**Cell Reports Methods, Volume *2***

## Supplemental information

# Development of an HIV reporter virus that identifies latently infected CD4$^+$ T cells

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## SUPPLEMENTAL INFORMATION

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## SUPPLEMENTARY FIGURES AND LEGENDS

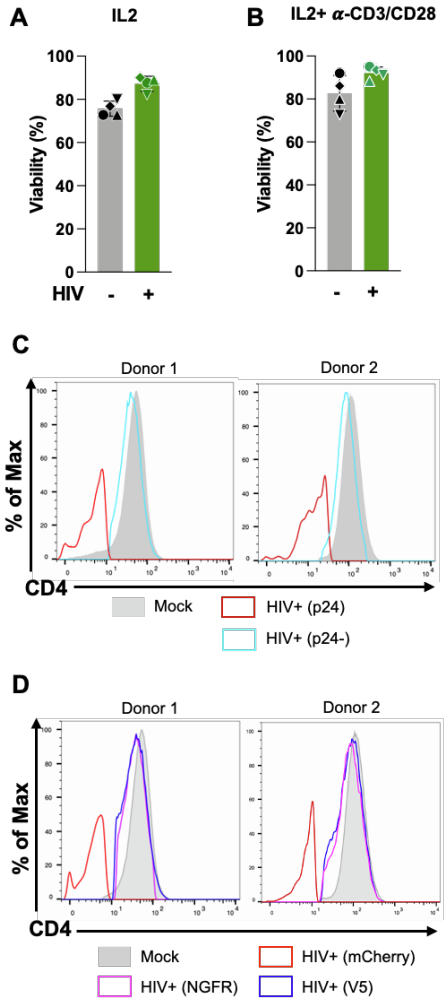



**Figure S1. Validation of pMorpheus-V5 virus by flow cytometry (Related to Figures 1 and 2)**

(A) CD4$^+$ T cells from four donors were stimulated with IL-2 for three days and then infected with pMorpheus-V5. Five days after infection, cell viability was determined using the LIVE/DEAD Fixable Dead Cell stain by flow cytometry. The bar graphs show the percentage of live cells after infection. Error bars correspond to SD. The average of four individual donors is shown (±SD). **$p$=0.003 (t-test).

(B) CD4$^+$ T cells from four donors were stimulated with IL-2 and α-CD3/CD28 for 3 days and infected with pMorpheus-V5. Five days after infection, cell viability was determined by flow cytometry. The bar graphs show the percentage of live cells after infection. The average of four individual donors is shown (±SD). The differences are not statistically different ($p$=0.1, t-test).

(C) $CD4^+$ T cells from healthy donors were stimulated with IL-2 and α-CD3/CD28 beads and infected with pMorpheus-V5. Flow cytometry was performed five days post-infection. Histogram plots show the CD4 expression for uninfected cells (shaded light grey), $p24_{POS}$ cells (red), or $p24_{NEG}$ cells (light blue). The data were analyzed by FlowJo. Results are from four independent donors.

(D) $CD4^+$ T cells from healthy donors were stimulated with IL-2 and α-CD3/CD28 beads and infected with pMorpheus-V5. Flow cytometry was performed five days post-infection. CD4 expression levels of NGFR (magenta), V5 (blue), and mCherry (red) are shown as histogram plots. The data were analyzed by FlowJo. These results are representative of data from four independent donors.

