replication
THOC4	Regulates transcriptional activity of basic leucine zipper proteins
MND1	Stimulates RAD51 during DSBR
CKS2	Regulates cyclin dependent kinases
CKS1B	Cell cycle regulation
CCNB2	Control of cell cycle at the G2/M transition
LMNB1	Involved in nuclear stability, chromatin structure and gene expression
UBASH3A	Negative regulator of T lymphocyte signaling
PROGRESSOR NETWORK	
APP	Function unknown, expressed in T lymphocytes
CASP8	T lymphocyte homeostasis and development
SRC	Maintains constitutive TCR phosphorylation and IL-2 production
FYB	Modulates expression of IL-2 and T lymphocyte activation
LYN	Maintains constitutive activation and phosphorylation of JAK/STAT
IL-7R	Regulates T lymphocyte homeostasis and development
AKAP13	Mediates response of T lymphocytes to osmotic stress
THRA	Regulates lymphocyte function and T cell receptor activation
CD44	Elevated in T lymphocytes previously exposed to antigen
CD74	Marker of activated T lymphocytes
HLA-DQA1	Increased expression following mitogenic or antigenic stimulation

Table S3, related to Figure 1: List of Direct Interacting Partners within the Controller and Progressor Networks as identified by DAPPLE Analysis. This table delineates the genes involved in the direct EC and CP networks which are involved in driving overall network connectivity.

