HIV-1-induced cytokines deplete homeostatic innate lymphoid cells and expand TCF7-dependent memory NK cells

Yetao Wang1, Lawrence Lifshitz1, Kyle Gellaty2, Carol L. Vinton3, Kathleen Busman-Sahay4, Sean McCauley1, Pranitha Vangala2, Kyusik Kim1, Alan Derr1, Smita Jaiswal1, Alper Kucukural2, Patrick McDonel2, Peter W. Hunt5, Thomas Greenough1, JeanMarie Houghton6, Ma Somsouk5, Jacob D. Estes4, Jason M. Brenchley3, Manuel Garber1,2, Steven G. Deeks5 and Jeremy Luban1,7*

Human immunodeficiency virus 1 (HIV-1) infection is associated with heightened inflammation and excess risk of cardiovascular disease, cancer and other complications. These pathologies persist despite antiretroviral therapy. In two independent cohorts, we found that innate lymphoid cells (ILCs) were depleted in the blood and gut of people with HIV-1, even with effective antiretroviral therapy. ILC depletion was associated with neutrophil infiltration of the gut lamina propria, type 1 interferon activation, increased microbial translocation and natural killer (NK) cell skewing towards an inflammatory state, with chromatin structure and phenotype typical of WNT transcription factor TCF7-dependent memory T cells. Cytokines that are elevated during acute HIV-1 infection reproduced the ILC and NK cell abnormalities ex vivo. These results show that inflammatory cytokines associated with HIV-1 infection irreversibly disrupt ILCs. This results in loss of gut epithelial integrity, microbial translocation and memory NK cells with heightened inflammatory potential, and explains the chronic inflammation in people with HIV-1.

Innate lymphoid cells (ILCs) are T cell counterparts that lack clonotypic antigen receptors or other lineage-defining cell-surface markers. ILCs carry out many biological functions that include host defense against pathogens and homeostatic maintenance in inflamed tissues. Among the many subsets of ILCs are natural killer (NK) cells, which are capable of lyzing tumor cells and virus-infected cells. ILCs are divided into three main subtypes based on the key transcription factors and cytokines that they produce. ILC1s and NK cells both express the transcription factor TBX21 and secrete interferon-γ (IFN-γ). ILC2s express the prostaglandin D2 receptor CRTH2, require the transcription factor GATA3 for their development, and produce the cytokines interleukin-4 (IL-4), IL-5, IL-9, IL-13 and amphiregulin. ILC3s express the transcription factor RORγt and produce IL-17 and IL-22.

Blood ILCs are irreversibly depleted by HIV-1 if ART is not started within a few weeks of infection. Simian immunodeficiency virus infection of macaques decreases homeostatic ILC3s in the intestine. The contribution of ILC deficiency to HIV-1-associated chronic inflammation remains unclear since abnormalities in homeostatic ILCs from human tissues such as the lamina propria have not been reported.

NK cells rapidly respond to virus-infected cells or tumors, independent of antigen-specific recognition. NK cells eliminate infected cells via antibody-dependent cell cytotoxicity. The clinical significance of NK cells for control of HIV-1 is supported by the influence of human leukocyte antigen (HLA) haplotype and NK cell killer cell immunoglobulin-like receptors (KIRs). Studies in mice, rhesus macaques and humans have shown that the response of NK cells is enhanced by a previous encounter with inflammatory cytokines, hapten or vaccination, or by certain viruses. How closely these memory-like NK cells share transcriptional and chromatin features with memory T cells, and whether HIV-1 infection affects this unique NK population, is not known.

Here, to better understand the etiology of the ongoing inflammation that is often observed with HIV-1 infection, despite effective suppression of viremia by ART, a detailed examination of the effect of HIV-1 on ILCs and NK cells was performed.

Results

HIV-1 infection decreases ILCs in the blood and in the colon lamina propria. To identify ILCs from among human peripheral...
blood mononuclear cells (PBMCs), 16 cell-surface proteins were used to exclude T cells, B cells, NK cells and other cells from defined lineages within the lymphoid gate (Extended Data Fig. 1a), and ILCs were defined as Lin–CD127– cells4. As previously described36, the majority of human blood ILCs were CRTH2+ and/or CD117+ (Extended Data Fig. 1b). Blood ILCs from people who were HIV-1– were then compared with those from people who were HIV-1+, either HIV-1 viremic, ART suppressed or spontaneous controllers of viremia who were not on ART (cohort described in Supplementary Table 1). Compared with people who were HIV-1–, ILCs were significantly decreased in all groups with HIV-1 infection (Fig. 1a and Extended Data Fig. 1c). The percentage of ILCs correlated with the number of CD4+ T cells, the CD4+ T cell nadir and the ratio of CD4+-to-CD8+ T cells (Fig. 1b,c and Extended Data Fig. 1d). In contrast, ILC percentage was inversely correlated with plasma sCD14 (Fig. 1d)—a protein released by monocytes in response to lipopolysaccharide that independently predicts clinical outcome of HIV-1 infection1. These results indicate that, despite repressed viral replication in people who are HIV-1+ and on ART, or in people who spontaneously control viremia, there is ongoing inflammation and a persistent decrease in blood ILCs.

Chronic systemic inflammation in people who are HIV-1+ has been associated with inappropriate translocation of bacterial products from the intestinal lumen12. Given the reduced number of ILCs in blood from people who are HIV-1+, and the fact that lamina propria ILCs contribute to the maintenance of gut epithelial integrity3, the effect of HIV-1 infection on colon lamina propria ILCs was examined next. In HIV-1+ controls, the majority of Lin–CD127– colon ILCs were CD117+RORγT+ ILC3s and capable of producing IL-22 in response to stimulation (Extended Data Fig. 1d). In people who were HIV-1+ and on effective anti-HIV-1 therapy (participants described in Supplementary Table 2), colon lamina propria ILCs were reduced in frequency (Fig. 1e,f and Extended Data Fig. 1g), despite undetectable viremia and no significant reduction in lamina propria CD4+ T cells (Extended Data Fig. 1h and Supplementary Table 2).

ILCs3 in the gut of people who were HIV-1+ and on ART were then assessed by immunohistochemistry. Compared with people who were HIV-1–, ILC3s were significantly decreased in rectosigmoid tissue from people who were HIV-1+ and on ART—a reduction that was even more pronounced when ILCs were normalized to total CD3+ T cells (Fig. 1g–i, Extended Data Fig. 1i and Supplementary Table 1). In samples from people who were HIV-1+ and on ART, CD3+ T cells were observed within large inflammatory infiltrates that originated in the submucosa and extended through the muscularis mucosae to the lamina propria, at times reaching the epithelium (Fig. 1g). This was rarely observed in people who were HIV-1–. Moreover, cells that were positive for the type I IFN-inducible protein MX1, or for the neutrophil marker myeloperoxidase (MPO), were increased in the gut of the people who were HIV-1+ and on ART, consistent with ongoing pathogenic inflammation in the gastrointestinal tract despite anti-HIV-1 therapy (Fig. 1j–m and Supplementary Table 1).

ILCs do not express CD4 or CCR5, they do not bear these proteins on their surface and they cannot be infected ex vivo by HIV-1 (Extended Data Fig. 1j). A reduction in ILCs is therefore unlikely to result from direct infection of these cells by HIV-1. To determine whether the decrease in CD127+ ILCs associated with HIV-1 infection might be a consequence of systemic elevation in cytokines, inflammatory metabolites, or leakage of microbes across the intestinal epithelium37, the effect on Lin–CD127+ PBMCs of exposure to these factors in vitro was assessed. No significant decrease in CD127 was observed after exposure to any of four cytokines (IL-6, IL-8, IL-10 or tumor necrosis factor), three chemokines (MCP-1, IP-10 or BCA-1), four Toll-like receptor agonists (R848, poly(I:C), Pam3CSK4 or lipopolysaccharide), or l-kyonurine (data not shown). However, as reported for T lymphocytes38, common γ-chain cytokines that are systemically elevated during HIV-1 infection1, including IL-2, IL-4 and IL-15, decreased the frequency of CD127+ cells (Fig. 1n and Extended Data Fig. 1k). A JAK3 inhibitor, CP-690550, prevented CD127 downregulation by IL-15 (Fig. 1n), consistent with a requirement for cytokine signaling via JAK3 (ref. 39). Neither the JAK1/2 inhibitor ruxolitinib nor the mTOR inhibitor rapamycin prevented CD127 downregulation (Fig. 1n). These results suggest that JAK3 signaling, in response to systemic elevation of common γ-chain cytokines, decreases ILCs in HIV-1 infection. This would deprive intestinal epithelium of homeostatic ILC3s, disrupt the integrity of the colon epithelium and explain ongoing inflammation associated with HIV-1 infection (Extended Data Fig. 1).

HIV-1 infection increases the proportion of CD94+ NK cells. Total NK cells (Lin–TBX21+ PBMCs), including CD56– NK cells and the CD56– NK cells that have been reported to increase with HIV-1 infection1, were assessed from people who were HIV-1+ and HIV-1+ who were either HIV-1 viremic, ART suppressed, or spontaneous controllers of viremia who were not on ART (Supplementary Table 1). No significant difference in the percentage of total NK cells was observed among any of the HIV-1+ groups (Fig. 2a,b).

CD94 is found on NK cell subsets with antiviral activity40 and associated with NK cell memory41. CD94+ NK cells were significantly increased in PBMCs from people who were HIV-1+ who are not on ART (Fig. 2c,d and Supplementary Table 1). However, people who were HIV-1+ and on ART, or people who spontaneously controlled viremia, had CD94 levels similar to those who were HIV-1–, indicating that ongoing viral replication, or inflammation associated with HIV-1 replication, contributes to the increase in CD94+ NK cells (Fig. 2c,d).

Stimulation of PBMCs from anonymous HIV-1– blood donors, with either PMA plus ionomycin, IL-15 (which is upregulated in HIV-1 infection) or IL-15 plus IL-12, increased the ratio of CD94+ to CD94– NK cells similar to that observed in the blood of people...
who were HIV-1+ (Extended Data Fig. 2a). When CD94− NK cells were sorted (Lin CD56−CD94− PBMCs in Extended Data Fig. 2b), CD94 levels increased in response to stimulation with PMA plus ionomycin or IL-12 plus IL-15 (Fig. 2e,f). When stimulated with PMA plus ionomycin, CD94+ NK cells showed higher degranulation activity (Extended Data Fig. 2c). In the absence of stimulation, sorted CD94+ NK cells had greater cytolytic activity than did CD94− NK cells (Extended Data Fig. 2d). Levels of Ki-67 and annexin V were comparable on the CD94− and CD94+ NK cells after stimulation, suggesting that the perturbed ratio of these cells in vivo did not result from intrinsic differences in rates of proliferation or apoptosis (Extended Data Fig. 2e).
TCF7 expression tracks with pseudotime trajectory from CD94−CD56dim NK cells to CD94+CD56hi NK cells. To better understand the molecular basis for the increase in CD94+ NK cells associated with HIV-1 infection, transcriptional profiles were obtained using sorted CD94+ and CD94− NK cells from HIV-1− anonymous blood donors (Lin−CD56−CD94+ and Lin−CD56−CD94− PBMCs as in Extended Data Fig. 2b). RNA sequencing (RNA-seq) revealed 210 genes that were differentially expressed in the two NK cell populations (Fig. 2g and Supplementary Table 3). Proteins encoded by differentially expressed genes that the literature indicates are important for NK cell function are highlighted in the heatmap (Fig. 2g) and were confirmed by flow cytometry (Fig. 2h and Extended Data Fig. 2f). CD94+ NK cells expressed higher levels of GZMK, TCF7, CXCR3, CD44, CD2 and SELL, the biological functions of which include NK cell survival, activation and memory. In contrast, gene expression and surface protein levels of KIR2DL1—an HLA-Cw4 ligand that inhibits NK cell cytotoxicity—were increased on CD94− NK cells (Fig. 2h and Extended Data Fig. 2f).