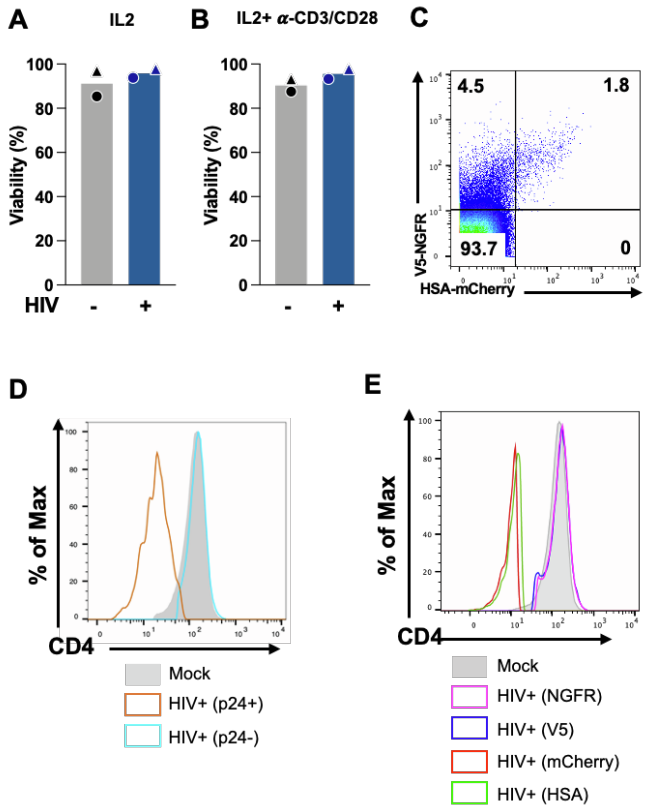



**Figure S2. Validation of pMorpheus-V5 by CyTOF (Related to Figures 1 and 4)**

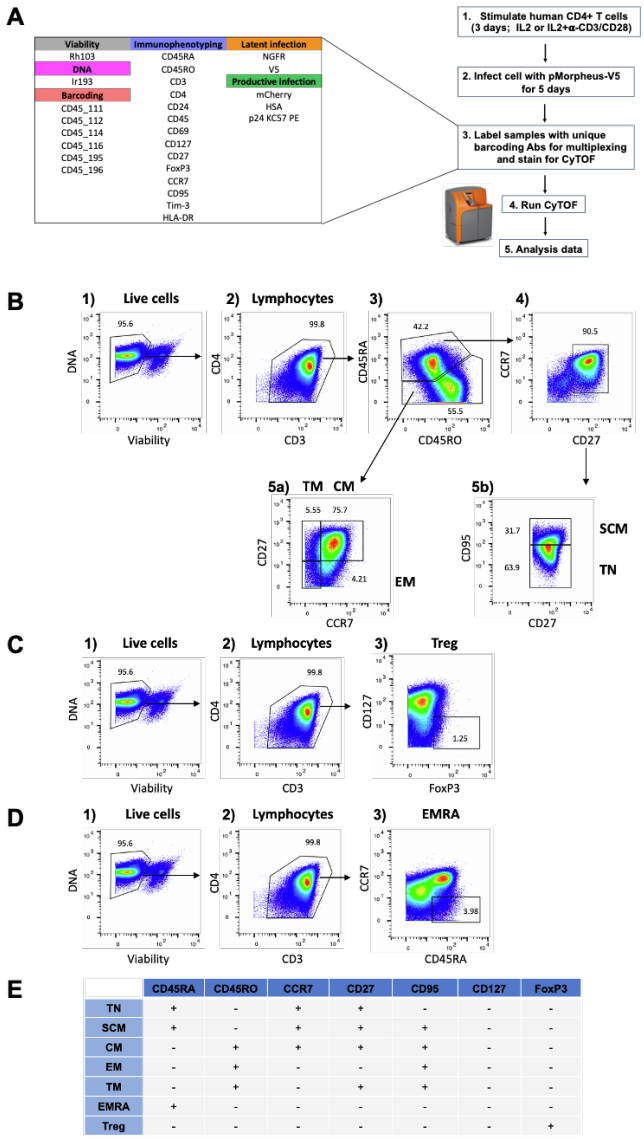
(A) $CD4^+$ T cells purified from PBMCs were cultured for three days in presence of IL-2 and then infected with pMorpheus-V5, which was pseudotyped with a dual-tropic HIV envelop. After 5 days of infection, cells were processed for CyTOF. pMorpheus-V5-infected primary human $CD4^+$ T cells stimulated with IL-2 were analyzed by CyTOF to determine viability. The average of two individual donors is shown (±SD). Each donor is indicated by a unique symbol in the figure.

(B) $CD4^+$ T cells from two donors were stimulated with IL-2 and α-CD3/CD28 beads for 3 days and then infected with pMorpheus-V5. Cell viability was determined by CyTOF. Bar graphs represent percentage of live cells after infection. Error bars correspond to SD. The average of two individual donors is shown (±SD). Each donor is indicated by a unique symbol in the figure.

