Supporting Information

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SI Materials and Methods

Cell Isolation and Culture. Adult peripheral blood (APB), obtained from healthy donors after informed consent, was collected in heparin-containing tubes. Umbilical cord blood (UCB) was obtained after informed consent, immediately after delivery of full-term infants. Thymi were removed during corrective cardiac surgery of patients in accordance with local ethics board regulations. CD4 T cells or total CD3 T cells were purified using negative-selection Rosette tetramers (StemCell Technologies). To subsequently isolate naive and memory T cell subsets by negative selection, CD4 lymphocytes were incubated with αCD45RO (Dako) or αCD45RA (Immunotech) mAbs, respectively, in combination with αHLA-DR and αCD69 mAbs, to eliminate preactivated T cells. The non-bound fractions were recovered following addition of α-mouse IgG-conjugated magnetic beads (Dynal). The purity of each cell population was monitored on a FACSCalibur (BD Pharmingen).

Cell Culture Conditions. Lymphocytes were cultured in RPMI medium 1640 (Gibco-BRL Invitrogen) supplemented with 10% (vol/vol) FCS, herein referred to as R10 medium. For IL-7 stimulation, human recombinant IL-7 (10 ng/mL; kindly provided by Cytheris) was replenished every 2–3 days. Cells were maintained in either a standard tissue culture incubator containing atmospheric (20%) O_2 or an incubator where 2.0–2.5% O_2 conditions were maintained by nitrogen injections (Heraeus incubator; Sanyo) and CO_2 was maintained at 5%. In indicated experiments, LY294002 (Sigma) was added to cultures at 15 μM 1 h before IL-7 stimulation.

Flow Cytometry. To detect expression of surface markers, cells were incubated with the appropriate fluorochrome-conjugated mAbs (BD Biosciences) and monitored compared with an Ig isotype antibody. The tyrosine phosphorylation state of STAT5 was assessed using an anti–phospho-STAT5 (Y694) antibody (BD Pharmingen). Cell cycle analysis was performed by DNA and RNA stains using 7-amino-actinomycin-D (7AAD; 20 μM; Sigma) and pyronin Y (PY; 5 μM; Sigma), respectively, or with an αKi67 mAb (Dako). Cells were analyzed on a FACSCalibur or FACSCanto (BD Biosciences) using BD Biosciences or FlowJo software (Tree Star).

Glucose Uptake Assays. Cells (2×10^6) were starved by incubation at 37 °C in serum/glucose-free RPMI for 30 min. Radiolabeled 2-deoxy-D-[1-^{3}H]glucose (Amersham Biosciences) was added to a final concentration of 0.1 mM (2 μCi/mL). Cells were incubated for 10 min at room temperature, washed in cold serum/glucose-free RPMI, and solubilized in 500 μL 0.1% SDS. Radioactivity was measured by liquid scintillation.

siRNA Transfections for Glut1 Inhibition. Purified T cells were transfected with the indicated synthetic siRNAs, complementary to the Glut1 3′ UTR (1), using the Human T Cell Nucleofector Kit (Amaxa) per the manufacturer's protocol.

Sequences of siRNAs for Glut1 inhibition: 5′-UGAUGUCCAGAAGAAUAUU-3′; 5′-UAUUAAAUACAGACACUAA-3′; 5′-AGCUUCUAUCCCAGGAGGU-3′. Sequence of control siRNAs (luciferase): 5′-CUUACGCUGAGUACUUCGA-3′.

Quantitative Analysis of Glut1 Transcripts. Total RNA was isolated using an RNeasy Kit (Qiagen). Quantitative PCR of cDNAs was performed using Quantitect SYBR Green PCR Master Mix (Roche). Primers were designed within a single exon, allowing the efficacy of primer pairs to be verified on genomic DNA samples, and standard curves for amplification products were established on genomic DNA. Amplification of cDNAs was performed using a LightCycler 480 (Roche). Cycling conditions comprised a denaturation step for 5 min at 95 °C, followed by 40 cycles of denaturation (95 °C for 10 s), annealing (63 °C for 10 s), and extension (72 °C for 10 s). After amplification, melting curve analysis was performed with denaturation at 95 °C for 5 s and continuous fluorescence measurement from 65 °C to 97 °C at 0.1 °C/s. Each sample was amplified in duplicate. Relative expression was calculated by normalization to 18S RNA.

Quantitative RT-PCR primers for Glut1: 5′-TGCTGATGATGAACCTGCTG-3′ (forward); 5′-GATGAGGATGCCGACGAC-3′ (reverse). Quantitative RT-PCR primers for 18S: 5′-CGGCTACCACATCCAAGGAA-3′ (forward); 5′-GGGCCTCGAAAGAGTCCTGT-3′ (reverse).

