Supporting Information

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SI Materials and Methods

Clinical Groups. Individuals were grouped according to clinical parameters as follows: HIV controllers [HIV-1 viral load <2,000 RNA copies/mL in the absence of antiretroviral therapy (ART)], chronic untreated (HIV-1 viral load >2,000 RNA copies/mL not receiving ART), and chronic treated (HIV-1 viral load <50 copies/mL receiving ART).

Fabrication of Nanowell Arrays. The nanowell arrays were fabricated using a combination of photolithography and replica molding, as described previously (1). Briefly, a silicon wafer supporting a pattern of SU-8 photoresist was prepared by photolithography and used as a template for molding. Nanowell arrays were produced by casting a biocompatible elastomer (polydimethylsiloxane, PDMS; 10:1 base:catalyst; Dow Corning) on this template and curing at 80 °C for 2 h. Each array comprising 72 × 24 blocks of wells was 1 mm thick and adhered directly to a 3 in ×1 in glass slide. In this study, the arrays comprised blocks of 7 × 7 nanowells with dimensions of 50 μm × 50 μm × 50 μm (with a center-to-center distance of 100 μm) for a total of 84,672 wells per array. Before use, the array was sterilized and rendered hydrophilic by treatment with an oxygen plasma (Harrick).

Preparation of Glass Slides for Microengraving. Functionalized glass slides were prepared as described (2). Briefly, poly-L-lysine–coated slides were incubated with 10 μg/mL of each capture antibody [IFN-γ (1-D1K; Mabtech), IL-2 (MAB602; R&D), TNF-α (MAb1; Biolegend), and human IgG (81-7100; Invitrogen)] for 1 h at 25 °C and then were stored at 4 °C until use. Borate buffer (pH 9) was used for diluting antibodies (3). Before microengraving, coated slides were blocked with 3% nonfat milk in PBS for 30 min, washed three times with PBS, and spun dry.

Intracellular Cytokine Staining. Intracellular staining (ICS) was performed as previously reported (4). Frozen peripheral blood mononuclear cells (PBMCs) were thawed and rested overnight. Then 1×10^6 cells in 1 mL of fresh R10 were transferred to 5-mL polycarbonate tubes and incubated with costimulatory antibodies (anti-CD28 and anti-CD49d) just before stimulation with the following conditions: no peptide (negative control), Gag overlapping peptides (OLPs) (0.5 μg/mL), and *Staphylococcus* endotoxin B (SEB) (0.25 μg/mL; positive control). After incubation at 37 °C for 1 h, brefeldin A was added at a concentration of 5 μg/mL, and the cells were transferred to an incubator (37 °C/5% CO_2) for 14 h. Cells then were washed with PBS, labeled first with viability dye (Invitrogen) for 30 min and stained for 15 min with the following fluorochrome-conjugated antibodies: CD3-APC H7, CD4-PerCP, CD8-APC, and CD14/19-Pacific Blue (BD Biosciences). The cells then were washed and permeabilized and labeled intracellularly with anti–IL-2-FITC, anti–IFN-γ-PE Cy7, and anti–TNF-α-Alexa 700 (BD Biosciences). Cells then were washed again and analyzed on an LSRII flow cytometer (BD Biosciences).

ELISpot. Briefly, frozen PBMCs were thawed and rested overnight, and 10^5 cells in 200 uL of R10 were loaded onto each well of a 96-well plate precoated with anti–human IFN-γ monoclonal antibody (2 μg/mL). Cells then were stimulated with Gag OLPs (14 μg/mL) and incubated overnight. Cells were stimulated separately with phytohemagglutinin (1.25 μg/mL) or no peptide as appropriate positive and negative controls. The plates then were incubated with a biotinylated IFN-γ secondary antibody (30 min) and then with streptavidin-alkaline phosphatase (30 min) and were developed colorimetrically. The frequencies of the responses are reported as the background-corrected spot-forming units per million PBMCs. A response at least two SDs above background was considered a positive response.

ELISpot also was used to map specificities of expanded clonal cells lines. These experiments were carried out as above, except that 5×10^4 cells were assessed in each well of a 96-well plate. Individual Gag peptides from the OLP pool (66 individual peptides total) or HLA-matched optimal Gag peptides were used to stimulate the lines. Positive responses were determined to be lines that demonstrated >400 spots per well (in all cases too numerous to count).