(C) Cells were stimulated with IL-2 and α-CD3/CD28 antibody-coated beads prior to infection. Latently ($V5_{POS}$, $NGFR_{NEG}$) or productively ($HSA_{POS}$, $mCherry_{POS}$+, $V5_{POS}$, $NGFR_{POS}$, $p24_{POS}$) infected cells were identified by CyTOF five days after infection. These results are representative of data from two independent donors.

(D) $CD4^+$ T cells were purified from PBMCs and cultured for three days in presence of IL-2 and α-CD3/CD28 beads before infection with pMorpheus-V5. After five days of infection, the CD4 levels were quantified by CyTOF. Histogram plots show CD4 levels for uninfected cells (shaded light grey), $p24_{POS}$ cells (orange), or $p24_{NEG}$ cells (light blue) T cells. These results are representative of data from two independent donors.

(E) IL-2 and α-CD3/CD28-stimulated $CD4^+$ T cells were infected with pMorpheus-V5 for 5 days and analyzed by CyTOF to measure *Nef*-mediated downregulation of CD4 levels. NGFR (magenta), V5 (blue), and mCherry (red) cells were analyzed for CD4 levels. These results are representative of data from two independent donors.




E

| | CD45RA | CD45RO | CCR7 | CD27 | CD95 | CD127 | FoxP3 |
|---|---|---|---|---|---|---|---|
| TN | + | - | + | + | - | - | - |
| SCM | + | - | + | + | + | - | - |
| CM | - | + | + | + | + | - | - |
| EM | - | + | - | - | + | - | - |
| TM | - | + | - | + | + | - | - |
| EMRA | + | - | - | - | - | - | - |
| Treg | - | - | - | - | - | - | + |

**Figure S3. Overview of the experimental approach and gating strategy for CyTOF (Related to Figure 4)**

(A) $CD4^+$ T cells from two donors were stimulated with IL-2 or a combination of IL-2 with α-CD3/CD28 antibody-coated beads for three days and then infected with pMorpheus-V5. After 5 days of infection, cells were processed for CyTOF. Representative latent ($NGFR_{POS}$, $V5_{POS}$) or productive ($HSA_{POS}$, $mCherry_{POS}$, $NGFR_{POS}$, $V5_{POS}$) infection profiles at five days post-infection for activated $CD4^+$ T cells stimulated with IL-2. Individual donor cells were first barcoded using CD45 antibody and then mixed prior to staining with the panel of 29 phenotypic markers shown at left of the figure.

(B) Outline of the gating strategy for analysis of the different $CD4^+$ T cell populations. All live $CD4^+$ T cell subsets were identified by DNA viability marker. $T_{CM}$ are defined as CD3+, CD4+, CD45RA-, CCR7+, CD27+; $T_{EM}$ are defined as CD3+, CD4+, CD45RA-, CCR7-, CD27-; $T_{TM}$ are defined as CD3+, CD4+, CD45RA-, CCR7-, CD27+; naïve T cells ($T_N$) are defined as CD3+, CD4+, CD45RA+, CCR7+, CD27+, CD95-; $T_{SCM}$ are defined as CD3+, CD4+, CD45RA+, CCR7+ CD27+, CD95+.

(C) $T_{reg}$ are defined as CD3+, CD4+, CD127-, Foxp3+.

(D) $T_{EMRA}$ are defined as CD3+, CD4+, CCR7-, CD45RA+.

(E) Table summarizing the phenotypic marker profile of each subset of $CD4^+$ T cells examined.