Virus Production and Infection. Self-inactivating single-round HIV-1 virions were generated by transient transfection of 293T cells with the GFP-encoding HIV-1 SIN vector pHIVSFFVGFP together with the Gag-Pol packaging construct 8.91. (2) For X4-HIV-1 envelope pseudotyping, 7 μg of HIVenv712 was cotransfected, whereas for VSV-G pseudotyping, 3 μg of pCMV-VSV-G plasmid was cotransfected. Viral supernatant was harvested 48 h post-transfection and concentrated by ultracentrifugation for 2 h at 4 °C at 25,000 rpm. To determine the HIV titers, serial dilutions of vector preparations were added to 293T cells or Jurkat cells ($CD4^+CXCR4^+$) for VSV-G and HIVenv712 pseudotypes, respectively. The titers are expressed as 293T or Jurkat ($CD4^+CXCR4^+$) transducing units (TU)/mL.

For single-round infections of CD4 T cells, viral supernatant containing 1×10^6 TU of HIVenv712-pseudotyped vector [multiplicity of infection (MOI) of 10] or $2–3 \times 10^6$ TU of VSV-G–pseudotyped HIV-1 vector (MOI of 20–30) was added to 1×10^5 lymphocytes. After overnight infection, cells were washed and reseeded in 1 mL of IL-7–supplemented R10 media for 48 h. Infection was determined as the percentage of GFP^+ cells by flow cytometry.

Statistics. Statistical analyses were performed using a paired, two-tailed Student's *t* test.

1. Montel-Hagen A, et al. (2008) Erythrocyte Glut1 triggers dehydroascorbic acid uptake in mammals unable to synthesize vitamin C. *Cell* 132:1039–1048.

2. Naldini L, et al. (1996) In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science* 272(5259):263–267.

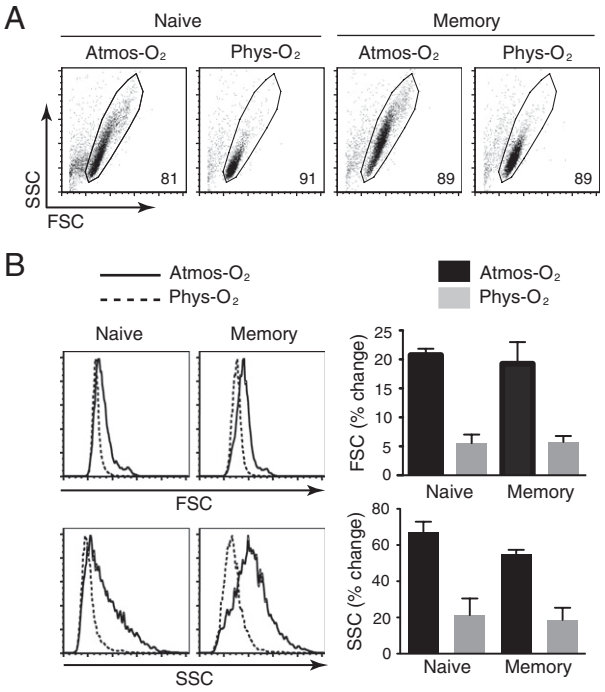

Fig. S1. IL-7 promotes CD4 T-cell survival at both atmospheric and physiological oxygen concentrations. Naive and memory CD4 T cell populations isolated from APB were stimulated with IL-7 (10 ng/mL) under 20% (Atmos-O_2) or 2.5% (Phys-O_2) O_2 conditions. (*A*) Cell size and granularity were assessed as a measure of forward scatter (FSC) and side scatter (SSC), respectively. The percentages of cells within the live gate are indicated. (*B*) Overlays of histograms showing the FSC and SSC profiles of CD4 T cells after 9 days of IL-7 stimulation at Atmos-O_2 (continuous line) and Phys-O_2 (dotted line) are shown (*Left*). Quantifications of the mean percentage changes in mean fluorescence intensity (MFI) relative to day 0 nonstimulated controls ± SD are shown (*Right*).

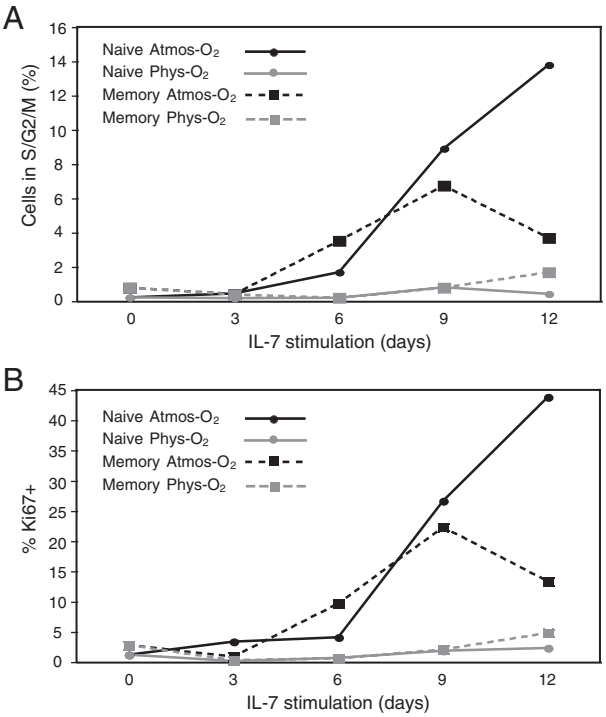

Fig. S2. Kinetics of cell cycle progression of IL-7–stimulated naive and memory CD4 T cells. Naive and memory APB CD4 T cell populations were stimulated with IL-7 (10 ng/mL) under 20% (Atmos-O_2) or 2.5% (Phys-O_2) oxygen conditions. Cell cycle progression was monitored at days 3, 6, 9, and 12 of culture under Atmos-O_2 and Phys-O_2 conditions, as a function of PY/7AAD (*A*) and Ki67 (*B*) staining. Data are representative of at least three independent experiments comprising three to four different donors.

