Proteomic profiling of HIV-1 infection of human CD4\(^+\) T cells identifies PSGL-1 as an HIV restriction factor

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Supplementary Fig. 1. Data analysis of proteomic and RNA-Seq profiling results and validation of mRNA levels of candidate genes. a, GO analysis of the genes with differential abundance among the V-, G-, and G+ cell populations in the proteomics profiling. The color indicates the log10(p-value) as shown on the scale bar on the right. The p-values are determined with Fisher’s exact test. b, Validation of protein abundance changes in Jurkat E6.1 cells. Vinculin (VCL) or GAPDH are used as loading controls. The numbers are the relative quantities of the intensities of the protein bands normalized to those of loading controls. The quantities of bands of the V- samples are then normalized as 1.00. c-d Quantification of mRNA levels of genes with differential protein levels in the three populations (V-, G- and G+) of primary CD4+ T cells (c) or Jurkat-E6.1 cells (d). The mRNA levels are normalized to that of GAPDH and then normalized to the V- level of each gene. Each symbol represents an
independent experiment in c-d. Data are representative of at least three independent experiments.
Supplementary Fig. 2. PSGL-1 is antagonized by HIV Vpu. a, MAGI cells overexpressing PSGL-1 were infected with HIV-IIIB at the indicated MOIs for two days and cells were collected and analyzed with q-RTPCR to quantitate PSGL-1 mRNA levels. MAGI cells overexpressing luciferase was used as a negative control. The data represent three independent experiments. b, Jurkat cells were infected with VSV-G-HIV-GFP at the indicated MOIs for two days before the cells being stained for PSGL-1 and analyzed with FACS. MFI: Median fluorescence intensity; G-: GFP low gate; G+: GFP high gate. c, 293T cells were transfected with plasmids expressing PSGL-1 with a Vpu-expressing plasmid or an empty vector at the indicated doses. Two days after transfection, cells were harvested and analyzed by Western blotting. d, Jurkat cells stably expressing luciferase, Nef or Vpu were lysed and blotted for PSGL-1 and GAPDH. e, Western blots of lysates of primary CD4+ T cells from a different donor than that of Fig. 3c. The cells were infected with NL4-3 (WT) or NL4-3 delVpu (delVpu) or mock infected for 48h. f, Primary CD4+ T were infected with wildtype VSV-G-HIV-GFP or the delVpu viruses at two MOIs for three days before the cells being stained for PSGL-1 and analyzed with FACS. g, 293T cells were transfected with siRNA (final concentration: 20 nM) targeting β-TrCP2. One day post siRNA transfection, cells were then transfected with plasmids expressing PSGL-1 and Myc-Vpu or an empty vector. Two days after plasmids transfection, cells were treated with MG132 and analyzed by Western blotting. h, 293T cells were transfected with siRNA targeting β-TrCP1 for one day before being transfected with FLAG-PSGL-1 and Myc-Vpu or an empty vector. Two days after transfection, cell lysates were analyzed by Western blotting. i, 293T cells were co-transfected with plasmids expressing HA-FLAG-β-TrCP1 or HA-FLAG-β-TrCP2 with plasmids expressing Myc-Vpu or Myc-Vpu S52/S6N. Two days after the transfection, β-TrCP1 and β-TrCP2 were immunoprecipitated with M2 anti-FLAG beads. The cell lysates and the precipitated proteins were analyzed by Western blotting. j, 293T cells were transfected with plasmids expressing FLAG-PSGL-1 and Myc-Vpu or Myc-Vpu S52/S6N mutation or an empty vector (-). Two days after the transfection, cells were treated with MG132 and PSGL-1 was immunoprecipitated with M2 anti-FLAG beads. The cell lysates and the precipitated proteins were analyzed by Western blotting. k, 293T cells were transfected with plasmids expressing PSGL-1 and Myc-Vpu or the indicated Vpu mutants or an empty vector. Two days after the transfection, the cell lysates were analyzed by Western blotting. l, 293T cells were transfected with plasmids expressing Tetherin and Myc-Vpu or the indicated Vpu mutants or an empty vector. Two days after the
transfection, the cell lysates were analyzed by Western blotting. m, 293T cells were transfected with plasmids expressing FLAG-PSGL-1 or FLAG-Tetherin. A different batch of 293T cells were transfected with plasmids expressing Myc-Vpu or Myc-Vpu A14L. Two days after the transfection, the two batch of cells lysates were mixed as indicated and Vpu was immunoprecipitated with 40µL anti-Myc beads. The cell lysates and the precipitated proteins were analyzed by Western blotting. b-m, Data are representative of at least three independent experiments.
Supplementary Fig. 3. PSGL-1 in target cells inhibits HIV-1 replication, starting from DNA synthesis. a, MAGI cells transfected with luciferase, PSGL-1 or Tetherin were infected with HIV-IIIB, NL4-3 delVpu or VSVG-HIV-GFP virus for 48h before being stained for p24 (green) and nuclei (blue). Nevirapine can block HIV-1 reverse transcription and was used to treat cells transfected with luciferase as a positive control. N=3. Scale bar: 50 µm. b, FACS quantification of BlaM assay of Jurkat-E6.1 cells stably expressing PSGL-1 or luciferase that were infected with NL4-3 HIV enclosing Vpr-BlaM for two hours before being washed with PBS and treated with CCF4-AM dye for overnight. The cells were fixed and analyzed by FACS the next day. T20 blocks HIV-1 entry and was used as a positive control. Pacific Blue: BlaM reaction product. AmCyan: BlaM reaction substrate. N=3. c, FACS quantification of BlaM assay of TZM-bl cells overexpressing PSGL-1 or luciferase that were infected with NL4-3 HIV enclosing Vpr-BlaM for two hours. Data are representative of at three independent experiments. d-h, MAGI cells transfected with luciferase, PSGL-1 and Tetherin were infected with HIV NL4-3 or NL4-3 delVpu virus for 12h to measured early RT copies (d, e) late RT copies (f) and 24h to measured 2-LTR circles (g) or infected for 72h and measured viral DNA integration by Alu qPCR (h). N=3 for d-h. Raltegravir can inhibit HIV-1 integration and was used as a positive control. i, Immunofluorescence staining of PSGL-1 (red) and p24 (green) in MAGI cells overexpressing PSGL-1 that are infected with HIV-IIIB for two days. Two representative images were shown for each sample. Scale bar: 20 µm.
Supplementary Fig. 4. PSGL-1 in producer cells inhibits the infectivity of progeny HIV-1 virions. **a**, Mock infection of A3R5 cells to show the low background signal of the GFP reporter cell line, PI: propidium iodide. This is a control for Fig. 5b. **b**, 293T cells were co-transfected with pNL4-3 or pNL4-3 delVpu and a PSGL-1 expressing vector or an empty vector as control. Two days post transfection, virion-particles-containing supernatants were pelleted and then subjected to velocity gradient centrifugation in 6 to 18% Optiprep solution. Fractions were collected, with fraction 1 referring to the top of the gradient. All: the unfractionated samples were shown as input. **c**, 293T cells in 12-well plates were co-transfected with 1 µg/well pNL4-3 or pNL4-3 delVpu proviral plasmids and 100 ng/well of plasmids expressing APEX2 or APEX2 tagged PSGL-1 or Tetherin or luciferase. The lysates of the transfected 293T cells and the supernatant were analyzed by Western blotting. Results of **a-c** are representative at least three independent experiments.
Supplementary Fig. 5. PSGL-1 mediates IFN-γ’s anti-HIV activity in activated primary CD4+ T cells. a, Western blot of PSGL-1 level of several cell lines and primary activated and resting CD4+ T cell samples. The activated CD4+ T cells were activated with PHA and IL-2.
b, Western blot of PSGL-1 of resting T cells or T cells activated by α-CD3/α-CD28 antibodies or PHA/IL-2. c, qRT-PCR quantification of T cell activation markers CD25 and CD38 in primary CD4+ T cell samples to validating the activation by PHA/IL-2 or CD3/28 antibodies.
d-e, qRT-PCR quantification of PSGL-1 and STAT1 mRNA in resting CD4+ T cells (d) and Jurkat T cells (e) treated with recombinant IFN-γ for 12h. The mRNA levels were first normalized to that of GAPDH and then normalized to the level of the untreated sample (IFN-
γ = 0 U/ml). f, Western blot of PSGL-1 in activated CD4+ T cells. Cells were treated with recombinant IFN-γ for 24h. g, qRT-PCR quantification of PSGL-1, Tetherin and STAT1 mRNA of activated CD4+ T cells treated with recombinant IFN-α for 12h. The mRNA levels were first normalized to that of GAPDH and then normalized to the level of the untreated sample. h, Western blots of PSGL-1 in different cell lines and primary CD4+ T cells. MAGI cells were transfected with the indicated amounts of PSGL-1 expressing vector (ng). The 300ng group of MAGI cells and the Jurkat cell lines stably expressing PSGL-1 used in Fig. 4 have comparable PSGL-1 levels as that of activated CD4+ T cells treated with 2000 U/ml IFN-γ. i-j, Activated primary CD4+ T cells from a different donor than that of Fig. 6 were treated or untreated with IFN-γ for 12h before being electroporated with two different siRNAs (final concentration: 20nM) targeting PSGL-1 or non-targeting control siRNA (siNT) for 48h. The cells were then either extracted for the Western blotting to measure the knockdown of PSGL-1 (i) or infected with HIV NL4-3 for 72h before the supernatants being collected to measure p24 release by ELISA (j), n=3. Data are representative of at least three independent experiments.