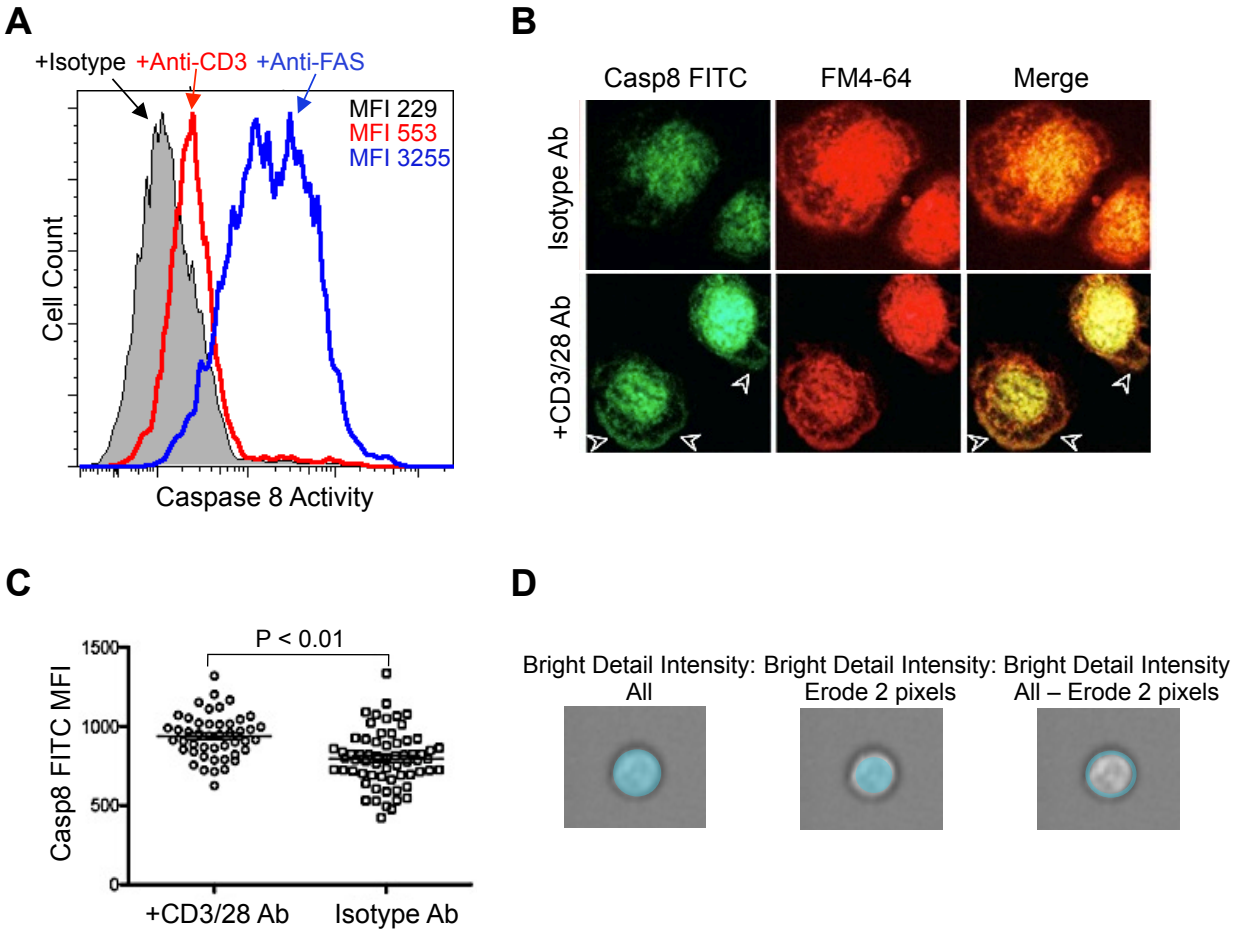

Figure S3, related to Figure 3: Active Caspase-8 upregulation and translocation upon TCR engagement in a KK10-specific CTL clone. (A) Histogram illustrating total MFI of Caspase-8 activity in KK10-specific CTL clones at 30 min stimulated with Isotype antibody (gray), anti-CD3 antibody (red) or anti-FAS antibody (blue). (B) KK10-specific CTL clones were imaged on Poly-L-Lysine coated coverslips at 30 min, and were stimulated with Isotype control antibody (upper panels) or anti-CD3 and anti-CD28 antibodies (lower panels). Active Caspase-8 (Green) and FM4-64 plasma membrane dye (Red) were acquired by confocal microscopy. Arrows illustrate active caspase-8 activity at the plasma membrane, indicating its translocation. (C) Quantitative measurement of MFI of active caspase-8 by confocal microscopy. The MFI of active caspase-8 per cell in the presence of anti-CD3 and anti-CD28 was increased as compared to isotype control stimulated CTLs. P-value was determined using unpaired t-test (two-tailed). (D) Schematic of Boolean approach to assess for cytoplasmic and membrane-associated caspase-8 activity utilized in Figure 3C-E.