Transcriptomes across the spectrum from CD94− to CD94+ NK cells sorted from HIV-1− PBMCs were then captured by single-cell RNA-seq (Fig. 3a, Extended Data Fig. 3a and Supplementary Table 4)11. Based on analysis of the 3,277 single-cell transcriptomes, CD94+ NK cells formed a homogeneous population (Fig. 3a, pink dots in upper right). CD94− NK cells formed two populations, one of which overlapped with CD94+ NK cells (Fig. 3a, aqua dots in upper right). Unbiased clustering of total Lin−CD56− PBMCs, irrespective of whether or not a cell was CD94+, also showed two distinct cell populations (Fig. 3b). The validity of clustering cells into two groups was confirmed by calculation prediction strength as a function of cluster number (Extended Data Fig. 3b). Cluster 1 was relatively homogeneous, consisting of 396 CD94+ NK cells and only 13 CD94− NK cells (Fig. 3b, red dots in lower left). Cluster 2 contained 371 CD94+ NK cells and 973 CD94− NK cells (Fig. 3b, blue dots in upper right). A heatmap based on all differentially expressed genes between the two clusters showed a shift in the pattern of gene expression along the continuum of CD94+ expression (see blue and yellow bars at the top of Fig. 3c and Supplementary Table 5).

To determine whether heterogeneity in the individual CD94− NK cell transcriptomes reflected different stages in the CD94− to CD94+ NK cell transition, a minimum spanning tree based on individual cell transcriptomes was constructed using Monocle23 (Fig. 3d). A heatmap utilizing the pseudotime ordering of single NK cell transcriptomes reflected different stages in the CD94− to CD94+ transition and maintenance 24–26), as determined by DESeq2).

Genes with the potential to regulate the transition from CD94− NK to CD94+ NK cells were identified by differential expression analysis based on the pseudotemporal ordering of cells from the minimum spanning tree. Candidate genes included TCF7 (Fig. 3e; false discovery rate (FDR) \( P < 7 \times 10^{-10} \)), a transcription factor activated by WNT signaling that is important for early T cell, NK cell and ILC development, as well as T cell memory establishment and maintenance21–26, PIM3 (a serine/threonine kinase that blocks apoptosis and promotes self-renewal27, CD44 (a gene important for T cell survival and establishment of memory cells24, CXCR3 and GZMK (genes highly expressed in memory CD8+ T cells28) and SELL (a marker for NK cells with the potential to proliferate and differentiate into effectors upon secondary stimulation29) (Fig. 3f).

When examined by flow cytometry, TCF7+ NK cells from HIV-1− blood donors were exclusively CD56hi—a marker for NK cells with high capacity for cytokine production and proliferation30. CD44, SELL and CXCR3 were also enriched in the TCF7+ NK cells (Fig. 3g and Extended Data Fig. 3d).

When total PBMCs or sorted CD94− NK cells from HIV-1− blood donors were treated with IL-15, or with IL-12 plus IL-15, CD94 was upregulated within 16 h (Fig. 2f and Extended Data Fig. 2a). After 5 d of IL-15 treatment, TCF7, CXCR3, CD44, GZMK and CD56 were upregulated (Fig. 3h and Extended Data Fig. 3e). CXCR6—a gene required for memory NK cell generation in response to hapten and viruses31—was also upregulated (Fig. 3h). These experiments in which cells were stimulated directly ex vivo substantiated the pseudotime analysis of the single-cell RNA-seq data, show that CD94+CD56hi NK cells are generated from CD94+CD56dim NK cells in response to inflammatory cytokines and suggest that the expanded CD94+ NK cell population in blood from people who are HIV-1− results from elevated inflammatory cytokines.

Distinct chromatin landscape of CD94−CD56hi NK cells. To further investigate the TCF7+CD56hi subset of CD94+ NK cells identified above (Fig. 3b, f, g), CD94+CD56dim, CD94−CD56hi and CD94−CD56dim NK cells were sorted from HIV-1− blood donors (Fig. 4a) and subjected to RNA-seq. The transcriptional profile of CD94+CD56hi NK cells was distinct from that of either CD94+CD56dim or CD94−CD56hi cells (Fig. 4b), with 275 and 162 differentially expressed genes, respectively (Fig. 4c, d and Supplementary Table 6). In contrast, only four genes distinguished CD94−CD56dim from CD94−CD56dim NK cells (Fig. 4e and Supplementary Table 6). Principal component analysis (PCA) revealed the CD94+CD56hi NK cell transcriptome to be a cluster distinct from the transcriptomes of the other NK cell subsets (Fig. 4f), and reactome pathway analysis showed it to be enriched for WNT signaling and TCF7 (Fig. 4g).

In addition to having a unique transcriptional profile, CD94+CD56hi NK cells had histone marks associated with transcriptional activation and accessiblet chromatin in chromosomal locations distinct from those in the other two NK cell subsets (Fig. 5a–c and Extended Data Fig. 4a,b). The chromatin landscapes for representative genes, including TCF7, a gene uniquely expressed in the CD94+CD56hi NK subset, and CD6, a gene only expressed in CD56hi NK cells, are shown (Fig. 5d, e and Extended Data Fig. 4c). De novo motif analysis showed enrichment for TCF7, RUNX, NK-xB and four other DNA-binding motifs within the accessible chromatin regions that were unique to CD94+CD56hi NK cells.
(Fig. 5f). In contrast, the IKZF1 binding motif was enriched within both CD56dim NK cell subsets (Fig. 5g). In CD94+CD56hi NK cells, TCF7 binding peaks mapped to 255 CD94+CD56hi NK cell ATAC-seq peaks located within genes encoding the cytokine receptors IL2RA, IL2RB and IL20RA, the transcription factors RUNX1, RUNX3 and NOTCH2, and the chromatin modifiers SETD5 and

![Image](image-url)
KMT3C, as well as the WNT signaling regulator AXIN1 (Fig. 5h and Extended Data Fig. 4d). These transcriptional and epigenetic profiles indicate that, among NK cells, CD94+CD56hi NK cell constituting a developmentally discrete subset, and that TCF7 is critical for establishment of this population.

CD94+CD56hi NK cells are bona-fide memory lymphocytes. CD94+CD56hi NK cells were enriched for transcripts associated with lymphocyte memory (Figs. 3f and 4b–d and Supplementary Table 6), including TCF7, CD44, SEL1L, CD70, IL7R, CCR1, CCR5, CCR7, BACH2 and DUSP4 (refs. 25,26,27,28). Chromatin accessibility and the density of H3K4me1 and H3K4me3 at these loci showed that these genes were specifically remodeled for heightened expression in the CD94+CD56hi NK cell subset (Figs. 5d and 6a and Extended Data Fig. 5a). In contrast, chromatin features at genes typical of effector lymphocytes, including PRDM1, CD57 and KLRG1 (refs. 29,30–32), indicated increased expression in CD56dim NK cell subsets (Fig. 6a and Extended Data Fig. 5a). Consistent with the discrete developmental stages revealed by the analysis of chromatin, pairwise comparison of the three NK cell subsets showed that only CD94+CD56hi NK cells were enriched for transcripts that distinguish CD8+ memory T cells (GSE9650; Fig. 6b,c and Supplementary Tables 7 and 8).

As expected for memory T lymphocytes, stimulation of CD94+CD56hi NK cells revealed a higher capacity for cytokine-induced proliferation, IFN-γ production and degranulation in response to either K562 or HIV-1-infected CD4+ T cells than was observed with the CD56dim NK cell subsets (Fig. 6d–g and Extended Data Fig. 5b). TCF7 was downregulated on sorted CD94+CD56hi NK cells that had undergone multiple rounds of replication in response to stimulation (Fig. 6d), as was reported for the transition to effector cell phenotype that follows stimulation of memory CD8+ T cells33–35. IFNγ-AS1, a long noncoding RNA required for epigenetic activation of IFN-γ production36–38, was expressed 30–200 times higher in CD94+CD56hi NK cells than in the CD56dim NK cell subsets (Fig. 6h).

The chromatin structure at the IFNG-AS1 locus, as well as at other genes contributing to IFN-γ induction, including CD28, RUNX3, ILX, GZMB and RAG2 (Fig. 6i and Extended Data Fig. 5c)36,37, indicates that robust IFN-γ production by CD94+CD56hi NK cells in response to stimulation is a developmental adaptation characteristic of memory lymphocytes.

By flow cytometry, CD94+CD56hi NK cells have higher levels of the memory NK cell marker CXCR6 (ref. 39), but lower levels of CD16, the effector and senescence marker CD57 and the inhibitory receptors KIR2DL1 and KIR3DL1 (ref. 40) (Extended Data Fig. 6a–c). The central memory character of CD94+CD56hi NK cells is also suggested by the fact that these cells proliferate and upregulate CD57 in response to inflammatory cytokines (Extended Data Fig. 6c). These results show that, as well as exhibiting typical memory features at the chromatin and transcriptional level, CD94+CD56hi NK cells functionally mimic memory T cells.

WNT signaling and TCF7 are required for the establishment of NK cell memory. The requirement for WNT signaling and TCF7 for the establishment of CD94+CD56hi NK memory cells was examined next (Figs. 3e–h, 4g and 5d,f,h and Extended Data Figs. 4d and 6d). First, the effect of pharmacologic inhibition of WNT signaling on the generation of memory cells from sorted CD94+CD56dim NK cells was assessed using established methods (Fig. 7a)39,40. In response to primary stimulation with IL-12, IL-15 and IL-18 for 16 h, followed by 5 d rest in low-dose IL-15 alone, CD94+CD56dim NK cells upregulated IFNG-AS1 (Fig. 7b) and acquired a global transcriptional profile characteristic of CD8+ memory T cells (as determined by comparison with GSE9650; Fig. 7c–e and Supplementary Tables 9 and 10)41–43. Upregulated genes not identified in the GSE9650 data, but that other datasets indicate are typical of memory lymphocytes, included DUSP4, CDK91A, RGS1, CCR1, IL2RA, CD70, CD74, STAT1 and ITGA1 (Fig. 7f)44–48. Downregulated genes characteristic of naive or effector lymphocytes included SEL1L, ITGA2 and KLRG1 (Fig. 7f).

All of these effects on stimulated CD94+CD56dim NK cells were prevented if the WNT signaling inhibitor LGK974 was present in the culture (Fig. 7g). No toxicity was detected with LGK974 for up to 8 d in culture, with or without stimulation, and the drug had no effect on CD94 dim levels in the absence of stimulation (Extended Data Fig. 7a) or on IFN-γ production in response to primary stimulation (Extended Data Fig. 7b). Importantly, the heightened IFN-γ production in response to secondary stimulation was inhibited by LGK974 or by knockdown of TCF7 (Fig. 7g and Extended Data Fig. 7c,d). These results show that WNT and TCF7 signaling are required for the establishment of memory after primary stimulation of CD94+CD56dim NK cells.

