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Table S1. Characteristics of people living with HIV-1.

Donor	Age	Sex	Nadir	CD4	CD8	Viral	Time since	Time on
	(915)						(ure)	AILT (JIS)
			(cens/µL)	(cens/µ∟)	(cens/µL)	(cp/mL)	(yrs)	
1	36	Male	154	461	941	<40	7	6.5
2	48	Male	195	662	658	<40	17	17
3	36	Male	156	882	1177	<40	14	9
4	55	Male	236	847	613	<40	29	14
5	21	Male	228	331	402	<40	4	4
6	60	Male	-	803	422	<40	25	20
7	36	Male	354	471	322	<40	12	5
8	57	Male	218	911	873	<40	24	21
9	47	Male	274	733	691	<40	13	7
10	50	Male	328	820	723	<40	22	16

Table S2. Mutagenized virus sequences used in this study.

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Backbone	Mutation	Original DNA sequence	Mutagenized sequence
			GCA GCT GAC ACA TAC
			ACC GCC GCC GAG GAG
	pNLAD8	GCA GCT GAC ACA GGA AAC AAC	CTG GCC GGC ATC GGC
PNLADO	ELA20	AGC	ATC CTG ACC GTG ATC
			CTG GGC GTG CTG GGA
			AAC AAC AGC
		GTG GGT CAC AGT CTA TTA TGG	
		GGT ACC <u>TGT GTG GAA AGA AGC</u>	
		AAC CAC CAC TCT ATT TTG TGC	
pNLAD8	PINLADO	ATC AGA [] AAC ATT AGT AGA	
	Aenv	<u>aca aaa tgg aat aac act tta</u>	
		<u>aat caa ata g</u> ct aca aaa tta	GAA CAA III GGG
		AAA GAA CAA TTT GGG	
	5 pJK7312AS ELA20		ACT GCA GAC AAA TAC
			ACC GCC GCC GAG GAG
n 11/7242AB		ACT GCA GAC AAA CCA GCA GCG	CTG GCC GGC ATC GGC
pJK/312A5		ACA	ATC CTG ACC GTG ATC
			CTG GGC GTG CTG CCA
			GCA GCG ACA
	AS pJK7312AS	GCT TAC TTA ATA TAT TGC ACC	
		AAA TAC <u>GTA ACT GTT TTC TAT</u>	
		<u>GGC GTG CCC GTG TGG AGA AAT</u>	
pJK7312AS		GCA TCC [] ACA TAT ATG TAT	
		<u>tgg cat agt aaa gat aat agg</u>	AGC TTG AAT AAG TAT
		ACT ATT ATA AGC TTG AAT AAG	TAT AAT TTA ACA
		TAT TAT AAT TTA ACA	
pNLAD8	pNLAD8 ELA20 ∆env	Both mutations from pNLAD8 ELA20 a	nd pNLAD8 ∆env
pJK7312AS	pJK7312AS ELA20 ∆env	Both mutations from pJK7312AS ELA2	20 and pJK7312AS ∆env

40 Table S3. Real-time qPCR primer sequences used in this study.

Strain	Amplification	Primer	Sequence	Annealing
				optimum
Human	β -globin	bglobin-f	CCCTTGGACCCAGAGGTTCT	65 °C
		bglobin-r	CGAGCACTTTCTTGCCATGA	65 °C
pJK7312AS	Late RT	hiv2-f2	GCAGGTAGAGCCTGGGTGTTC	65 °C
		hiv2jk-psi-rev	GTTCCGAAGACTCTCACTCTTCTC	65 °C
pNLAD8	Late RT	hiv1-3'U3-fwd	GCATGGAATGGATGACCCTGAGA	65 °C
		hiv1-psi-rev2	CGTCGAGAGATCTCCTCTGGCTTTA	65 °C