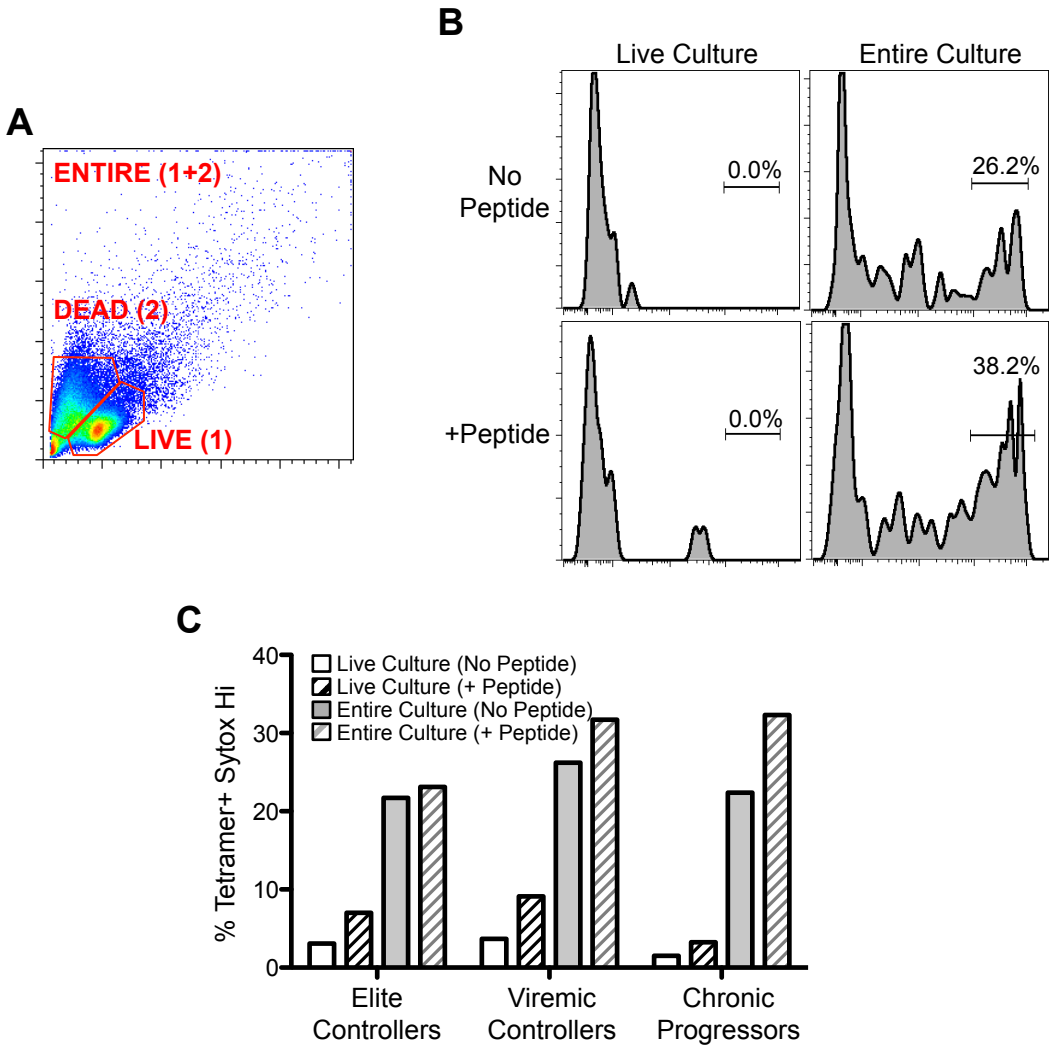

Figure S4, related to Figure 4: Assessment of necrotic death by Sytox Green staining. (A) Representative plot of purified $CD8^+$ T cells with gating of live and dead populations based on forward and side scatter properties. (B) $CD8^+$ T cells were stimulated in the presence or absence of HIV peptide and stained with Sytox Green dye, in addition to tetramer and anti-CD8 antibody. As shown, gating on live cells results in relatively low levels of necrotic Sytox Hi cells. However, gating on the entire culture reveals a significant increase in the number of Sytox Hi cells. (C) Summary data of percentage of Sytox Hi cells in the live and entire cultures within each patient group.

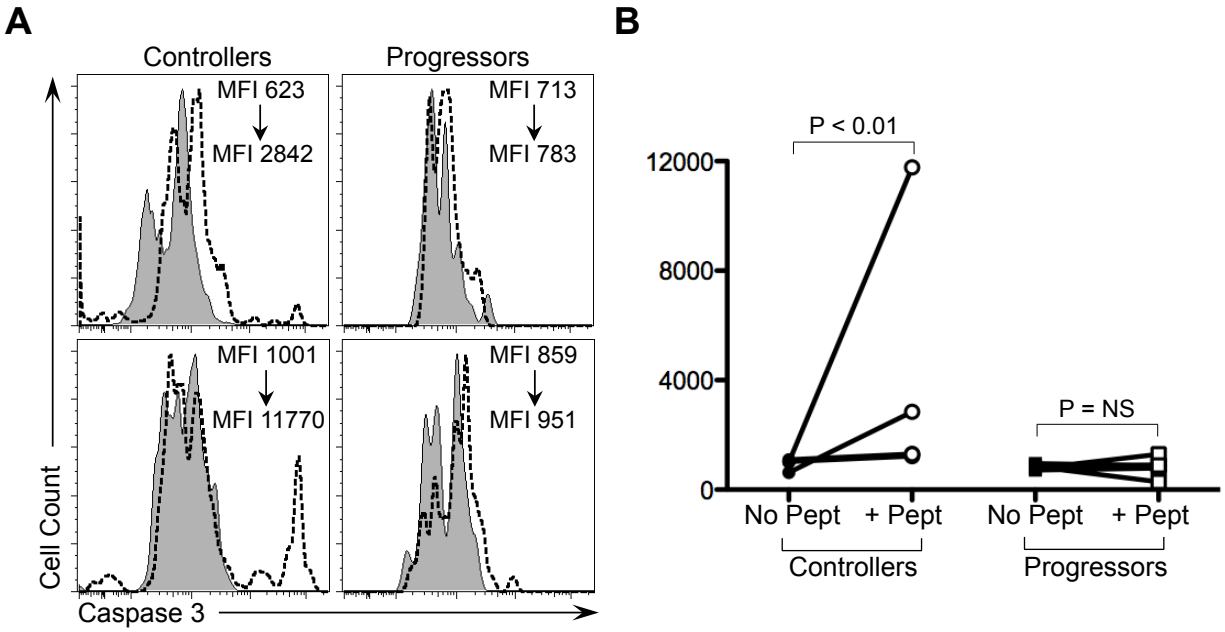

Figure S5, related to Figure 4: Assessment of caspase-3 activity within peptide-stimulated HIV-specific CD8⁺ T cells from controllers and progressors. (A) Representative data showing modulation in MFI of caspase-3 activity following 3d peptide stimulation (1 ug/mL) in KK10-specific CD8⁺ T cell responses from an 2 HLA-B*2705 elite controllers and 2 HLA-B*2705 chronic progressors. Filled plot (gray) represents caspase-3 MFI in the absence of KK10 peptide and dashed line represents caspase-3 MFI in the presence of KK10 peptide. (B) Summary data of change in caspase-3 activity following peptide stimulation in controllers (n = 5) and chronic progressors (n = 5). Statistical comparisons were made using the Wilcoxon matched pairs test.