Expansion of TCF7+ NK cells in HIV-1 infection. Finally, the effect of HIV-1 on the percentage of TCF7+ NK cells in blood was assessed. Compared with people who were HIV-1−, TCF7+ NK cells were significantly upregulated in HIV-1 viremic and ART-suppressed individuals, but not in spontaneous controllers (Fig. 8a and Supplementary Tables 1 and 2). These results indicate that TCF7+ NK cells may be increased due to the inflammation that persists, despite suppression of viremia by ART, and that these cells may themselves contribute to the ongoing inflammation under these conditions. In contrast with people who were HIV-1−, in whom TCF7 was almost exclusively expressed in CD94+CD56hi NK cells (Fig. 3f,g), people who were HIV-1+ with uncontrolled viremia or under ART also had increased TCF7 levels on CD94−CD56dim NK cells (Fig. 8b and Supplementary Table 1,2). The percentage of either CD56− cells or TCF7− NK cells correlated inversely with the percentage of blood ILCs, indicating that HIV-1-associated inflammation had opposite effects on NK cells and ILCs (Fig. 8c, Extended Data Fig. 7e and Supplementary Table 1). A similar increase with HIV-1 infection was observed for another memory NK marker, NKG2C (Fig. 8d and Supplementary Table 1,2). Analogous to the expansion of memory T cells associated with the chronic inflammation that accompanies HIV-1 infection, CD94+ NK cells and CD94+TCF7+ memory NK cells are both likely to have expanded from CD94− NK cells in response to the elevated systemic cytokines that accompany HIV-1 infection (Fig. 8e).
Discussion
ART suppresses HIV-1 viremia, preserves CD4+ T cells and prevents progression to AIDS. Nonetheless, non-AIDS inflammatory pathology has been observed in such individuals. This inflammation has been attributed to disruption of the intestinal epithelium, the integrity of which is maintained by homeostatic...
cytokines produced by lamina propria CD4+ T_{h}17 cells and ILC3s. Experiments here, with tissues from people who were HIV-1+ and on ART, with undetectable viremia, show reduced ILC3s, neutrophil infiltration and MX1+ cells in the intestine, and elevated plasma sCD14. Given that blood ILCs are disrupted soon after HIV-1 infection, it is likely that colon lamina propria ILCs are also depleted during acute HIV-1 infection, with attendant loss of epithelial barrier function, and chronic inflammation despite pharmacologic suppression of viremia.

ILCs do not express CD4 or CCR5 (the essential HIV-1 entry receptors), and infection of ILCs ex vivo is undetectable with either CCR5-tropic or CXCR4-tropic HIV-1 (ref. 4). It is therefore more likely that ILCs are eliminated by stimulation with the common γ-chain cytokines associated with acute HIV-1 infection, rather than

Fig. 4 | TCF and WNT signaling pathway enrichment in CD94+CD56hi NK cells. a, Sorting strategy for CD94-CD56dim, CD94+CD56dim and CD94+CD56hi NK cell subsets. b, Heatmap of differentially expressed genes, as determined by RNA-seq (log₂[fold change of normalized counts]>1; P<0.05, determined by DESeq2), for the indicated NK cell subsets sorted from four HIV-1+ blood donors. c, c-e, Pairwise comparison of the indicated NK cell subsets based on differentially expressed genes. f, PCA based on RNA-seq data from the indicated NK cell subsets. g, Reactome pathway analysis based on 152 differentially expressed genes in CD94+CD56hi NK cells. Gene Ontology-produced P values (as determined by Fisher’s exact test) with FDR correction (Benjamini–Hochberg method) are shown in parentheses. All data were generated using blood from HIV-1+ donors.
by direct HIV-1 infection. In contrast with the CD4+ T cell recovery often observed with ART, ILC reduction appears permanent, perhaps due to ablation of a dedicated ILC precursor. Alternatively, ILC depletion might be an ongoing response to inflammation that could be interrupted with JAK3 inhibitors such as the one used here to protect ILCs from cytokine stimulation ex vivo.

CD94+NKG2C+ NK cells were increased in people infected with HIV-1, but the total percentage of NK cells among PBMCs was not altered by HIV-1, showing that CD94+NK cells increase at the expense of CD94− NK cells. Pseudotime analysis of single-cell transcriptomes mapped a TCF7-dependent trajectory from CD94− NK cells to CD94+ NK cells, and stimulation of CD94+ NK cells ex vivo gave rise to a homogeneous population of CD94+TCF7+ NK cells, indicating that the increase in CD94+ NK cells is in response to HIV-1-associated inflammation. The inverse correlation between TCF7+CD56hi NK cells and ILCs indicates that the expansion of TCF7+CD56hi NK cells in people who are HIV-1+ may occur secondary to the inflammation that results from loss of gut-resident, homeostatic ILC3s.

Fig. 5 | Distinct chromatin landscape in CD94+CD56hi NK cells. a–c, Heatmap showing differential enrichment (>2 fold change of normalized counts) for H3K4me1 (a) and H3K4me3 (b) by CUT&RUN, as well as accessible chromatin by ATAC-seq (c), in sorted CD94−CD56dim, CD94+CD56dim and CD94+CD56hi NK cell subsets. Data are representative of two HIV-1− blood donors. d,e, H3K4me1, H3K4me3 and ATAC-seq signals on TCF7 (d) and CD6 (e) in the indicated NK cell subsets. f,g, De novo analysis of transcription factor binding motifs enriched in open chromatin from CD56hi NK cells (f) and CD56dim NK cells (g), using HOMER. h, ATAC-seq and TCF7 CUT&RUN signal at the indicated loci from CD94+CD56hi NK cells. IgG, immunoglobulin G.

All data were generated using blood from HIV-1− anonymous donors. Numbers on the right side of d,e and h are the range of values for the indicated tracks, multiplied by 106 and divided by the total number of mapped reads.
Fig. 6 | Chromatin, transcriptome and phenotype define CD94+CD56hi NK cells as memory cells. a, CUT&RUN and ATAC-seq showing memory gene-associated loci in sorted CD94+CD56dim, CD94+CD56dim and CD94+CD56hi NK cells. b, c, GSEA comparing the memory CD8+ T cell expression signatures (GSE9650) from sorted CD94+CD56hi and CD94+CD56dim NK cells (b) or from sorted CD94+CD56dim and CD94+CD56hi NK cells (c). P values and FDRs were determined using the GSEAPreranked module from GenePattern with 1,000 permutations. NES, normalized enrichment score. d, Sorted NK cells labeled with carboxyfluorescein succinimidyl ester (CFSE) and cultured for 5 d in IL-15 (5 ng ml−1). CFSE and TCF7 were detected by flow cytometry. e, Percentage of IFN-γ+ cells by flow cytometry after stimulation of sorted NK cell populations with IL-12+IL-15 (n = 4). f, Fold increase of surface CD107a on bead-enriched NK cells after incubation with K562 cells (n = 4). g, Bead-enriched NK cells incubated with IL-15 (5 ng ml−1) for 5 d, then with NL4-3-nef-GFP-infected CD4+ T cells. The fold increase of surface CD107a on the indicated NK populations is shown, as in f (0 h: n = 5; 0.5, 1 and 2 h: n = 3; 3 h: n = 5). h, IFNG-AS1 expression relative to GAPDH by quantitative reverse transcription PCR for the indicated NK cell populations sorted from three donors. Each population contains two technical replicates. i, IFNG-AS1 chromatin analysis as in a. Data are means ± s.e.m. Statistical significance was determined by two-way ANOVA (e–g) or two-tailed unpaired t-test (h). *P < 0.05; **P < 0.01; ***P < 0.001. All data were generated using blood from HIV− anonymous donors.
The CD94+TCF7+CD56hi NK cells expanded with HIV-1 infection satisfy criteria for bona-fide memory cells, including increased rates of proliferation and production of cytokines, although, unlike memory NK cells in human cytomegalovirus infection, they did not bear markers typical of senescence or effector function, such as CD57, PRDM1 and KLRG1 (ref. 8). Instead, global assessment of transcription, chromatin marks and chromatin accessibility, the magnitude and kinetics of cytokine production, and degranulation in response to stimulation with K562 cells or HIV-1-infected CD4+ T cells, showed that these CD94+TCF7+CD56hi NK cells resemble memory T cells, except for the lack of clonotypic antigen receptors.

TCF7 is required for the establishment and maintenance of memory T cells85. In a similar fashion, TCF7 was shown here to be required for the establishment and maintenance of memory NK cells. TCF7 may act as a transcription factor in the WNT signaling pathway or as a histone deacetylase48. The fact that the TCF7 knockdown and WNT inhibitor had similar phenotypes indicates that, by acting as a transcription factor in the WNT signaling pathway, TCF7 is an essential regulator of a memory state in NK cells.

The NKG2C+CD94+CD56hiTCF7+ NK cells that are increased with HIV-1 infection may provide necessary antiviral protection. People who inherit a deletion in the gene encoding NKG2C are at greater risk of HIV-1 infection, and have higher pre-treatment viral loads and faster disease progression49. Specific KIR and HLA genotype combinations influence rates of HIV-1 acquisition and disease progression, perhaps due to KIR-mediated NK cell licensing or recognition of HIV-1-derived peptides by non-clonotypic receptors. Interestingly, high...
percentages of CD56\(^+\) NK cells have been associated with nearly undetectable viral load\(^{10}\), suggesting that TCF7\(^+\)NKG2C\(^+\)CD94\(^+\)CD56\(^+\) NK cells can control HIV-1. It is hoped that better understanding of these memory NK cells will lead to new approaches for preventing and controlling HIV-1 and other infections.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at [https://doi.org/10.1038/s41590-020-0593-9](https://doi.org/10.1038/s41590-020-0593-9).

Received: 11 January 2019; Accepted: 28 December 2019; Published online: 17 February 2020

