Supplementary Figure 1

Alternative gene ranking method reveals an identical set of HIV HDFs.

Gene scores were calculated by using mean log2 fold change in the abundance of all sgRNAs for each gene.
Supplementary Figure 2

mRNA expression analysis of *SLC35B2* and *TPST2* in GXRCas9 cells subcloned following CRISPR-based knockout.

Wild-type *SLC35B2* and *TPST2* mRNA expression levels in WT GXRCas9 cells and *TPST2* and *SLC35B2*-knockout clones as assessed by qRT-PCR, using primers that overlap the sgRNA target site to selectively amplify wild-type cDNA. Error bars, s.d. from triplicate reactions.
Supplementary Figure 3

*ALCAM*-null GXRCas9 cells are protected from a multi-round, spreading JR-CSF infection.

Low MOI (MOI = 0.1) virus challenge. Six days following JR-CSF infection, viable, GFP− cells were counted and cell number was normalized to that under a mock-infected condition. Error bars, s.d. from triplicate wells; *P < 0.0001, Welch's t test.
Supplementary Figure 4

Validation of SLC35B2 as an HIV HDF in primary human CD4+ T cells.

Sulfation of surface CCR5 in primary CD4+ T cells following JR-CSF or Rejo.C challenge. Intracellular HIV Gag (p24) and total and sulfated surface CCR5 expression are shown as assessed by flow cytometry. Error bars, s.d. from triplicate wells; *P < 0.01, Welch’s t test. All P < 0.0001, except as follows: Donor 1 uninfected vs. Rejo.C p24–, P = 0.0005; Donor 2 uninfected vs. JRCSF p24–, P = 0.0003; uninfected vs. Rejo.C p24–, P = 0.0033; Rejo.C p24– vs. p24+, P = 0.0001.

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Supplementary Figure 5

mRNA expression analysis of selected genes involved in T cell adhesion.

mRNA expression of ALCAM, LFA-1, and the ICAM family in primary CD4+ T cells and GXRCas9 cells as assessed by RNA sequencing.
Antibody blockade of cell adhesion factors attenuates HIV spread in primary human CD4⁺ T cells.

(a) CRISPR-mediated knockout of the LFA-1 subunit (encoded by ITGAL) only blocks cell-to-cell transmission if donor and acceptor cells are both CD11a-null. (b,c) Cell-to-cell HIV transmission assay in primary CD4⁺ T cells following blockade with antibody to ICAM-1/LFA-1 (b) or CD45, as a control (c). The assay is set up as in Figure 5 except that donor cells are infected 36 h prior to co-culture and co-culture is for 2 d. Readout is by flow cytometry following intracellular staining for HIV Gag. Antibodies against ICAM-1, CD11a, and CD18 are added 15 min prior to co-culture. Error bars, s.d. from triplicate wells; *P < 0.01, Welch’s t test. P values were as follows: (a) n.s., P = 0.836; *P = 0.0036; (b) *P = 0.002; (c) n.s., P = 0.72.
Supplementary Figure 7

Loss of RELA, a candidate HDF identified by three previous RNAi screens, does not protect GXR cells from JR-CSF viral challenge.

Virus challenge assay (JR-CSF, MOI = 1) of GXRCas9 cells transduced with sgRELA (left) and immunoblot demonstrating depletion of RelA (right). RagC is a loading control. Error bars, s.d. from triplicate wells; n.s., $P = 0.787$, Welch’s $t$ test.
Supplementary Figure 8

Essentiality analysis of screen hits and selected putative HIV HDFs.

Screen for essential genes in the Raji B cell line (Wang et al., 2015). For every gene in the human genome, the mean of the individual log2-transformed fold change values in the abundance of each of the sgRNAs targeting that gene is shown. Screen hits and selected putative HIV HDFs that are among the 10% most depleted (i.e., cell-essential) genes are highlighted.
mRNA expression analysis of paralogous sulfation pathway genes.