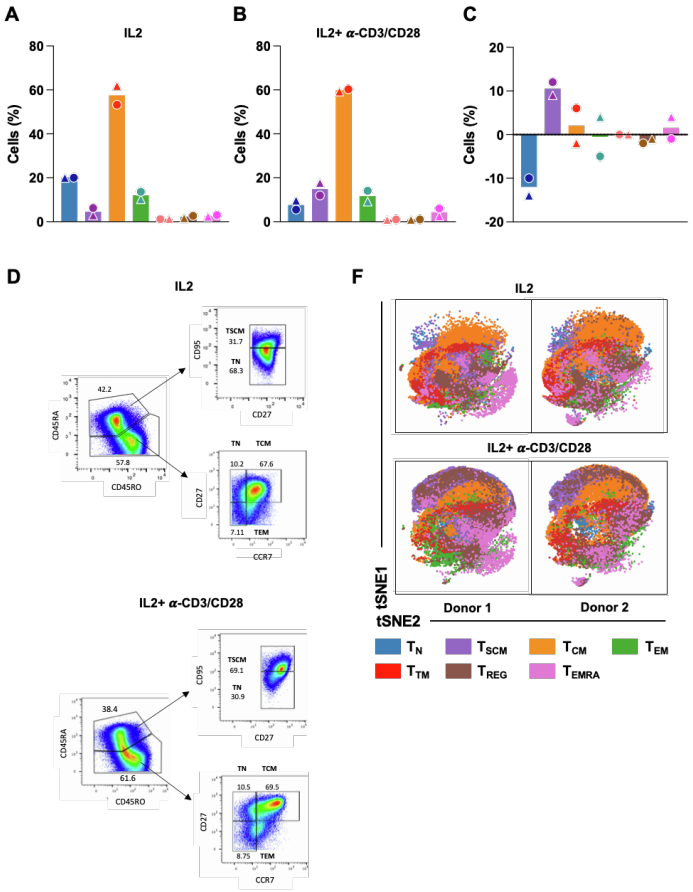



**Figure S4. Distribution of CD4⁺ T cell subsets in presence of IL-2 stimulation or activation (in the absence of HIV infection) (Related to Figure 4)**

(A) The bar graphs show the distribution of CD4⁺ T cell subsets from two different donors after three days of IL-2 stimulations as measured by CyTOF. These results are from two independent donors. Each donor is identified by a symbol. The legend shows the color code for each CD4⁺ T cell subset. The average of two individual donors is shown.

(B) Primary human CD4⁺ T cells stimulated with IL-2 and αCD3/CD28 were analyzed by CyTOF. The distribution of CD4⁺ T cell subpopulations are shown as bar graphs. The results are from two independent donors. Each donor is indicated by a unique symbol.

(C) Bar graph shows the fold change of CD4⁺ T cell subsets following IL-2 or IL-2 and α-CD3/CD28 bead stimulation as measured by CyTOF. The average of two individual donors is shown (±SD). Each donor is indicated by a unique symbol.

(D) Outline of the gating strategy for analysis of the naïve T cells, and $T_{SCM}$. Naïve T cells ($T_N$) are defined as CD3+, CD4+, CD45RA+, CCR7+, CD27+, CD95-; $T_{SCM}$ are defined as CD3+, CD4+, CD45RA+, CCR7+ CD27+, CD95+, $T_{EM}$ are defined as CD3+, CD4+, CD45RA-, CCR7-, CD27-.

(E) viSNE analysis showing pMorpheus-V5 infected CD4⁺ T cells from two donors stimulated with IL-2 and α-CD3/CD28 beads for three days. The tSNE1 and tSNE2 axes are based on relevant markers of cell subsets defined by analysis with the Cytobank program. The legend shows the color code for each CD4⁺ T cell subset. viSNE analysis arranged different CD4⁺ T cell subpopulations in presence of IL-2 and α-CD3/CD28 beads. The tSNE1 and tSNE2 axes are based on relevant markers of cell subsets defined by analysis with the Cytobank program. These results are from two independent donors. The legend shows the color code for each CD4⁺ T cell subset.