**References**

1. Deeks, S. G., Tracy, R. & Douek, D. C. Systemic effects of inflammation on health during chronic HIV infection. *Immunity* **39**, 633–645 (2013).
2. Brenchley, J. M. et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat. Med.* **12**, 1365–1371 (2006).
3. Vivier, E. et al. In Vitro activation of lymphocytes: 10 years on. *Cell* **174**, 1054–1066 (2018).
4. Kla-verpris, H. N. et al. In Vitro activation of lymphocytes: 10 years on. *Cell* **174**, 1054–1066 (2018).
5. Mudd, J. C. et al. Hallmarks of primate lentiviral immunodeficiency infection recapitulate loss of innate lymphoid cells. *Nat. Commun.* **9**, 3967 (2018).
6. Bruel, T. et al. Elimination of HIV-1-infected cells by broadly neutralizing antibodies. *Nat. Commun.* **7**, 10844 (2016).
7. Alter, G. et al. HIV-1 adaptation to NK-cell-mediated immune pressure. *Nature* **476**, 96–100 (2011).
8. Cerwenka, A. & Lanier, L. L. Natural killer cell memory in infection, inflammation and cancer. *Nat. Rev. Immunol.* **16**, 112–123 (2016).
9. Lim, A. I. et al. Systemic human ILC precursors provide a substrate for tissue ILC differentiation. *Cell* **168**, 1086–1100.e10 (2017).
10. Colonna, M. Innate lymphoid cells: diversity, plasticity, and unique functions in immunity. *Immunity* **48**, 1104–1117 (2018).
11. Mazzucchelli, R. & Durum, S. K. Interleukin-7 receptor expression: intelligent design. *Nat. Rev. Immunol.* **7**, 144–154 (2007).
12. Leonard, W. J. & O'Shea, J. J. JAKS and STATs: biological implications. *Annu. Rev. Immunol.* **16**, 293–322 (1998).
13. Nadl, D. et al. Characterization of CD56\(^-\)/CD16\(^+\) natural killer (NK) cells: a highly dysfunctional NK subset expanded in HIV-infected viremic individuals. *Proc. Natl Acad. Sci. USA* **102**, 2886–2889 (2005).
14. Fang, M. et al. CD94 is essential for NK cell-mediated resistance to a lethal viral disease. *Immunity* **34**, 579–589 (2011).
15. Jeevan-Raj, B. et al. The transcription factor Tcfl contributes to normal NK cell development and function by limiting the expression of granymes. *Cell Rep.* **20**, 613–626 (2017).
16. Wendel, M., Galani, I. E., Suri-Payer, E. & Cervera, A. Natural killer cell accumulation in tumors is dependent on IFN-\(\gamma\) and CXCR3 ligands. *Cancer Res.* **68**, 8437–8445 (2008).
17. Scovocchia, G., Titus, J. A. & Segal, D. M. Signaling pathways regulating CD44-dependent cytosis in natural killer cells. *Blood* **90**, 716–725 (1997).
18. Liu, L. L. et al. Critical role of CD2 co-stimulation in adaptive natural killer cell responses revealed in NKG2C-deficient humans. *Cell Rep.* **15**, 1088–1099 (2016).
19. Juelke, K. et al. CD2L2 expression identifies a unique subset of polyfunctional CD56\(^+\) NK cells. *Blood* **116**, 1299–1307 (2010).
20. Gazit, R. et al. Expression of KIR2DL1 on the entire NK cell population: a possible novel immunodeficiency syndrome. *Blood* **103**, 1963–1966 (2004).
21. Klein, A. M. et al. Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. *Cell* **161**, 1187–1201 (2015).
22. Tishkharini, R., Wulthier, G. Cluster validation by prediction strength. *J. Comput. Graph. Stat.* **14**, 511–528 (2005).
23. Trapnell, C. et al. The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. *Nat. Biotechnol.* **32**, 381–386 (2014).

24. Utzschneider, D. T. et al. T cell factor 1-expressing memory-like CD8+ T cells sustain the immune response to chronic viral infections. *Immunity* **45**, 415–427 (2016).

25. Jeannet, G. et al. Essential role of the Wnt pathway effector Tcf-1 for the establishment of functional CD8 T cell memory. *Proc. Natl Acad. Sci. USA* **107**, 9777–9782 (2010).

26. Yang, Q. et al. TCF-1 upregulation identifies early innate lymphoid progenitors in the bone marrow. *Nat. Immunol.* **16**, 1044–1050 (2015).

27. Aksoy, I. et al. Self-renewal of murine embryonic stem cells is supported by the serine/threonine kinases Pim-1 and Pim-3. *Stem Cells* **25**, 2996–3004 (2007).

28. Baaten, B. J. G. et al. CD44 regulates survival and memory development in Th1 cells. *Immunity* **32**, 104–115 (2010).

29. Weng, N.-P., Araki, Y. & Subedi, K. The molecular basis of the memory T cell response: differential gene expression and its epigenetic regulation. *Nat. Rev. Immunol.* **12**, 306–315 (2012).

30. Moretta, L. Dissecting CD56dim human NK cells. *Blood* **116**, 3689–3691 (2010).

31. Paust, S. et al. Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigen-specific memory of haptens and viruses. *Nat. Immunol.* **11**, 1127–1135 (2010).

32. Roychoudhuri, R. et al. BACH2 regulates CD8+ T cell differentiation by controlling access of AP-1 factors to enhancers. *Nat. Immunol.* **17**, 851–860 (2016).

33. Shin, H. M. et al. Epigenetic modifications induced by Blimp-1 regulate CD8+ T cell memory progression during acute virus infection. *Immunity* **39**, 661–675 (2013).

34. Kamimura, Y. & Lanier, L. L. Homeostatic control of memory cell progenitors in the natural killer cell lineage. *Cell Rep.* **10**, 280–291 (2015).

35. Brenchley, J. M. et al. Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. *Blood* **101**, 2711–2720 (2003).

36. Wherry, E. J. et al. Molecular signature of CD8+ T cell exhaustion during chronic viral infection. *Immunity* **27**, 670–684 (2007).

37. Lin, W.-H. W. et al. CD8+ T lymphocyte self-renewal during effector cell determination. *Cell Rep.* **17**, 1773–1782 (2016).

38. Vigneau, S., Rohrlich, P.-S., Brahic, M. & Bureau, J.-F. Tnfrsf1d1, a candidate gene for the control of Theiler's virus persistence, could be implicated in the regulation of gamma interferon. *J. Virol.* **77**, 5632–5638 (2003).

39. Gomez, J. A. et al. The NeST long ncRNA controls microbial susceptibility and epigenetic activation of the interferon-γ locus. *Cell* **152**, 743–754 (2013).

40. Walker, W., Aste-Amezaga, M., Kastelein, R. A., Trinchieri, G. & Hunter, C. A. IL-18 and CD28 use distinct molecular mechanisms to enhance NK cell production of IL-12-induced IFN-γ. *J. Immunol.* **162**, 5894–5901 (1999).

41. Schoenborn, J. R. & Wilson, C. B. Regulation of interferon-γ during innate and adaptive immune responses. *Adv. Immunol.* **96**, 41–101 (2007).

42. Björkström, N. K. et al. Expression patterns of NKG2A, KIR, and CD57 define a process of CD56dim NK-cell differentiation uncoupled from NK-cell education. *Blood* **116**, 3853–3864 (2010).

43. Romee, R. et al. Cytokine activation induces human memory-like NK cells. *Blood* **120**, 4751–4760 (2012).

44. Cooper, M. A. et al. Cytokine-induced memory-like natural killer cells. *Proc. Natl Acad. Sci. USA* **106**, 1915–1919 (2009).

45. Henning, A. N., Roychoudhuri, R. & Restifo, N. P. Epigenetic control of CD8+ T cell differentiation. *Nat. Rev. Immunol.* **18**, 340–356 (2018).

46. Hu, G. & Chen, J. A genome-wide regulatory network identifies key transcription factors for memory CD8+ T-cell development. *Nat. Commun.* **4**, 2830 (2013).

47. O'Sullivan, T. E., Sun, J. C. & Lanier, L. L. Natural killer cell memory. *Immunity* **43**, 634–645 (2015).

48. Xing, S. et al. Tcf1 and Lef1 transcription factors establish CD8+ T-cell development. *Nat. Commun.* **4**, 844–851 (2013).

49. Thomas, R. et al. NKG2C deletion is a risk factor of HIV infection. *AIDS Res. Hum. Retroviruses* **28**, 844–851 (2012).

50. Fregni, G. et al. High number of CD56bright NK-cells and persistently low T-cell differentiation during T cell exhaustion during chronic viral infection. *Hum. Immunol.* **77**, 5632–5638 (2013).
Methods
Clinical samples. Blood, plasma samples and PFA-fixed and paraffin-embedded rectosigmoid tissue blocks were from the University of California, San Francisco (UCSF) Observational Study of the Consequences of the Protease Inhibitor Era (SCOP-E) cohort. Individuals in the SCOP-E cohort included those who were HIV-1+, HIV-1+ viremic, HIV-1- and on ART and HIV-1+ controllers, the clinical characteristics of which are provided in Supplementary Table 2. The clinical cohort used for each specific experiment is indicated in the Results and in the figure captions.

All human blood and colon samples were collected from participants who had provided written informed consent for protocols that included the study of cellular immunity in HIV-1 infection, in accordance with procedures approved by the University of Massachusetts Medical School (UMMS) and the UCSF institutional review boards. Routine screening colonoscopy was scheduled as needed, as assessed by the patient and his/her treating physicians.

Immunohistochemistry and fluorescence microscopy. Formaldehyde-fixed, paraffin-embedded tissue blocs were sectioned (5 μm thick) from the clinical specimens were placed on Fisher Plus microscope slides, deparaffinized in xylene, rehydrated through graded ethanol and subjected to heat-induced epitope retrieval (HER) with citrate pH 6.0 (HIER) buffer for 10 min at 95–100°C to remove epithelial cells. The remaining tissue containing the LPL cells was washed with complete RPMI 1640 (1% fetal bovine serum, 10% dextran-coated charcoal-removed fetal bovine serum (Invitrogen; SA5-10171) at a 1:500 dilution for 30 min at room temperature. Mononuclear cells were washed three times with MACS buffer (0.5% bovine serum albumin and 2 mM EDTA in phosphate-buffered saline (PBS)) and either used immediately or frozen in fetal bovine serum containing 10% dimethyl sulfoxide.

Lineage antibodies. Antibodies against CD3, CD4, TCRαβ, TCRγδ, CD19, CD20, CD22, CD34, FcεRI, CD11c, CD303, CD123, CD1a and CD14 were used to identify mononuclear cell populations. The clinical cohort used for each specific experiment is indicated in the Results and in the figure captions.

Human intestinal lamina propria lymphoid (LPL) cell preparation. Human intestinal biopsies were incubated with PBS containing 5 mM EDTA, 150 μg/ml−1 dithiothreitol and 0.1% β-mercaptoethanol on a shaker for 15 min at 37°C to remove epithelial cells. The remaining tissue containing the LPL cells was washed with complete RPMI 1640 (1% fetal bovine serum, 1:100 Glutamax and 1:1,000 PMA and ionomycin (eBioscience; 00-4970-03) for 3–6 h. In both cases, protein transport inhibitor (eBioscience; 0-4980-03) was present during the stimulation. For intracellular staining of transcription factors or cytokines, cells were fixed and permeabilized using a FoxP3 Staining Buffer kit (eBioscience; 00-5552-00) and target intracellular molecules were stained as for surface staining.

NK cell sorting. PBMCs were stained with a panel of lineage markers. The LIN population was sorted based on cell-surface CD56 and CD94, as indicated in the captions to Extended Data Fig. 2b and Fig. 4a, using a BD FACSAria IIu.

Degranulation assay. For the response to PMA/ionomycin, PBMCs were seeded in 24-well plates at 2×106 cells per well in RPMI 1640 with anti-CD107a antibody (Biolgend; 328630) at 1:200 dilution. Then, cells were stimulated with PMA/ ionomycin (Invitrogen; 00-4970-03; 1:500) for 5 h, and surface CD107a on NK cells (Lin− CD56+CD16-) was detected by flow cytometry. For the response to K562 cells, NK cells were enriched from PBMCs with magnetic beads (BD; 557987) and incubated in V-bottomed 96-well plates with K562 cells (NK versus K562, 2:1) for the indicated time, and surface CD107a on NK cells was detected. For the response to HIV-1-infected CD4+ T cells, CD4+ PBMCs were enriched from individuals with high variability in tissue autofluorescence were excluded before breaking the code. A V-bottomed 96-well plate (5,000 cells in 100 μl) was then added to a 96-well solid black microplate. The fluorescence released by labeled target cells was monitored in a Beckman Coulter Access System (using excitation filter: 485/20 nm; band-pass filter: 528/20 nm). Specific lysis was determined as: ((test fluorescence release – spontaneous fluorescence release)/(maximum fluorescence release – spontaneous fluorescence release))×100.
Plasma sCD14. The concentration of sCD14 in the plasma was detected using an enzyme-linked immunosorbent assay according to the manufacturer’s instructions (R&D Systems; DC140).