Supplementary Fig. 6. Analysis of the protein abundance alterations of known ubiquitination substrates of the Cul5-Rbx1 E3 ligases. Plot of Log2 ratio of calculated protein abundances in G+ population and G- population of the known ubiquitination substrates of Cul5-Rbx1 E3 ligases.
Supplementary Table 1: Statistics of proteins identified with significant abundance changes between the three cell populations (V-, G- and G+).

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<th>Upregulated</th>
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<th>Downregulated</th>
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<td></td>
<td>In at least 2 MS runs</td>
<td>In both donors</td>
<td>In at least 2 MS runs</td>
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<td>G+/G-</td>
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<td>G-/V-</td>
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<td>616</td>
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The numbers indicate the number of proteins that have significant abundance changes between the two indicated cell populations in either two mass spectrometry (MS) runs or in both donors’ samples (at least one run of each donor). For example, G+/G- upregulated means the proteins have higher abundance in G+ cell population than in G- cell population. The different proteomic coverages of the two donors’ samples are due to the different cell numbers/infection rate of the two donors’ samples, in addition to person-to-person variation in protein expression, therefore, we did not restrict our analysis to proteins that were differentially expressed in both donors.

List of Supplementary Data Sets

Data Set 1. Raw mass spectrometry intensities of all identified proteins;
Data Set 2. List of Proteins with differential abundances among cell populations;
Data Set 3. Gene Ontology analysis of proteins with differential abundances among cell populations;
Data Set 4. List of interferon stimulated genes;
Data Set 5. List of read counts of RNA-Seq analysis;
Data Set 6. List of genes with differentially mRNA levels among cell populations;
Data Set 7. List of positive selected genes.