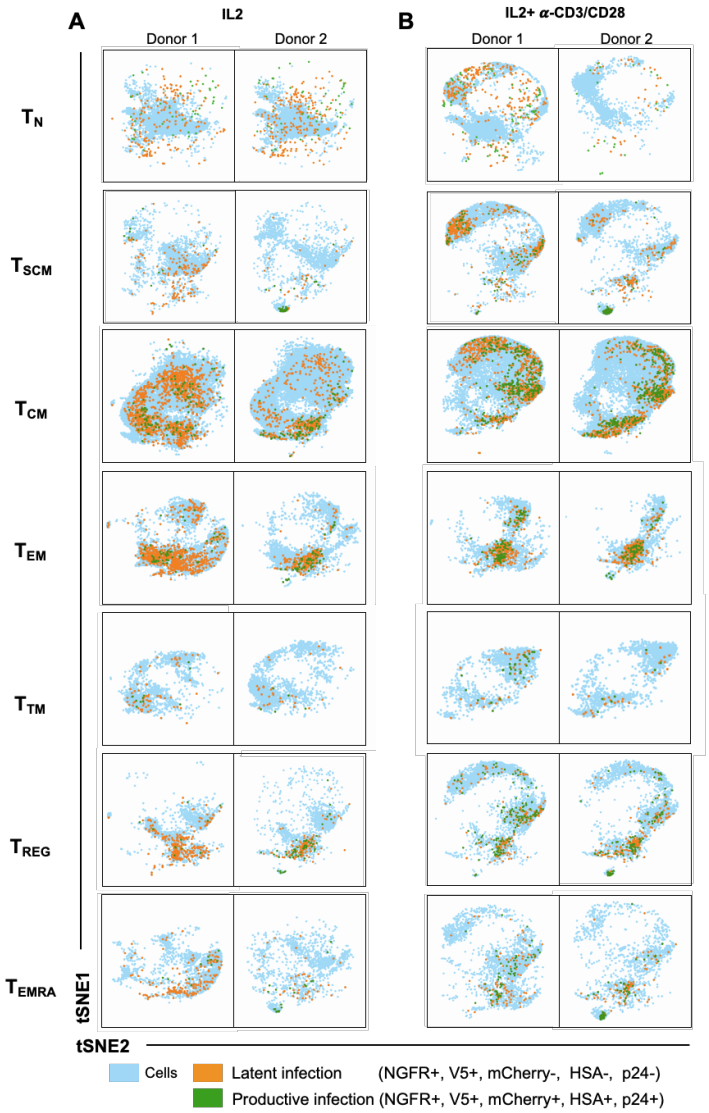



**Figure S5. High dimensional CyTOF analysis reveals that distribution of CD4⁺ T cell subsets with and without activation upon pMorpheus-V5 infection (Related to Figure 5).**

(A) The infection of CD4⁺ T cell subsets from two different healthy donors after 3 days of IL-2 stimulation followed by pMorpheus-V5 infection as measured by CyTOF. viSNE analysis arranged latently and productively infected CD4⁺ T cell subpopulations in presence of IL-2. Latent and productively infected cells are identified by yellow and green symbols. These results are from two independent donors.

(B) CD4⁺ T cells from two healthy donors were stimulated with IL-2 and α-CD3/CD28 beads for three days and infected with pMorpheus-V5 for 5 days. The latently and productively infected cells in each subset were analyzed by CyTOF. viSNE analysis arranged latent and productive infection in different CD4⁺ T cell subpopulations following IL-2 and α-CD3/CD28 bead stimulation. The tSNE1 and tSNE2 axes are based on relevant markers of cell subsets defined by analysis with the Cytobank program. Latent and productively infected cells are identified by yellow and green symbols. These results are from two independent donors.

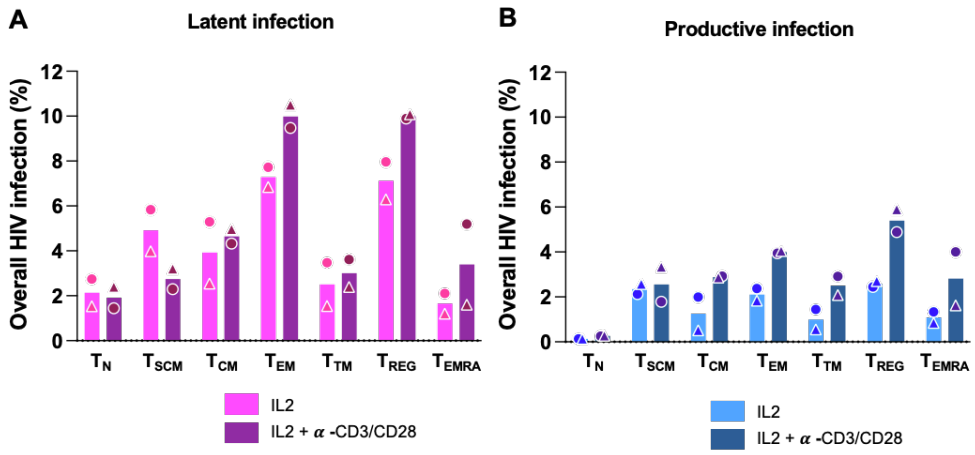



**Figure S6. Determination of latent and productive infection in seven different CD4⁺ T cell subsets (Related to Figure 5).**

(A) CD4⁺ T cell subset specific latency establishment. CD4⁺ T cells from healthy donors were stimulated with IL-2 or with IL-2 and α-CD3/CD28 antibody-coated beads and infected with pMorpheus-V5. Latently infected cells ($NGFR_{POS}$, $V5_{POS}$) in seven different CD4⁺ T cell subsets were determined by CyTOF. The bar graphs represent percentage of latent infection for each T cell subsets. The average of two individual donors is shown (±SD). Each donor is indicated by a unique symbol.