44 Table S4. List of genes.

Gene Symbol	FP	RP	Full Gene Name
ACTB	CCAACCGCGAGAAGATGAC	TAGCACAGCCTGGATAGCAA	actin beta
AKT1	CACACACTCACCGAGAACC	TCGTGGGTCTGGAAAGAGTA	AKT serine/threonine kinase 1
BAG6	GCCTCAGTGGATCCAGCAA	AGAGAAAGCAAGGCCCCAAA	BCL2-associated athanogene 6
BATF	AGCAGTGACTCCAGCTTCA	CTCTTCTGGGCGGCAATAC	basic leucine zipper ATF-like transcription factor
BAX	GGGTTGTCGCCCTTTTCTAC	TCTTGGATCCAGCCCAACA	BCL2 associated X, apoptosis regulator
BCL2 ATGTGTGTGGAGAGCGTCAA		GTGCCGGTTCAGGTACTCA	BCL2 apoptosis regulator
BCL2L1	CTGCCTGCCTTTGCCTAA	CCAAAACACCTGCTCACTCA	BCL2 like 1
BCL6	GATGGAGCATGTTGTGGACAC	AGGAGGCTTGATGGCAGAAA	BCL6 transcription repressor
BECN1	GAGACCCAGGAGGAAGAGACTA	AATCTGCGAGAGACACCATCC	beclin 1
CASP8	GGAAATCTCCAAATGCAAACTGG	CAGGATGACCCTCTTCTCCAT	caspase 8
CCL3	ATGGCTCTCTGCAACCAGTT	CCGGGAGGTGTAGCTGAAG	C-C motif chemokine ligand 3
CCL3L1	ACGCAGGCAGCAAAGAGTA	ATTGGGAGCAGGTGATGGAA	C-C motif chemokine ligand 3 like 1
CCNH	GAACAGAACTTGCCTGTCAC	CTTCAGATCTGGGTGGTTCA	cyclin H
CCR5	TGAGACATCCGTTCCCCTACA	TGGCAGGGCTCCGATGTATA	C-C motif chemokine receptor 5 (gene/pseudogene)
CCR7	GTGGTGGCTCTCCTTGTCA	CTGTGGTGTTGTCTCCGATGTA	C-C motif chemokine receptor 7
CD244	AACCACAGCCCTTCCTTCAA	GAGCAGGGTTCTGGGCTTTA	CD244 molecule
CD27	CACTACTGGGCTCAGGGAAA	TGCTGGTCACAGTCCTTCA	CD27 molecule
CD300A	GAAAAGCCAGCACCACCAA	TCAAACACCACCGAGGCATA	CD300a molecule
CD38	ACCTCACATGGTGTGGTGAA	GTTGCTGCAGTCCTTTCTCC	CD38 molecule
CD69	TCACCCATGGAAGTGGTCAA	ACACACTTGTCAGACCCTGTA	CD69 molecule
CD8A	CCATCATGTACTTCAGCCACTTCG	GCTGCGACGCGATGGT	CD8a molecule
CDC42	GCTGTTGGTAAAACATGTCTCC	AACTGTGACTGCATAGTTGTCA	cell division cycle 42
CDK2	GTTGTACCTCCCTGGATGAA	ATCCGCTTGTTAGGGTCGTA	cyclin dependent kinase 2
CDK4	GACATGTGGAGTGTTGGCTGTA	TGGTCGGCTTCAGAGTTTCC	cyclin dependent kinase 4
CDK6	TTGCCCGCATCTATAGTTTCCA	CAAGACTTCGGGTGCTCTGTA	cyclin dependent kinase 6
CDK7	TGAAACTTTGGGCACACCAA	GATGCAAAGGTATTCCAGGGAAA	cyclin dependent kinase 7
CDKN1A	TGGAGACTCTCAGGGTCGAAAA	CGGCGTTTGGAGTGGTAGAA	cyclin dependent kinase inhibitor 1A
CDKN1B	GCAATGCGCAGGAATAAGGAA	TTGGGGAACCGTCTGAAACA	cyclin dependent kinase inhibitor 1B