Lentivirus production and TCIF7 knockdown. TCIF7-specific gPZIP short hairpin RNA sequences (Pharmacon) were subcloned into a modified pAGM plasmid and this was used to transfect HEK 293 cells to generate vesicular stomatitis virus G protein-pseudotyped lentivirus, as described previously. Lin CD56+ NK cells were subjected to three rounds of transduction before 1st stimulation.

Cytokine-induced NK cell memory assay. PBMCs or sorted CD94– NK cells were pre-stimulated with IL-15 (5 ng ml−1; Peprotech; 200-15) + IL-12 (50 ng ml−1; R&D Systems; DC140). Enzyme-linked immunosorbent assay according to the manufacturer’s instructions. PBMCs or sorted CD94– NK cells were pre-stimulated with IL-15 (5 ng ml−1; Peprotech; 200-15) + IL-12 (50 ng ml−1; R&D Systems; DC140). In vitro transcribed RNA was fragmented reagents (Ambion) and underwent a second reverse transcription reaction. CD94– NK cell libraries were amplified with indexed primers PE1-RP1 and hydrogel microspheres carrying primers for barcoding each captured cell. The single-cell RNAseq data for each cell was sequenced in individual droplets with unique barcodes, the droplet was broken and cDNA from all cells was pooled. A single-cell sequencing library was prepared using essentially CEL-seq2 (ref. 55). Previously described CRISPR-Cas9 methods for targeting the CD56 gene were introduced into pre-stimulated NK cells with a repaired pluripotent stem cell method (ref. 56). The single-cell sequencing library was sequenced using 45 cycles of read 1, eight cycles of index 1 and 32 cycles of read 2. The second reverse transcription reaction. CD94– NK cell libraries were amplified with indexed primers PE1-RP1 or RP12 (for donor 2; 5’-CAAGCAGAAGACGGCATACGAGATCTACACGGTCTCGGCA-3’). Libraries from donor 2 were paired-end sequenced on a NextSeq 500 version 2 (Illumina) using 45 cycles of read 1, eight cycles of index 1 and 32 cycles of read 2. Useful Illumina NextSeq 500 version 2 with custom sequencing primers added to the cartridge: R1(5’-GGACTTCTTGGAACGCTTCCGGAACGGCAAG-3’); R2(5’-CTTTGTCTTCCATTGGAAGCTGG-3’). The single-cell sequencing library was sequenced using 45 cycles of read 1, eight cycles of index 1 and 32 cycles of read 2. The second reverse transcription reaction. CD94– NK cell libraries were amplified with indexed primers PE1-RP1 or RP12 (for donor 2; 5’-CAAGCAGAAGACGGCATACGAGATCTACACGGTCTCGGCA-3’). The single-cell sequencing library was sequenced using 45 cycles of read 1, eight cycles of index 1 and 32 cycles of read 2.

Throughout this study, the focus was on identifying differential gene expression patterns between different populations of NK cells. The single-cell sequencing library was sequenced using 45 cycles of read 1, eight cycles of index 1 and 32 cycles of read 2. The second reverse transcription reaction. CD94– NK cell libraries were amplified with indexed primers PE1-RP1 or RP12 (for donor 2; 5’-CAAGCAGAAGACGGCATACGAGATCTACACGGTCTCGGCA-3’). The single-cell sequencing library was sequenced using 45 cycles of read 1, eight cycles of index 1 and 32 cycles of read 2.

Library preparation for single-cell RNA-seq. The single-cell sequencing library was prepared using InDrop barcoding. Individual, pre-sorted cells were captured in droplets containing lysis buffer, reverse transcription reactions and hydrogel microspheres carrying primers for barcoding each captured cell. The single-cell RNAseq data for each cell was sequenced in individual droplets with unique barcodes, the droplet was broken and cDNA from all cells was pooled. A single-cell sequencing library was prepared using essentially CEL-seq2 (ref. 55). The second reverse transcription reaction. CD94– NK cell libraries were amplified with indexed primers PE1-5’-CAAGCAGAAGACGGCATACGAGATCTACACGGTCTCGGCA-3’. Libraries from donor 2 were paired-end sequenced on a NextSeq 500 version 2 (Illumina) using 45 cycles of read 1, eight cycles of index 1 and 32 cycles of read 2.

Library preparation for single-cell RNA-seq. The single-cell sequencing library was prepared using InDrop barcoding. Individual, pre-sorted cells were captured in droplets containing lysis buffer, reverse transcription reactions and hydrogel microspheres carrying primers for barcoding each captured cell. The single-cell RNAseq data for each cell was sequenced in individual droplets with unique barcodes, the droplet was broken and cDNA from all cells was pooled. A single-cell sequencing library was prepared using essentially CEL-seq2 (ref. 55). The second reverse transcription reaction. CD94– NK cell libraries were amplified with indexed primers PE1-5’-CAAGCAGAAGACGGCATACGAGATCTACACGGTCTCGGCA-3’. Libraries from donor 2 were paired-end sequenced on a NextSeq 500 version 2 (Illumina) using 45 cycles of read 1, eight cycles of index 1 and 32 cycles of read 2.

Library preparation for single-cell RNA-seq. The single-cell sequencing library was prepared using InDrop barcoding. Individual, pre-sorted cells were captured in droplets containing lysis buffer, reverse transcription reactions and hydrogel microspheres carrying primers for barcoding each captured cell. The single-cell RNAseq data for each cell was sequenced in individual droplets with unique barcodes, the droplet was broken and cDNA from all cells was pooled. A single-cell sequencing library was prepared using essentially CEL-seq2 (ref. 55). The second reverse transcription reaction. CD94– NK cell libraries were amplified with indexed primers PE1-5’-CAAGCAGAAGACGGCATACGAGATCTACACGGTCTCGGCA-3’. Libraries from donor 2 were paired-end sequenced on a NextSeq 500 version 2 (Illumina) using 45 cycles of read 1, eight cycles of index 1 and 32 cycles of read 2.
RNA-seq processing and analysis. The pooled sets of RNA-seq reads were separated by CEL-seq barcodes and mapped to the HG19 genome using TopHat (version 2.0.14; default parameters). Aligned reads were quantified by ESAT61 using a transcript annotation file containing all RefSeq genes filtered to select only ‘NM’ transcripts and extending the transcripts up to 1,000 bases past the annotated 3’ end (-task score3p), discarding multimapped reads (-multimap ignore) and requiring a single prime5). The most varied genes were analyzed using DESeq2. Differential expression analysis was performed using DESeq2 (ref. 65) and MA plots were created using values from lfcShrink within DESeq2. Any gene differentially expressed in these three tests in Fig. 4b was shown. For PCA, data were transformed using log fold within DESeq2, and preproc was used to calculate the principal components.

Single-cell RNA-seq processing and analysis. Reads were mapped to the HG19 genome using TopHat (version 2.0.14; default parameters). To assign each read to a given cell and collapse duplicate reads into single UMIs, alignments were processed using ESAT’s single-cell analysis module (-scPrep) with the same transcript annotation file as was used for the bulk RNA-seq analysis, extending the transcripts up to 1,000 bases past the annotated 3’ end (-wExt 1,000, -task score3p), discarding multimapped reads (-multimap ignore) and requiring a single observation of a UMI for a transcript to be counted (-umiMin 1).

The final output of ESAT is a table in which rows are genes, cells are columns, and values represent the number of UMIs detected in each cell. The dataset was loaded into R and, if not part of base R, the packages used are noted. First, data were normalized using the TMM method from EdgeR64. On this matrix, PCA was run to determine the number of dimensions that contribute variance to the data and to select genes that were highly variable in the dataset. Independent component analysis (ICA) was run using the fastICA algorithm to reduce the data to the number of dimensions that contained variance. To determine the optimum number of cell clusters in the dataset, the methods of Tibshirani and Walther were utilized. This analysis showed that two clusters gave the highest predictive strength, and that further subdividing of the cells into additional clusters severely lowered the score. Spectral clustering was then run with two centers and using the symmetrical method on the cell’s ICA components using the knn package. Differential expression analysis was used to determine the genes that were most significantly different between the two clusters65. For visualization, the cells were then reduced to two dimensions using the Rtsne package, which used the ICA components as input65. Lastly, the minimum spanning tree was constructed on the t-distributed stochastic neighbor embedding (t-SNE) plot utilizing Monocle3. For pseudotime analysis, Monocle’s built-in differential expression tools were utilized. For visualization purposes, t-SNE mapped cells were color coded by the expression of given genes; a weighted density map was created that takes into account both the number of cells in a region and their expression values. Heatmaps were generated using the list of genes that were found to be differentially expressed in both the spectral clustering analysis and with the pseudotime analysis. Rows of the heatmap were grouped by similarity (heatmap.2) and columns were ordered based on the pseudotime provided by the pseudotime analysis.

Data availability. The data that support the findings of this study are available within the manuscript and its Supplementary Information, and from the corresponding author upon request. Source data for Figs. 1, 2 and 6–8 and Extended Data Figs. 1–3 and 5–7 are provided with the paper. Bulk and single-cell RNA-seq, CUT&RUN and ATAC-seq datasets can be found under SuperSeries GSE122326 at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE122326; CD56hi and CD94–CD56dim, CD56dim and CD94+ NK cells bulk and single-cell RNA-seq (GSE122323); CD4+ and CD94+ NK cell bulk and single-cell RNA-seq (GSE122324); CD56dim NK cells, 1st stim and 5-culture RNA-seq (GSE122325); CD4+ and CD94+ NK cell ATAC-seq (GSE122548); and CD4+ and CD94+ NK cell CUT&RUN (GSE122549).

References
51. Davis, Z. B. et al. A conserved HIV-1-derived peptide presented by HLA-E renders infected T-cells highly susceptible to attack by NKG2A/CD94-bearing natural killer cells. PLoS Pathog. 12, e1004521 (2016).
52. Neri, S., Mariani, E., Meneghetti, A., Cattini, L. & Facchini, A. Calcein-acetoxyxymethyl cytotoxicity assay: standardization of a method allowing additional analyses on recovered effector cells and supernatants. Clin. Diagn. Lab. Immunol. 8, 1131–1135 (2001).
53. Peltt, T. et al. TRIM5 is an innate immune sensor for the retrovirus capsid lattice. Nature 472, 361–365 (2011).
54. Poli, A. et al. CD56(dim) natural killer (NK) cells: an important NK cell subset. Immunology 126, 458–465 (2009).
55. Hashimshony, T. et al. CEL-Seq2: sensitive highly-multiplexed single-cell RNA-Seq. Genome Biol. 17, 77 (2016).
56. Shen, P. J. & Henkoff, S. An efficient targeted nuclease strategy for high-resolution mapping of DNA binding sites. Elife 6, e21856 (2017).
57. Hainer, S. J., Boskovic, A., Rando, O. J. & Fazzio, T. G. Profiling of gene expression with high efficiency for low cell numbers. Nat. Protoc. 13, 1006–1019 (2018).
58. Buenrostro, J. D., Wu, B., Chang, H. Y. & Greenleaf, W. J. ATAC-Seq–a method for assaying chromatin accessibility genome-wide. Curr. Protoc. Mol. Biol. 109, 21.29.1–21.29.9 (2015).
59. Buenrostro, J. D., Giresi, P. G., Zaba, L. C., Chang, H. Y. & Greenleaf, W. J. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. Nat. Methods 10, 1213–1218 (2013).
60. Kim, D. et al. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. 14, R36 (2013).
61. Derr, A. et al. End Sequence Analysis Toolkit (ESAT) expands the extractable information from single-cell RNA-Seq data. Genome Res. 26, 1397–1410 (2016).
Acknowledgements
We thank the study participants who provided blood and colon biopsy samples, as well as their caretakers, J. Daly, S. Cheeseman and M. Wessolossky of the UMMS. C. Mannarino, A. Foley, M. McManus (UMMS) and M. Krome (UCSF) provided Institutional Review Board regulatory assistance, sample preparation and record keeping. K. Luzuriaga (UMMS) supported the patient sample database and repository. A. Ratner, S. Boswell and A. Klein (Harvard Medical School) contributed technical assistance and barcoded hydrogel beads. T. Fazzio and T. Wu provided technical support and protein A MNase for CUT&RUN. D. Artis, L. Berg, M. Colonna, J. Huh, J. Kang, R. Rutishauser and S. Swain offered invaluable advice. This research was supported by NIH grants U01HG007910 (to M.G. and J.L.), R37Al147868 (to J.L.), R01Al111809 (to J.L.), DP1DA034990 (to J.L.), R21Al119885 (to M.G.), R01DK105837 (to M.G.) and P51OD01192 (to J.D.E. at the Oregon National Primate Research Center), and the Translational Medicine Core of the University of Massachusetts Center for AIDS Research (P30 AI042845). The UCSF-based SCOPE cohort was supported by the UCSF/Gladstone Institute of Virology and Immunology CFAR (P30 AI027763) and the CFAR Network of Integrated Systems (R24 AI087039). Additional support was provided by the Delaney AIDS Research Enterprise (AI096109 and AI127966). Funding for this study was provided in part by the Division of Intramural Research/NIAID/NIH (to J.M.B.). The content of this publication does not necessarily reflect the views or policies of DHHS, nor does the mention of trade names, commercial products, or organizations imply endorsement by the US Government.