Viral-Inhibition Assay. Assays were performed as previously described (5). Autologous $CD4^+$ T cells were negatively enriched from PBMCs using Dynal magnetic bead separation (Invitrogen; 15 uL per 10^6 cells) and activated with anti-CD3.8 antibody (0.5 μg/mL) in R10/50 for 3 d. Activated $CD4^+$ T cells were infected with HIV-1 strain JRCSF at a multiplicity of infection of 0.05 for 4 h and were washed with R10. Then 1×10^5 HIV-exposed cells were plated with bulk CD8 or Gag-specific CD8 clones at a ratio of 1:1 in 200 uL of R10/50. Infected and noninfected CD4 cells were cultured by themselves as positive and negative controls, respectively. HIV replication was assessed by p24 ELISA (Perkin-Elmer) with culture supernatants at day 3, 5, and 7.

1. Ogunniyi AO, Story CM, Papa E, Guillen E, Love JC (2009) Screening individual hybridomas by microengraving to discover monoclonal antibodies. *Nat Protoc* 4:767–782.
2. Han Q, Bradshaw EM, Nilsson B, Hafler DA, Love JC (2010) Multidimensional analysis of the frequencies and rates of cytokine secretion from single cells by quantitative microengraving. *Lab Chip* 10:1391–1400.
3. Ronan JL, Story CM, Papa E, Love JC (2009) Optimization of the surfaces used to capture antibodies from single hybridomas reduces the time required for microengraving. *J Immunol Methods* 340:164–169.
4. Nomura L, Maino VC, Maecker HT (2008) Standardization and optimization of multiparameter intracellular cytokine staining. *Cytometry A* 73:984–991.
5. Yang OO, et al. (1997) Suppression of human immunodeficiency virus type 1 replication by CD8+ cells: Evidence for HLA class I-restricted triggering of cytolytic and noncytolytic mechanisms. *J Virol* 71:3120–3128.

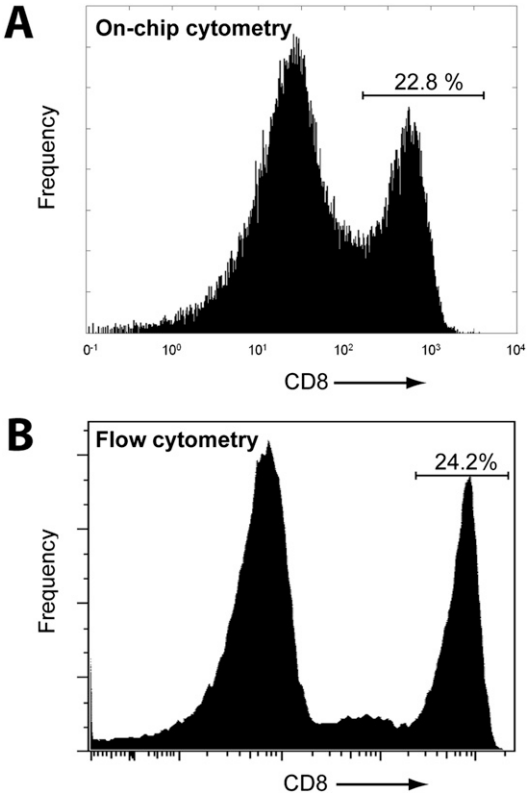

Fig. S1. Histograms of the distribution of PBMCs expressing CD8 as determined by on-chip imaging cytometry (*A*) and comparative analysis by flow cytometry (*B*).

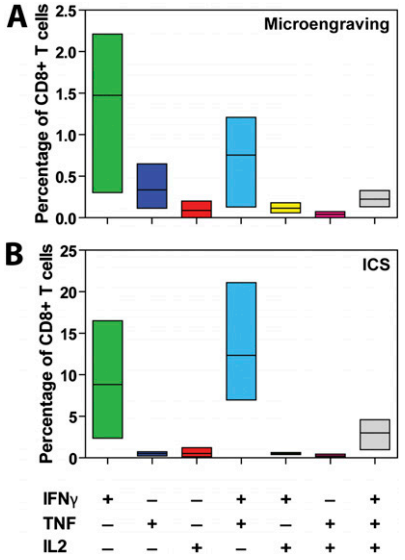

Fig. S2. Box plots of the relative distributions of functional responses measured for SEB-activated T cells by microengraving (*A*) and by ICS (*B*) (n = 3).