CDKN2A	CACCGCTTCTGCCTTTTCA	CCCACATGAATGTGCGCTTA	cyclin dependent kinase inhibitor 2A
CPT1A	TCCATGCCATCCTGCTTTACA	AGTGGAATCGTGGATCCCAAA	carnitine palmitoyltransferase 1A
CTLA4	CATGGACACGGGACTCTACA	AATCTGGGTTCCGTTGCCTA	cytotoxic T- lymphocyte
ERCC1	AACCCGGGGCAAAATCCAA	CACATTGCGCACGAACTTCA	ERCC excision repair 1, endonuclease non- catalytic subunit
ERCC3	GACTGGAGTCCCTGATGGAA	TGCTTCAAGACCAGCTTGAC	ERCC excision repair 3, TFIIH core complex helicase subunit
ERCC4	CTTCTGGAATCTCTGAGAGCAA	GAGGTGCTGGAGTCAAGAAA	ERCC excision repair 4, endonuclease catalytic subunit
ESRRA	GGAGCGAGAGGAGTATGTTCTAC	CTTCTCGCAGCTGCTCCA	estrogen related receptor alpha
FAS	GCATCTGGACCCTCCTACC	CCTTGGAGTTGATGTCAGTCAC	Fas cell surface death receptor
FASLG	TGGGGATGTTTCAGCTCTTCC	CTGTGTGCATCTGGCTGGTA	Fas ligand
FOXO1	GGTGTCAGGCTGAGGGTTA	TTCTCTCAGTTCCTGCTGTCA	forkhead box O1
GAPDH	GAACGGGAAGCTTGTCATCAA	ATCGCCCCACTTGATTTTGG	glyceraldehyde-3- phosphate dehydrogenase
GATA3	CACGGTGCAGAGGTACCC	AGGGTAGGGATCCATGAAGCA	GATA binding protein 3
GLS	AAGGCACAGACATGGTTGGTA	CACTGGCTGATTCACAAGTCAC	glutaminase
GLS2	TTAGACCCACGGCGTGAA	TCGCCACTATAGGCAGCAA	glutaminase 2
GZMB	CCCCATCCAGCCTATAATCCTAA	CTGGGCCTTGTTGCTAGGTA	granzyme B
GZMK	CACATTTCATCTGGGCTTCTTAAA	GTGACACTTCTTTCCCTCCA	granzyme K
HIF1A	CAGTCGACACAGCCTGGATA	TTCTTCTGGCTCATATCCCATCAA	hypoxia inducible factor 1 subunit alpha
HK1	TCATTTCCCTGCCAGCAGAC	CGCAGTCTGTTGCCTTAAAACC	hexokinase 1
HLA-DRB1	AGAATGGAGACTGGACCTTCC	GCTCTGTGCAGATTCAGACC	major histocompatibility complex, class II, DR beta 1
ID2	CTCAACACGGATATCAGCATCC	CACACAGTGCTTTGCTGTCA	inhibitor of DNA binding 2
IFITM1	ACACCCTCTTCTTGAACTGGT	CCAACCATCTTCCTGTCCCTA	interferon induced transmembrane protein 1
IFNA1	TGACTCATACACCAGGTCAC	CAGGGGTGAGAGTCTTTGAA	interferon alpha 1
IFNB1	ATGAGCAGTCTGCACCTGAA	GACTGTACTCCTTGGCCTTCA	interferon beta 1
IFNG	ACTGCCAGGACCCATATGTAA	GTTCCATTATCCGCTACATCTGAA	interferon gamma
IFNGR1	AAGCCAGGGTTGGACAAAA	GATATCCAGTTTAGGTGGTCCAA	interferon gamma receptor 1
IL7R	GGAGAAAGTGGCTATGCTCAA	CTGCGATCCATTCACTTCCA	interleukin 7 receptor
IRF1	AACAAGGATGCCTGTTTGTTCC	TGGGATCTGGCTCCTTTTCC	interferon regulatory factor 1
KLRD1	AGCATTTACTCCAGGACCCAAC	TAACAGTTGCACCGGTACCC	killer cell lectin like receptor D1
LAG3	TGGAGCCTTTGGCTTTCAC	GAGGGTGAATCCCTTGCTCTA	lymphocyte activating 3