Author contributions
Y.W. and J.L. designed the experiments. Y.W. performed the experiments with assistance from C.L.V., K.B.-S., S.J., K.G., A.D., L.L., S.M., K.K., P.V., P.W.H., S.G.D., J.M.B., J.D.E. and P.M. Y.W. and J.L. analyzed the experimental data. Y.W., L.L., K.G., P.V., A.K., M.G. and J.L. analyzed the expression data. T.G., J.H., M.S. and S.G.D. obtained and provided the clinical samples. Y.W. and J.L. wrote the manuscript, which was revised and approved by all authors.

Competing interests
The authors declare no competing interests.

Additional information
Extended data is available for this paper at https://doi.org/10.1038/s41590-020-0593-9.
Supplementary information is available for this paper at https://doi.org/10.1038/s41590-020-0593-9.
Correspondence and requests for materials should be addressed to J.L.
Peer review information Zoltan Fehervari was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.
Reprints and permissions information is available at www.nature.com/reprints.
Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | ILC_gating_and_effect_of_HIV-1.  

a, Lymphoid, singlet, live, CD45+ PBMCs from HIV-1- individuals were stained with lineage antibodies (see Methods), CD56, and CD16, and the percent ILCs were Lin−CD56−CD16−CD127+ cells.  

b, CD117 and CRTH2 on Lin−CD56−CD16−CD127+ cells from HIV-1- PBMCs (n = 11).  

c, ILCs from HIV-1-, HIV-1+ viremic, HIV-1+ on ART, and HIV-1+ spontaneous controllers, as in a (Supplementary Table 1).  

d, Correlation of ILCs (Lin−CD56−CD16−CD127+PBMCs) with CD4+ nadir (Supplementary Table 1). Correlation coefficient (R) by Pearson, zero slope p value determined by F-test (n = 80).  

e, CD127, CD117, and RORγt in Lin− colon lamina propria from HIV-1-.  

f, IL-22 and CD127 on Lin− colon lamina propria from HIV-1-.  

g, CD127, CD117, and RORγt in Lin− colon lamina propria from HIV-1-.  

h, IL-22 produced by gut lamina propria ILC3s maintains epithelium integrity (left). Irreversible decrease in ILC3s with HIV-1 infection (right). Data are mean ± s.e.m.; h,i, two tailed unpaired t-test; b,k, two tailed paired t-test. ns, not significant, *p < 0.05, **p < 0.001.
Extended Data Fig. 2 | HIV-1 infection increases CD94+ NK cells. a, Fraction of CD94+NK cells among Lin-TBX21+ PBMCs after stimulation with PMA and ionomycin (n = 10) or with IL-15 (n = 10), or with IL-12+IL15 (n = 4). b, Sorting strategy for CD94− and CD94+NK cells. c, Percent CD107a among CD94− and CD94+NK cells after PBMCs were stimulated with PMA/iono (n = 5). d, Percent specific lysis of K562 or Jurkat cells by sorted CD94−NK cells and CD94+NK cells (n = 8). e, Percent Ki67 and Annexin V among CD94− or CD94+NK cells after the indicated treatment (n = 4). f, Representative flow cytometry for indicated genes as detected in Fig. 2h. Data are mean ± s.e.m. Each dot represents a unique sample. two-tailed paired t-test, lines connect cells from common donor. ns, not significant, *p < 0.05, **p < 0.01, ***p < 0.001. All data are from HIV-1− anonymous blood donors.
Extended Data Fig. 3 | Single_cell_analysis_of_CD94−_and_CD94+NK_cells. a, Heatmap of 1,729 CD94− (blue) and 1,548 CD94+NK cells (yellow) sorted from 2 donors using all differentially expressed genes based on CD94 positivity. b, Plot of predictive strength as a function of the number of clusters in Fig. 3b shows that 2 clusters yield stable and significant groupings, while separation into additional clusters artificially segregates the cells. The predictive strength based on 1753 single cells was calculated using spectral clustering on the ICA components. c, Heatmap from Fig. 3c was reconstructed utilizing the pseudotime ordering of single cells based on the minimum spanning tree. d, Flow cytometry for CD44, CXCR3 and SELL on TCF7− and TCF7+NK cells. e, Flow cytometry for GZMK after sorted Lin−CD56−CD94−NK cells were treated as in Fig. 3h. f,g, PBMCs were treated with or without IL-15 for 5 days, Lin−CD56− cells were gated on CD56 and CD94 (f) and percent CD56+ NK cells (g) (n=10). Data are mean ± s.e.m. two-tailed paired t-test, *p<0.001. All data are from HIV-1− anonymous blood donors.
Extended Data Fig. 4 | Distinct chromatin landscape of CD94+CD56hiNK cells. a, PCA based on H3K4me3 CUT&RUN of the indicated NK cell subsets (n = 2). b, Correlation between differentially expressed genes and enriched H3K4me3 regions by CUT&RUN (log2 fold change) p < 0.001. The correlation coefficient (R) was determined by Pearson, p value for the slope being zero was determined by the F-test. c, Differential signals for H3K4me3 CUT&RUN and ATAC-Seq at the indicated loci in the indicated NK cell subsets. d, Overlapping signal for TCF7 CUT&RUN and ATAC-Seq at the indicated loci. Data are from HIV-1 blood donors.
Extended Data Fig. 5 | Memory-associated gene loci are accessible in the CD94+CD56hiNK cells. a, H3K4me1 and H3K4me3 CUT&RUN and ATAC-Seq signal on genes associated with memory T and NK cells, except for effector marker KLRG1, on the indicated NK cell subsets. b, IFN-γ production among CD56dim and CD56hiNK cells after stimulation with IL-12 + IL-15 for 16 hr (n = 4), mean ± s.e.m.; two tailed paired t-test, *p < 0.01. c, H3K4me1 and H3K4me3 CUT&RUN and ATAC-Seq signal at loci for IFN-γ signaling related genes. Data are from HIV-1 blood donors.
Extended Data Fig. 6 | Surface markers and WNT-associated gene loci comparing CD56dim and CD56hi NK cells. a, Detection of CD16 (n = 4), KIR2DL1 (n = 7), KIR2DL2/3 (n = 8), KIR3DL1 (n = 8) and CD57 (n = 8) in CD56dim and CD56hi NK cells from PBMCs, mean ± s.e.m.; two tailed paired t-test, *p < 0.001. c, Sorted, CFSE labelled CD94+CD56hi NK cells were cultured in IL-12 (10 ng/ml) and IL-15 (10 ng/ml) for 5 days. Proliferation, CXCR6, and CD57 were detected as indicated. CD56dim and CD56hi NK cells from fresh, unstimulated PBMCs were used as control. d, H3K4me3 CUT&RUN and ATAC-Seq signals for gene loci of WNT signaling components and WNT target genes in the indicated NK cell subsets. AXIN1, in contrast, is a WNT inhibitory gene. Data are from HIV-1+ blood donors.
Extended Data Fig. 7 | WNT_inhibition_blocks_cytokine_induced_NK_cell_memory. a, PBMCs were treated with or without LGK974 for 16 hrs. Percentage of Lin-TBX21+ cells, and the CD94– and CD94+ cells among the Lin–TBX21+ population, are indicated (left); data are representative of 4 anonymous HIV-1– blood donors. PBMCs were stimulated with IL-12 and IL-15 for 16 hrs in the absence or presence of LGK974. Live cells and Lin–CD56+ cells were examined (right); data are representative of 10 anonymous HIV-1– blood donors. b, NK cells without primary stimulation as in Fig. 7a were stimulated with IL-12+IL-15, then IFN-γ production of cells with or without LGK974 was detected (n=8), samples are from HIV-1– donors. c, Magnetic beads enriched NK cells from HIV-1– donors were transduced with lentivectors expressing GFP and shRNAs targeting either TCF7 or control, the TCF7 level in GFP+ cells was detected by flow cytometry. d, Control or TCF7 knockdown NK cell in c were treated as in Fig. 7a, percent IFN-γ among GFP+ cell were detected after secondary stimulation (n=3). e, Correlation of ILCs with TCF7+NK cells. Samples are from HIV-1+ viremic individuals, ART suppressed HIV-1+ individuals (ART), and HIV-1+ individuals who spontaneously control viremia without ART. Cohort characteristics are described in Supplementary Table 1 (n=53). The correlation coefficient (R) was determined by Pearson, p value for the slope being zero was determined by the F-test. Data are mean±s.e.m; b,d, two tailed paired t-test, ns, not significant, *p < 0.01.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

☐ n/a
☐ Confirmed
☐ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐ The statistical test(s) used AND whether they are one- or two-sided
☐ Only common tests should be described solely by name; describe more complex techniques in the Methods section.
☐ A description of all covariates tested
☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
☐ Give P values as exact values whenever suitable.
☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐ Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Sequencing data was collected using Nextseq 500, Illumina. Flow cytometry data were collected using FACSCelesta flow cytometer, BD.