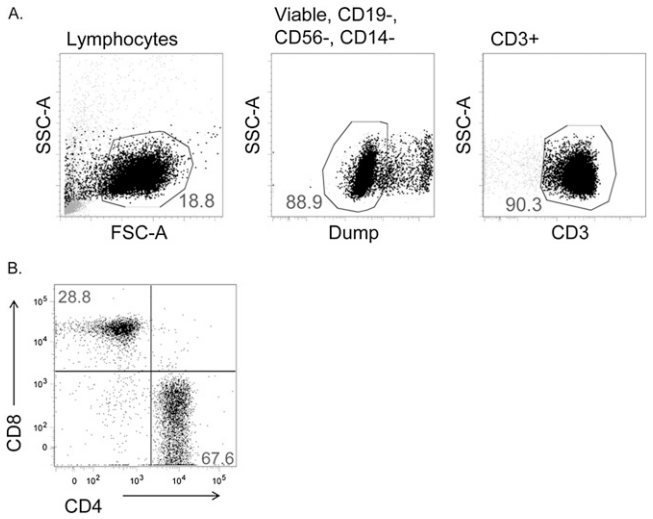

Fig. S3. Representative flow cytometry plots of the gating strategy used to identify (*A*) $CD3^+$ cells and (*B*) $CD3^+$ $CD8^+$ and $CD3^+CD4^+$ cells.

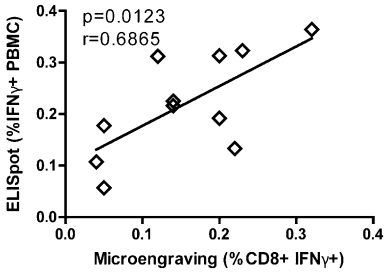

Fig. S4. Scatterplots of responses measured from HIV^+ subjects by ELISpot and microengraving. Each diamond indicates one subject. Correlations were assessed by Spearman's rank correlation.

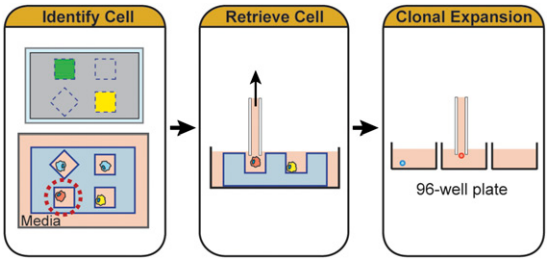

Fig. S5. Schematic illustration of the enumeration of IFN-γ^+ $CD8^+$ cells by microengraving and subsequent retrieval by micromanipulation.

Table S1. Clinical parameters for HIV$^+$ subjects used for microengraving with CD4$^+$ and CD8$^+$ T-cell percentage as determined by flow cytometry

Patient ID	Sex	Age	Race	Classification	Viral load (copies/mL)	CD4$^+$ T-cell count (cells/μL)	CD8%	CD4%
CR0648z	Male	41	White	Treated	<50	649	58.1	36.5
CR0712Y	Male	40	White	Treated	<50	791	47.2	40.7
013113J	Male	43	African American	Untreated	10,6000	230	68.6	22.2
505402	Male	37	White	Untreated	39,795	807	53.2	39.8
CR0541v	Male	31	African American	Untreated	4,240	320	50.1	44.9
CR0555w	Female	34	White	Untreated	24,400	875	53.9	40.6
FEN007	Male	45	African American	Untreated	1,420	510	45.1	51.1
013646a	Male	45	White	Controller	<50	871	22.0	75.3
CR0462R	Male	26	White	Controller	<50	1290	37.3	57.0
CR0509m	Male	45	African American	Controller	3,740	364	50.5	36.3
CR0520z	Male	38	White	Controller	929	1181	32.3	61.6
CR0559m	Male	47	White	Controller	<50	1026	40.6	55.2
CR0757	Male	44	White	Controller	10,800	1041	33.6	62.1
CR0772T	Male	40	African American	Controller	<50	696	28.8	67.6
CTR0174	Male	46	African American	Controller	110	1024	43.0	49.5
CTR0278c	Female	51	White	Controller	252	271	63.8	28.3
FW23	Male	49	White	Controller	1,104	829	30.2	63.3

Table S2. Fine epitope specificities of 12 CD8$^+$ T-cell clones identified from an HIV$^+$ elite controller by microengraving

Clone number	Gag OLP	Optimal peptide	HLA allele	Protein
CTR0278.1	41, 42	REPWDEWVV	Cw8	p24
CTR0278.2	59	FLGKIWPSYK	A2	p15
CTR0278.3	59	FLGKIWPSYK	A2	p15
CTR0278.4	41	DRFYKTLRA	B14	p24
CTR0278.5	59	FLGKIWPSYK	A2	p15
CTR0278.6	59	FLGKIWPSYK	A2	p15
CTR0278.7	11, 12	SLYNTVATL	A2	p17
CTR0278.8	41	DRFYKTLRA	B14	p24
CTR0278.9	41, 42	REPWDEWVV	Cw8	p24
CTR0278.10	11, 12	SLYNTVATL	A2	p17
CTR0278.11	25	TPQDLNTML	Cw8	p24
CTR0278.12	25	TPQDLNTML	Cw8	p24