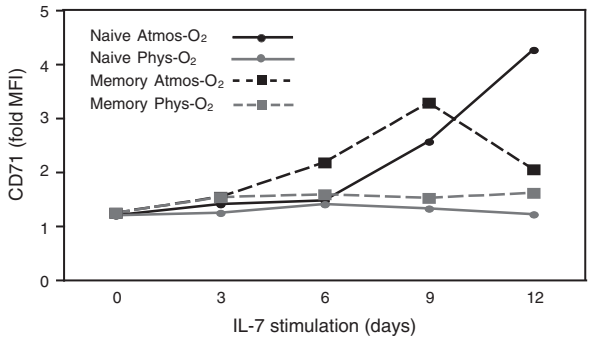

Fig. S3. Kinetics of IL-7–induced CD71 up-regulation at physiological and atmospheric oxygen concentrations. Naive and memory APB CD4 T cells remained unstimulated or were stimulated with IL-7 (10 ng/mL) under 20% (Atmos-O_2) or 2.5% (Phys-O_2) oxygen conditions. Quantification of the relative levels of CD71, monitored as a function of the fold change in ΔMFI, is presented for days 0, 3, 6, 9, and 12 of IL-7 stimulation. Data are representative of three independent experiments comprising four different donors.

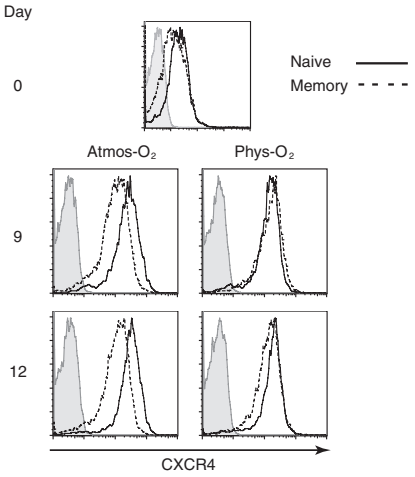

Fig. S4. IL-7–induced CXCR4 expression is maintained at 2–20% oxygen concentrations. CXCR4 expression was monitored on naive and memory APB CD4 T cell populations following IL-7 stimulation. Histograms show CXCR4 staining (open histograms) compared with isotype controls (closed histograms) at 0, 9, and 12 days of IL-7 stimulation at 20% (Atmos-O_2) and 2.5% (Phys-O_2) oxygen conditions. Data are representative of three independent experiments comprising eight different donors.

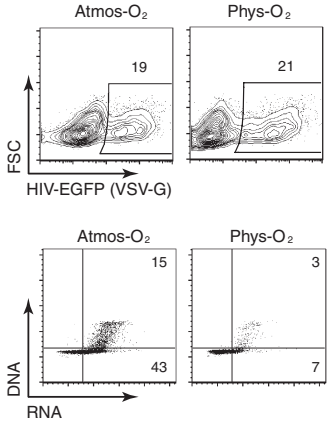

Fig. S5. Permissivity of cord blood T cells to single-round infection with VSV-G–pseudotyped HIV-1 virions is independent of cell cycle entry under physiological oxygen conditions. UCB CD4 T cells were stimulated with IL-7 for 4 days and then infected with VSV-G envelope-pseudotyped HIV-1 virions expressing EGFP. Cell cycle entry was assessed at the time of infection by PY/7AAD staining, and the percentages of cells in the G1b (lower right quadrant) and S/G2/M (upper right quadrant) phases of the cell cycle are indicated (*Lower*). The percentages of HIV-EGFP$^+$ cells are indicated. Data are representative of five independent experiments comprising five different donors.

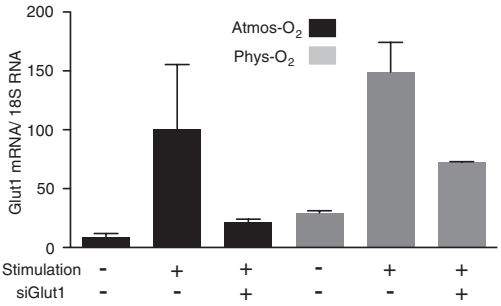

Fig. S6. Inhibition of Glut1 transcription by Glut1-specific siRNAs. CD4 T cells from APB were transfected with Glut1-specific siRNAs (siGlut1) and then stimulated under 20% (Atmos-O_2) or 2.5% (Phys-O_2) conditions for 24 h. Glut1 transcripts were quantified by quantitative RT-PCR; cDNAs were amplified with Glut1-specific primers and normalized to 18S transcripts. The means ± SD for triplicate samples are shown.