Data analysis

Data analysis used software:
FlowJo, LLC, https://www.flowjo.com/ (FlowJo_V10)
GraphPad Prism, GraphPad Software, Inc, https://www.graphpad.com/scientific-software/prism/ (GraphPad Prism 8)
Tophat, Kim et al., 2013, https://ccb.jhu.edu/software/tophat/index.shtml
SAMtools, Li et al., 2009, http://www.htslib.org
R Statistical Computing Software, The R Foundation, https://www.r-project.org/
ESAT, Derr et al. 2016, https://github.com/garber-lab/ESAT
DESeq2, Love et al., 2014, https://bioconductor.org/packages/release/bioc/html/DESeq2.html
EdgeR, Robinson and Olshack, 2010, https://bioconductor.org/packages/release/bioc/html/edgeR.html
fastCA, Hyvärinen and Oja, 2000, https://cran.r-project.org/web/packages/fastCA/index.html
Rtsne, Van Der Maaten, 2014, https://cran.r-project.org/web/packages/Rtsne/index.html
Monocle, Trapnell et al., 2014, https://bioconductor.org/packages/release/bioc/html/monocle.html
kknn, Samworth, 2012, https://cran.r-project.org/web/packages/kknn/index.html
HOMEr, Heinz S, 2010, http://homer.ucsd.edu/homer/index.html
GSEA, Aravind S, 2005, https://genepattern.broadinstitute.org/gp/pages/index.jsf
DEBrowser, Kucukural A, 2019, https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-018-5362-x.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Accession codes and links related with sequencing data in this study was provided as follows:
Datasets can be found under SuperSeries GSE122326 at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE97727.
GSE97727: CD94- and CD94+ NK cell bulk and single cell RNA-Seq
GSE122324: CD94+CD56dim, CD94+CD56dim, and CD94+CD56hi NK cells RNA-Seq
GSE122325: CD94+CD56dim NK cells, 10 stim and 5 day culture RNA-Seq
GSE125458: CD94+CD56dim, CD94+CD56dim, and CD94+CD56hi NK cells ATAC-Seq
GSE125459: CD94+CD56dim, CD94+CD56dim, and CD94+CD56hi NK cells CUT&RUN

Figures associated with raw data:
Fig. 2g; Fig. 3a-f; Fig. 4b-g; Fig. 5a-h; Fig. 6a-c and i; Fig. 7b-f; Extended Data Fig. 3a-c; Extended Data Fig. 4a-d; Extended Data Fig. 5a-c; Extended Data Fig. 6d.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [x] Life sciences
- [] Behavioural & social sciences
- [] Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-list.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
Sample size was determined based on previous studies of similar nature. No statistical methods were used to predetermine sample sizes.

Data exclusions
For PFA-fixed and paraffin-embedded rectosigmoid tissues blocks from UCSF, samples with small tissue size or high variability in tissue autofluorescence were blindly excluded. The submucosa and tunica muscularis were blindly excluded. It is common for highly vascularized tissues that are collected by biopsy to contain residual RBC, which, when fixed with formalin, for histologic evaluation, do not autofluoresce. Thus, when this occurs, a sample with high variability in tissue autofluorescence were blindly excluded. The submucosa and tunica muscularis were blindly excluded. It is common for highly vascularized tissues that are collected by biopsy to contain residual RBC, which, when fixed with formalin, for histologic evaluation, do not autofluoresce. Thus, when this occurs, in lieu of quantifying the entire mucosa, we are excluding regions around the sequent regions to ensure the RBCs, and other autofluorescent regions of the tissue, are not included in the quantification. In addition, because tissue sections, depending on the position of the tissue in the block and the residual amount of tissue in the block, do vary in the amount of tissue in any given section, and because the confidence in tissue quantification increases with the number of total cells quantified (i.e the denominator), we excluded samples from quantification with a low number of total mucosal cells.

Replication
Flow cytometry experiments were replicated using 3-113 independent donors for each treatment, condition, marker or cell subset. Bulk and single cell RNA-Seq experiments were replicated using 2-4 independent donors. RT-PCR experiments were replicated using 2 independent donors. Histology experiments were replicated using 8-16 independent donors. The number of donors and repeats times of specific figure were included in the figure legend, all attempts at replication were successful.

Randomization
Leukopaks used to PBMCs were randomly picked. Samples of HIV-1 negative and positive individuals were randomly pulled out from the storage.

Blinding
Samples from University of California, San Francisco SCOPE Cohort were tested blindly, sample information was known from Translational Medicine Core of the University of Massachusetts Center for AIDS Research Cohort, when samples were picking up or obtained.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description
Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).

Research sample
State the research sample (e.g. Harvard university undergraduates, villagers in rural (India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.

Sampling strategy
Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to
### Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Study description**
Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.

**Research sample**
Describe the research sample (e.g. a group of tagged Passer domesticus, all Steroceros turbeni within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.

**Sampling strategy**
Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.

**Data collection**
Describe the data collection procedure, including who recorded the data and how.

**Timing and spatial scale**
Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken.

**Data exclusions**
If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.

**Reproducibility**
Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.

**Randomization**
Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.

**Blinding**
Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.

**Did the study involve field work?**
- [ ] Yes
- [ ] No

### Field work, collection and transport

**Field conditions**
Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).

**Location**
State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).

**Access and import/export**
Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).

**Disturbance**
Describe any disturbance caused by the study and how it was minimized.

### Reporting for specific materials, systems and methods
### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑️  | Antibodies            |
| ☑️  | Eukaryotic cell lines |
| ☑️  | Palaeontology         |
| ☑️  | Animals and other organisms |
| ☑️  | Human research participants |
|   | Clinical data         |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑️  | ChIP-seq              |
| ☑️  | Flow cytometry        |
| ☑️  | MRI-based neuroimaging|

### Antibodies

| Antibodies used | Cell line and dilution |
|-----------------|------------------------|
| Anti-Human CD3  | Biolegend Cat# 317306 Clone: OKT3 (FITC) [1:200 dilution] |
| Anti-Human CD4  | Biolegend Cat# 317408 Clone: OKT4 (FITC) [1:200 dilution] |
| Anti-Human CD9  | Biolegend Cat# 317198 Clone: B1 (FITC) [1:200 dilution] |
| Anti-Human CD19 | Biolegend Cat# 302206 Clone: HIB19 (FITC) [1:200 dilution] |
| Anti-Human CD20 | Biolegend Cat# 302304 Clone: 2H7a (FITC) [1:200 dilution] |
| Anti-Human CD22 | Biolegend Cat# 363508 Clone: S-HCl-1 (FITC) [1:200 dilution] |
| Anti-Human CD14 | Biolegend Cat# 325604 Clone: HCD14 (FITC) [1:200 dilution] |
| Anti-Human CD16 | Biolegend Cat# 300103 Clone: 3G8 (PerCP) [1:200 dilution] |
| Anti-Human CD16 | Biolegend Cat# 300103 Clone: 3G8 (PerCP/Cy5.5) [1:200 dilution] |
| Anti-Human CD34 | Biolegend Cat# 343504 Clone: S81 (FITC) [1:200 dilution] |
| Anti-Human CD94 | Biolegend Cat# 305504 Clone: DX22 (FITC) [1:200 dilution] |
| Anti-Human CD94 | Biolegend Cat# 305504 Clone: DX22 (PE) [1:200 dilution] |
| Anti-Human CD94 | Biolegend Cat# 305516 Clone: DX22 (PE/Cy7) [1:200 dilution] |
| Anti-Human CD94 | Biolegend Cat# 305516 Clone: DX22 (PerCP/Cy5.5) [1:200 dilution] |
| Anti-Human CD94 | Biolegend Cat# 305508 Clone: DX22 (APC) [1:200 dilution] |
| Anti-Human FcrRIα | Biolegend Cat# 334608 Clone: AER-37 (FITC) [1:200 dilution] |
| Anti-Human CD1a | Biolegend Cat# 300014 Clone: HI149 (FITC) [1:200 dilution] |
| Anti-Human CD11c | Biolegend Cat# 301604 Clone: 3.9 (FITC) [1:200 dilution] |
| Anti-Human CD123 | Biolegend Cat# 306014 Clone: 8H6 (FITC) [1:200 dilution] |
| Anti-Human B2C1 | Biolegend Cat# 354208 Clone: 201A (FITC) [1:200 dilution] |
| Anti-Human TCRβ | Biolegend Cat# 306706 Clone: IP26 (FITC) [1:200 dilution] |
| Anti-Human B2C1 | Biolegend Cat# 103206 Clone: RA3-682 (FITC) [1:200 dilution] |
| Anti-Human RORγT | ebioscience Cat# 12-6988-82 Clone: AFK5-9 (PE) [1:50 dilution] |
| Anti-Human RORγT | ebioscience Cat# 17-6988-82 Clone: AFK5-9 (APC) [1:50 dilution] |
| Anti-Human TRX1 | ebioscience Cat# 25-5825-82 Clone: ebio4B10 (PE/Cy7) [1:200 dilution] |
| Anti-Human CRTH2 | ebioscience Cat# 25-9966-42 Clone: TWAI (PE/Cy7) [1:200 dilution] |
| Anti-Human CRTH2 | ebioscience Cat# 350110 Clone: BM16 (APC) [1:200 dilution] |
| Anti-Human IL-22 | ebioscience Cat# 12-7129-42 Clone: 22URTI (PE) [1:200 dilution] |
| Anti-Human IFN-γ | Biolegend Cat# 502512 Clone: 45.B3 (APC) [1:200 dilution] |
| Anti-Human IFN-γ | Biolegend Cat# 502509 Clone: 45.B3 (PE) [1:200 dilution] |
| Anti-Human IFN-γ | Biolegend Cat# 502528 Clone: 45.B3 (PE/Cy7) [1:200 dilution] |
| Anti-Human CD2 | Biolegend Cat# 309207 Clone: TS1/8 (PE) [1:200 dilution] |
| Anti-Human CD6 | Biolegend Cat# 313906 Clone: BL-C06 (PE) [1:200 dilution] |
| Anti-Human CD244 | Biolegend Cat# 338806 Clone: B18 (APC) [1:200 dilution] |
| Anti-Human CD44 | Biolegend Cat# 338806 Clone: B18 (PE) [1:200 dilution] |
| Anti-Human CD45 | BD Cat# 560178 Clone: 2D1 (APC/H7) [1:200 dilution] |
| Anti-Human CD56 | Biolegend Cat# 318306 Clone: HCD56 (PE) [1:200 dilution] |
| Anti-Human CD56 | Biolegend Cat# 318310 Clone: HCD56 (APC) [1:200 dilution] |
| Anti-Human CD56 | Biolegend Cat# 318322 Clone: HCD56 (PerCP/Cy5.5) [1:200 dilution] |
| Anti-Human CD56 | Biolegend Cat# 318332 Clone: HCD56 (APC/Cy7) [1:200 dilution] |
| Anti-Human CD57 | Biolegend Cat# 359623 Clone: HNK-1 (PE/Cy7) [1:200 dilution] |
| Anti-Human CD62L | Biolegend Cat# 304805 Clone: DREG-56 (PE) [1:200 dilution] |
| Anti-Human CD62L | Biolegend Cat# 304810 Clone: DREG-56 (APC) [1:200 dilution] |
| Anti-Human CD62L | Biolegend Cat# 304822 Clone: DREG-56 (PE/Cy7) [1:200 dilution] |
| Anti-Human CD107a | Biolegend Cat# 328619 Clone: HA43 (APC) [1:200 dilution] |
| Anti-Human CD107a | Biolegend Cat# 328607 Clone: HA43 (PE) [1:200 dilution] |
| Anti-Human CD117 | Biolegend Cat# 313213 Clone: 10020 (PerCP/Cy5.5) |
| Anti-Human CD117 | Biolegend Cat# 313206 Clone: 10020 (APC) [1:200 dilution] |
| Anti-Human CD117 | Biolegend Cat# 351316 Clone: A019D5 (APC) [1:200 dilution] |
| Anti-Human CD117 | Biolegend Cat# 351304 Clone: A019D5 (PE) [1:200 dilution] |
| Anti-Human CD117 | Biolegend Cat# 351320 Clone: A019D5 (PE/Cy7) [1:200 dilution] |
| Anti-Human Granzyme K | Biolegend Cat# 370503 Clone: GM26E7 (Alexa Fluor 647) [1:200 dilution] |
| Anti-Human Granzyme K | Biolegend Cat# 370513 Clone: GM26E7 (PerCP/Cy5.5) [1:200 dilution] |
| Antibody | Clone | Dilution | Source |
|----------|-------|----------|--------|
| Anti-Human NKG2A | MACS | 130-098-813 Clone: REA110 (PE) | 1:200 dilution |
| Anti-Human FGFR2 | Biologend | Cat# 346603 Clone: TDA3 (PE) | 1:200 dilution |
| Anti-Human KIR2DL1 | Biologend | Cat# 339505 Clone: HP-MA4 (PE) | 1:200 dilution |
| Anti-Human KIR2DL2/L3 | Biologend | Cat# 312613 Clone: DX27 (PerCP/Cy5.5) | 1:200 dilution |
| Anti-Human KIR3DL1 | Biologend | Cat# 31719 Clone: DX9 (PE/Cy5) | 1:200 dilution |
| Anti-Human TCF7 | Cell Signaling Cat# 14456 Clone: CD69 (PE) | 1:200 dilution |
| Anti-Human CXCRL3 | Biologend | Cat# 353705 Clone: G02H17 (PE) | 1:200 dilution |
| Anti-Human CXCRL3 | Biologend | Cat# 353708 Clone: G02H17 (APC) | 1:200 dilution |
| Anti-Human CXCRL6 | Biologend | Cat# 356006 Clone: K041ES (APC) | 1:200 dilution |
| Anti-Human CXCRL6 | Biologend | Cat# 356003 Clone: K041ES (PE) | 1:200 dilution |
| Anti-Human CXCRL6 | Biologend | Cat# 356009 Clone: K041ES (PerCP/Cy5.5) | 1:200 dilution |
| Anti-Human Ki67 | Biologend | Cat# 350519 Clone: Ki-67 (PerCP/Cy5.5) | 1:200 dilution |
| Anti-Human Ki67 | Biologend | Cat# 350514 Clone: Ki-67 (APC) | 1:200 dilution |
| Anti-TCF7 | Cell Signaling Cat# 22035 Clone: CD699 | 1:200 dilution |
| Anti-Human Annexin V ebioscience Cat# 88-8007-74 (APC) | 1:200 dilution |
| Anti-Human Annexin V Biologend Cat# 640936 (PerCP/Cy5.5) | 1:200 dilution |