(a,b) mRNA expression of TPST2, SLC35B2, PAPSS1, and select paralogs in GXRCas9 cells (a) and activated, primary CD4⁺ T cells and GXRCas9 cells (b) as assessed by RNA sequencing.
Supplementary Note

Development of a CD4+ T cell line model for pooled, CRISPR/Cas9 screens for HIV

Because CD4+ T cells are the primary target cells for HIV infection, we selected the CD4+ T cell leukemia line, CCRF-CEM. In previous work\(^1\), CCRF-CEM cells were engineered to express high levels of CCR5 and GFP under the control of the HIV-1 LTR promoter, which is driven in \textit{trans} by the HIV protein \textit{tat}\(^2\). Productive HIV infection leads to GFP expression, allowing the cellular infection state to be monitored at the single cell level by flow cytometry.

We stably transduced these cells, termed “GXR”, with a Cas9 expression vector (Fig. 1a). A sub-clone, hereafter termed “GXRCas9”, was selected based on the following criteria: (1) high CD4, CCR5, and CXCR4 surface expression, (2) low basal GFP expression and high induced GFP expression upon HIV infection, and (3) high Cas9 activity as demonstrated by transducing the cells with a lentiviral sgRNA construct targeting \textit{CCR5}. Following HIV infection, a large and clearly distinguishable GFP-high population was induced in GXRCas9 cells, while GFP expression remained at baseline following HIV infection in GXRCas9 cells transduced with a \textit{CCR5}-targeting sgRNA (Fig. 1b). Our experimental system thus allows identification and sorting of cells that are resistant to HIV infection, enabling pooled genetic screening for HIV dependency factors.

Identification of host genes that inhibit rather than facilitate HIV infection

As our primary interest was in identifying candidates for novel host-directed anti-HIV therapies, we optimized our screen to identify host genes whose loss conferred a high degree of protection against productive, multi-round HIV infection. In order to accomplish this, we (1) identified a cell line that was highly permissive to HIV infection (2) infected cells with replication-competent HIV at a low MOI and cultured for an extended period of time, and (3) re-challenged the surviving cells with HIV. We note that this stringent selection protocol is unsuited for identifying genes whose loss would enhance HIV infection (i.e. host restriction factors). To identify genes that inhibit the HIV infection process, significant modifications to the screen, such as sorting soon after viral challenge to isolate the most readily infected cells and using a cell line that is more resistant to HIV infection, would be required.

Role of sulfation in HIV latency and virion stability

Sulfation may affect HIV in ways not related to entry. A recent study demonstrated that knockdown of a cytosolic sulfotransferase in monocyte-derived macrophages disrupted HIV reverse transcription through an unknown mechanism\(^3\), and chemical inhibition of sulfation decreased latent HIV reactivation in primary T cell and cell line models of latency\(^4\).

Additionally, inhibition of the sulfation pathway may reduce the virulence of HIV virions. High levels of tyrosine sulfation on gp120 are seen in virions released from activated primary CD4+ T cells, consistent with the high expression of \textit{TPST2} we observed. Inhibiting this tyrosine sulfation de-stabilizes the native envelope trimer\(^5\). This suggests that targeting \textit{TPST2} or \textit{SLC35B2} in primary T cells may confer an additional protective effect by de-stabilizing gp120 on intact HIV virions.
Disruption of T cell aggregation as a therapeutic approach for HIV

Therapeutic approaches to induce immune tolerance by inhibiting the heterotypic and homotypic adhesion of a wide range of immune cell types have already been developed\textsuperscript{6}. A randomized, multicenter clinical trial demonstrated that the safety and efficacy of such an approach in patients undergoing transplantation\textsuperscript{7}. Though we have demonstrated that disrupting interactions between ICAM-1 and LFA-1 through CRISPR-mediated knockout and antibody blockade can confer partial protection from cell-to-cell HIV transmission, more efficient methods to disrupt these aggregates would be needed to confer strong protection against cell-to-cell transmission akin to what we observed in the ALCAM-null GXRCas9 cell line. Strategies should also be developed to disrupt T cell aggregation specifically in tissue sites of HIV transmission, such as the rectum and female genital tract, or in sites of high T cell contact, such as lymphoid organs, to extend these \textit{in vitro} findings to the clinical setting.
Supplementary Note References