LAMP1	TCCAGGCTTTCAAGGTGGAA	CCACAGCGATGGGGATCA	lysosomal associated membrane protein 1
LEF1	AAGAAAGTGCAGCTATCAACCA	GCTGTCTTTCTTTCCGTGCTA	lymphoid enhancer binding factor 1
MIF	TGCCATCATGCCGATGTTCA	GGTGAGCTCGGAGAGGAAC	macrophage migration inhibitory factor
MLST8	TGCAGGCTACGACCACAC	TGTGACCTCCAAGGCATTCA	MTOR associated protein, LST8 homolog
MTOR	CCAAACCCAGGTGTGATCAA	TCCTCATTTCCAGGCCACTA	mechanistic target of rapamycin kinase
MX1	ATGCTACTGTGGCCCAGAAA	GGCGCACCTTCTCCTCATA	MX dynamin like GTPase 1
МҮС	CCTGGTGCTCCATGAGGA	CCTGCCTCTTTTCCACAGAAA	MYC proto- oncogene, bHLH transcription factor
NDUFA5	ACCAACTTCAAGGCGGTCAA	TGGCTCCCATAGTTTCCATTCC	NADH:ubiquinone oxidoreductase subunit A5
NFATC1	TCCTCTCCAACACCAAAGTCC	AGGATTCCGGCACAGTCAA	nuclear factor of activated T cells 1
NT5E	ATGAACGCCCTGCGCTAC	GTGGCTCGATCAGTCCTTCC	5'-nucleotidase ecto
OAS1	TACCCTGTGTGTGTGTCCAA	AGAGGACTGAGGAAGACAACC	2'-5'-oligoadenylate synthetase 1
OPA1	CCAAAGTAGACCTGGCAGAGAA	TCATTGGGAAGAGCTTTCCTTCA	OPA1, mitochondrial dynamin like GTPase
PCNA	TCTGAGGGCTTCGACACCTA	CATTGCCGGCGCATTTTAGTA	proliferating cell nuclear antigen
PDCD1	GCAGCCTGGTGCTGCTA	GTGCGCCTGGCTCCTA	programmed cell death 1
PFKM	AGGAAGAATGTGCTTGGTCAC	CGCCCATCTTAGTGGCAAA	phosphofructokinase, muscle
РКМ	CGGGTGAACTTTGCCATGAA	CATCCGGTCAGCACAATGAC	pyruvate kinase M1/2
POLR2A	CTCGCCTCTTCTACTCCAACA	ATGGAGTCCCCAATGCCAATA	RNA polymerase II subunit A
PRDM1	CCTGGTACACACGGGAGAAAA	TTGAGATTGCTGGTGCTGCTA	PR/SET domain 1
PRF1	CATCTGTGTAGCCGCTTCTCTA	TGCCCAGGAGGAGCAGAC	perforin 1
PRKAA1	CCAACTATGCTGCACCAGAA	AGAATAACCCCACTGCTCCA	protein kinase AMP- activated catalytic subunit alpha 1
PRKCA	ACCATCCGCTCCACACTAAA	AGTCGTCGGTCTTTGTCTGAA	protein kinase C alpha
RHOA	GTGCCCACAGTGTTTGAGAA	TGTGTCCCACAAAGCCAAC	ras homolog family member A
RICTOR	CTTCCGTGTCGGAGGTTCATA	ACACAGCCTCTGCTTCTTCA	RPTOR independent companion of MTOR complex 2
RPTOR	GCTCAGAGCTGGAGGATGAA	AGGGTCCACACCAACATTCA	regulatory associated protein of MTOR complex 1
RUNX1	ACCACAGAGCCATCAAAATCAC	CGGGCTTGGTCTGATCATCTA	RUNX family transcription factor 1
SH2D1A	GAAGTCCTCAGCTAGAAGTACACA	GGGCTTTCAGGCAGACATCA	SH2 domain containing 1A
SLC2A1	ATTGTGGGCATGTGCTTCC	AGAACCAGGAGCACAGTGAA	solute carrier family 2 member 1
SLC2A3	CCGTCGGACTCTTCGTCAA	TAAAGCAGCCACCAGTGACA	solute carrier family 2 member 3
STAT3	GGAAATAATGGTGAAGGTGCTGAAC	CCGAGGTCAACTCCATGTCAAA	signal transducer and activator of transcription 3

TBX21	GGGCGTCCAACAATGTGAC	CCGTCGTTCACCTCAACGATA	T-box transcription factor 21
TCF7	CTGCACATGCAGCTATACCC	TGGATTCTTGGTGCTTTTCCC	transcription factor 7
TERT	CCAGAACGTTCCGCAGAGAAAA	ACGCTGAACAGTGCCTTCAC	telomerase reverse transcriptase
TFAM	GAAGACTGTAAAGGAAAACTGGAA	CATTTGTTCTTCCCAAGACTTCA	transcription factor A, mitochondrial
TNF	CCCAGGGACCTCTCTCTAATCA	ATGGGCTACAGGCTTGTCAC	tumor necrosis factor
TNFSF10	AGAAGGAAGGGCTTCAGTGAC	CCTGGACCTCCATCATAGCC	TNF superfamily member 10
TP53	GACTGTACCACCATCCACTACA	AAAGCTGTTCCGTCCCAGTA	tumor protein p53
VHL	TGTTTAGGGGCAAACATCACAA	AAAATGCCACCACCTTCTCC	von Hippel-Lindau tumor supressor
XCL1	ACTGCCGGTTAGCAGAATCAA	CCCATGTGGCTTGTGGATCA	X-C motif chemokine ligand 1
XPC	CTCTGCTCGAGATGATGAGGAA	ACAATACCAGCCGGGTCAA	XPC complex subunit, DNA damage recognition and repair factor