(B) CD4⁺ T cell subset specific productive infections. pMorpheus-V5-infected primary human CD4⁺ T cells from healthy donors stimulated with IL-2 or with IL-2 and α-CD3/CD28 antibody-coated beads were analyzed by CyTOF to determine levels of $NGFR_{POS}$, $V5_{POS}$, $mCherry_{POS}$, $HSA_{POS}$, and $p24_{POS}$ in different CD4⁺ T cell subsets. The bar graphs represent percentage of productive infection of each T cell subsets. The average of two individual donors is shown (±SD). Each donor is indicated by a unique symbol.

**Supplemental Table 1. Summary of the frequency of latently and productively infected CD4+ T cells from six different healthy human donors as identified by pMorpheus-V5 (Related to Figures 2 and 4).** The relative frequency of latent, productive and total infected cells is listed for each donor.

| Individual donor | IL-2 | | | IL2+CD3/28 | | | Method |
|---|---|---|---|---|---|---|---|
| | Latent | Productive | Total | Latent | Productive | Total | |
| | *(%)* | *(%)* | *(%)* | *(%)* | *(%)* | *(%)* | |
| **D1** | 2.8 | 2.3 | **5.1** | 6.5 | 4.7 | **11.2** | Flow cytometry |
| **D2** | 3.7 | 2.4 | **6.1** | 5.4 | 8.3 | **13.7** | Flow cytometry |
| **D3** | 2.6 | 2.1 | **4.7** | 7.3 | 7.6 | **15.0** | Flow cytometry |
| **D4** | 4.6 | 2.7 | **7.3** | 4.8 | 3.1 | **7.9** | Flow cytometry |
| **D5** | 6.1 | 1.6 | **7.7** | 4.9 | 4.3 | **9.2** | CyTOF |
| **D6** | 2.2 | 1.4 | **3.6** | 5.9 | 5.7 | **11.6** | CyTOF |
| **average** | 3.6 | 2.1 | **5.7** | 5.8 | 5.6 | **11.4** | |

**Supplemental Table 2. Oligonucleotide primers and probes used in this study (Related to Figure 3)**

| Assay | Primer /Probe | Sequence |
|---|---|---|
| *Alu*-1 | LM667[a] | 5'-ATGCCACGTAAGCGAAACTCTGGCTAACTAGGGAACCCACTG-3' |
| | Alu-1[a] | 5'-TCCCAGCTACTGGGGAGGCTGAGG-3' |
| | LR1[II] | 5'-CCACTGCTAGAGATTTTCCA-3' |
| | LR2[II] | 5'-ATGCCACGTAAGCGAAACT-3' |
| | ZXF-P | 5'FAM-TGTGACTCTGGTAACTAGAGATCCCTCAGACCC-TAMRA-3' |
| MS | ks1-F[a] | 5′-CTTAGGCATCTCCTATGGCAGGAA −3′ |
| | mf83-R[a,II] | 5'-GGATCTGTCTCTGTCTCTCTCTCCACC-3' |
| | mf84-R[II] | 5'-ACAGTCAGACTCATCAAGTTTCTCTATCAAAGCA-3' |
| | ks2-tq | 5′-FAM-TTCCTTCGGGCCTGTCGGGTCCC-TAMRA-3′ |
| Housekeeping | BGR | 5'-CAACCTCAAACAGACACCATG-3' |
| | BGF | 5'-TCCACGTTCACCTTGCCC-3' |
| | BGX-P | 5-FAM-CTCCTGAGGAGAAGTCTGCCGTTACTGCC-TAMRA-3' |

[a] Primer sets for first PCR

[II] Primer sets for second q-PCR