48 Figure S1. Virus production and late RT analysis.

50 (A) Insertion of the ELA20 sequence into HIV-1 and HIV-2. (B) Western blot of Gag, capsid 51 (CA), cyclophilin A (CypA), and tubulin in virus-producing 293FT cells and VLPs. Data are representative of three independent experiments. (C) Concentrations of p24 or p27 in crude 52 53 preparations of pNLAD8 (n = 3) or pJK7312As (n = 6) and crude or ultracentrifuged 54 preparations of pNLAD8 ELA20 Δ env (n = 3) or pJK7312As ELA20 Δ env (n = 9), respectively. Each dot represents one experiment. Bars indicate mean values. **p < 0.01, ***p < 0.00155 (unpaired repeated measures ANOVA). (**D**) Titers of crude pNLAD8 (n = 3) or pJK7312As (n56 57 = 5) on 293FT cells and crude or ultracentrifuged pNLAD8 ELA20 Δ env (n = 3) or pJK7312As ELA20 Δ env (n = 5) on GHOST X4R5 cells. Each dot represents one experiment. Bars indicate 58 59 mean values. ****p < 0.0001 (unpaired repeated measures ANOVA). (E) Quantification of pJK7312As or pJK7312As ELA20 Δ env (top, n = 4) and pNLAD8 or pNLAD8 ELA20 Δ env 60 late RT products (bottom, n = 4) 24 h after infection of MDDCs. Cells were left untreated or 61 treated with azidothymidine (AZT) and nevirapine (NVP) at the time of the infection. SIVmac 62 VLPs were added to provide Vpx. NI, no infection. Each dot represents one experiment. Bars 63 indicate mean values. ***p < 0.001, ****p < 0.0001, ns = not significant (paired repeated 64 65 measures ANOVA using log-transformed data).

Figure S2. Memory phenotype of primed CD8⁺ T cells.



Representative flow cytometry plots showing the expression of CCR7 and CD45RA among
ELA-specific CD8⁺ T cells primed in the presence of ELA20 Δenv HIV-1 (top) or ELA20 Δenv

- 70 HIV-2 (bottom). Numbers indicate percentages in the drawn gates.

73 Figure S3. Differentially expressed genes in CD8⁺ T cells primed with HIV-1 or HIV-2.



77 Plots show genes that were differentially expressed between ELA-specific CD8⁺ T cells primed

- 78 with ELA20 Δenv HIV-1 (red) and ELA-specific CD8⁺ T cells primed with ELA20 Δenv HIV-
- 79 2 (blue). Each dot represents one donor. Results are plotted as $\log 2^{-\Delta ct}$ normalized to *GAPDH*.
- 80 Data are shown as median \pm IQR. Samples lacking a *GAPDH* signal were excluded from the
- 81 analysis. *p < 0.05, **p < 0.01, ***p < 0.001 (pairwise t-test).
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84 Figure S4. Schematic representation of the *in vitro* priming approach using cognate

peptide and adjuvants.



88 (A) Priming conditions using HIV-uninfected HLA-A2⁺ donor PBMCs. (B) Priming conditions

89 using HLA-A2⁺ PBMCs from PLWH.

- 92 Figure S5. Differentially expressed genes in CD8⁺ T cells primed in the presence of
- 93 proinflammatory cytokines, IFN-α, or cGAMP.



Plots show genes that were differentially expressed among ELA-specific CD8⁺ T cells primed in the presence of proinflammatory cytokines (IL-1 β , TNF, and PGE2, n = 7), IFN- α (n = 7), or cGAMP (n = 7). Each dot represents one donor. Results are plotted as log 2^{- Δ ct} normalized to *GAPDH*. Data are shown as median ± IQR. Samples lacking a *GAPDH* signal were excluded

100 from the analysis. *p < 0.05, **p < 0.01, ***p < 0.001 (pairwise t-test).

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Figure S6. Impact of cGAMP on CD8⁺ T cell priming with ELA in the presence of
 proinflammatory cytokines or with ELA20 Δenv HIV-1.



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Expression of granzyme B, perforin, and T-bet among ELA-specific CD8⁺ T cells primed with ELA20 in the presence of proinflammatory cytokines (IL-1 β , TNF, and PGE2) (**A**) or ELA20 Δ env HIV-1 in the absence or presence of cGAMP (**B**). Each connected dot represents one donor (n = 7). *p < 0.05 (Wilcoxon signed rank test).

- 113 Figure S7. Secretion of soluble factors after stimulation of PBMCs with proinflammatory
- 114 cytokines or cGAMP.



116 Concentrations of various chemokines/cytokines in culture supernatants 3 days after 117 stimulation of PBMCs from HUDs (n = 8) (A) or PLWH on ART (n = 8) (B) with 118 proinflammatory cytokines (IL-1 β , TNF, and PGE2) or cGAMP. Each dot represents one 119 donor. Bars indicate median values. ***p < 0.001, ****p < 0.0001 (Mann-Whitney U test).