Validation
No customized antibodies were used. All antibodies used to detect human proteins were validated in human, please refer to manufacture's website for validation details, antibody profiles and relevant citations.

Eukaryotic cell lines

**Policy information about cell lines**

**Cell line source(s)**
K562 and Jurkat cells were purchased from ATCC.

**Authentication**
Cells were confirmed for mycoplasma negative by mycoplasma detection kit (Lonza. Cat#LT27-318)

**Mycoplasma contamination**
Cells were confirmed for mycoplasma negative by mycoplasma detection kit (Lonza. Cat#LT27-318)

**Commonly misidentified lines**

| Cell line source | Authentication | Mycoplasma contamination | Commonly misidentified lines |
|------------------|----------------|--------------------------|----------------------------|
| K562 and Jurkat cells | Not independently authenticated by our lab. | Cells were confirmed for mycoplasma negative by mycoplasma detection kit (Lonza. Cat#LT27-318) | Cell lines used in this study was not in commonly misidentified lines list of iCLAC database. |

Palaeontology

**Specimen provenance**
Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).

**Specimen deposition**
Indicate where the specimens have been deposited to permit free access by other researchers.

**Dating methods**
If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

Animals and other organisms

**Policy information about studies involving animals; ARRIVE guidelines**
Recommended for reporting animal research.

**Laboratory animals**
For laboratory animals, report species, strain, sex and age OR state that the study did not involve laboratory animals.

**Wild animals**
Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.

**Field-collected samples**
For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.

**Ethics oversight**
Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

**Policy information about studies involving human research participants**

**Population characteristics**
Age and gender of participants were deidentified by cohort provider.

**Recruitment**
All human blood and colon samples were collected from participants who had provided written informed consent for protocols.
Recruitment

that included study of cellular immunity in HIV-1 infection, in accordance with procedures approved by the University of California, San Francisco (UCSF) Institutional Review Boards (clinical information were described in Supplementary Table 1) and the University of Massachusetts Medical School (UMMS) (clinical information were described in Supplementary Table 2). Routine screening colonoscopy was scheduled as medically indicated at UMMS. HIV-1 control individuals undergoing colonoscopy the same day were matched for gender and age. No selection were used in participants recruitment. For PFA-fixed and paraffin-embedded rectosigmoid tissues blocks from UCSF, samples with small tissue size or high variability in tissue autofluorescence were blindly excluded, the submucosa and tunica muscularis were blindly excluded (please see the rational above), therefore any relevent selection bias should be excluded.

Ethics oversight

University of Massachusetts Medical School

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration

Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.

Study protocol

Note where the full trial protocol can be accessed OR if not available, explain why.

Data collection

Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.

Outcomes

Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

GSE122549: CD94-CD56dim, CD94+CD56dim, and CD94+CD56hi NK cells CUB&RUN

For reviewers:

To review GEO accession GSE122549:
Enter token adyhowaipbuvh into the box.

Files in database submission

GSM3473668 cut and run sample1 CD94-CD56dim Rabbit IgG
GSM3473669 cut and run sample1 CD94-CD56dim H3K4me1
GSM3473671 cut and run sample1 CD94-CD56dim H3K4me3
GSM3473672 cut and run sample1 CD94-CD56dim TCF7
GSM3473674 cut and run sample1 CD94+CD56dim Rabbit IgG
GSM3473675 cut and run sample1 CD94+CD56dim H3K4me1
GSM3473677 cut and run sample1 CD94+CD56dim H3K4me3
GSM3473678 cut and run sample1 CD94+CD56dim TCF7
GSM3473680 cut and run sample1 CD94+CD56hi Rabbit IgG
GSM3473681 cut and run sample1 CD94+CD56hi H3K4me1
GSM3473683 cut and run sample1 CD94+CD56hi H3K4me3
GSM3473684 cut and run sample1 CD94+CD56hi TCF7
GSM3473686 cut and run sample2 CD94-CD56dim Rabbit IgG
GSM3473687 cut and run sample2 CD94-CD56dim H3K4me1
GSM3473688 cut and run sample2 CD94-CD56dim H3K4me3
GSM3473690 cut and run sample2 CD94-CD56dim TCF7
GSM3473692 cut and run sample2 CD94+CD56dim Rabbit IgG
GSM3473693 cut and run sample2 CD94+CD56dim H3K4me1
GSM3473695 cut and run sample2 CD94+CD56dim H3K4me3
GSM3473696 cut and run sample2 CD94+CD56dim TCF7
GSM3473698 cut and run sample2 CD94+CD56hi Rabbit IgG
GSM3473699 cut and run sample2 CD94+CD56hi H3K4me1
GSM3473701 cut and run sample2 CD94+CD56hi H3K4me3
GSM3473702 cut and run sample2 CD94+CD56hi TCF7

GSE122549_RAW.tar TDF

Genome browser session

(e.g. UCSC)

To review GEO accession GSE122549: enter token adyhowaipbuvh into the box.

Methodology

Sorted CD94-CD56dim, CD94+CD56dim, and CD94+CD56hi NK cells were processed as described94–96. Cells were lysed in nuclear extraction buffer [20 mM HEPES-KOH, pH 7.9; 10 mM KCl; 0.5 mM spermidine; 0.1% Triton X-100; 20% glycerol].
Nuclei were precipitated by centrifugation and then resuspended and bound to Bio-Mag Plus Concavalin A coated beads (Polysciences, cat# 86057), and incubated for 5 mins in blocking buffer (20 mM HEPES, pH 7.5; 150 mM NaCl; 0.5 mM spermine; 0.1% BSA; 2 mM EDTA). Then nuclei were incubated overnight at 4°C with the following antibodies at 1:100 dilution: rabbit anti-human IgG (Control, abcam, cat# ab2410), rabbit anti-H3K4me1 (diagnode, cat# c15410194), rabbit anti-H3K27ac (diagnode, cat# c15410196), rabbit anti-H3K4me3 (diagnode, cat# c15410003), rabbit anti-TCF7 (cell signaling technology, cat# 22033), or rabbit anti-H3K27me3 (diagnode, cat# c15410195). Protein A-micrococcal nuclease fusion protein, a gift from Thomas Fazzio, was added at 600 ug/ml for 1 hr at 4°C. CaCl2 was added to a final concentration 2 mM to activate the micrococcal nuclease, and 2X stop buffer (200 mM NaCl; 20 mM EDTA; 4 mM EGTA; 50 ug/ml RNase A; 40 ug/ml glycerol) was added 30 min later. Supernatant containing the released chromatin was subjected to phenochloroform isomyan extraction and DNA was precipitated with ethanol. The sequencing library was constructed according to the NEBNext Ultra II DNA library Prep kit for Illumina-based sequencing (NEB, cat# 7665L), using NEBNext Multiplex Oligos (NEB, cat# E6095S). For donor 1, primers D1-D6 were used to amplify each antibody-enriched DNA preparation from CD94-CDS6dimNK cells according to the antibody order mentioned above. Accordingly, primers D7-D12 were used for sorted CD94+CDS6dimNK cells, and primers E1-E6 were used for sorted CD94+CDS6hNK cells. For donor 2, primers E7-E12 were used for sorted CD94+CDS6dimNK cells, primers F1-F6 were used for sorted CD94+CDS6dimNK cells, and primers F7-F12 were used for sorted CD94+CDS6hNK cells. Libraries were paired-end sequenced on a Nextseq 500 V2 (illumina) using 45 cycles Read 1, 8 cycles Index 1, and 32 cycles Read 2.

Sequencing depth

The sequencing depth of CUT&RUN was around 5-10 million paired-end reads for each sample. Since the CUT&RUN typically requires ~1/10th the sequencing depth as ChIP-Seq [Peters J Skene, 2017, eLife], the sequencing depth in this study was sufficient to generate high quality data for downstream analysis.

Antibodies

- Anti-Human IgG (abcam Cat# ab2410)
- Anti-H3K4me1 (diagnode Cat# c15410194)
- Anti-H3K4me3 (diagnode Cat# c15410003)
- Anti-TCF7 (Cell Signaling Cat#22033 Clone: C63D9)

Peak calling parameters

For CUT&RUN, paired-end reads were removed where the average quality scores in window size 10 are less then 15 and trimmed where leading and trailing bases with quality scores less than 15 using trimmomatic version 0.32. Reads that were longer than 25 bases after trimming were kept for further analysis. The reads were then aligned to human reference genome hg19 using Bowtie2 with options --un-conc to filter out reads that align un-concordantly. Duplicated reads were filtered out using Picard’s MarkDuplicates version 0.32. Peaks 2b were then called using MACS2. Alignment files were also converted to tif format using igvtools count function version 2.3.31 using -w 5 parameter.

Data quality

The methods to ensure data quality was mentioned in "Peak calling parameters" section (see above).

Software

Software used for CUT&RUN was listed in "Software and Code" section (see above).

Flow Cytometry

Plots

- Confirm that:
  - The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
  - The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
  - All plots are contour plots with outliers or pseudocolor plots.
  - A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Live and dead cells were discriminated using the Live and Dead violet viability kit [Invitrogen, L-34963]. For cell surface molecule detection, the cells were resuspended in antibody-containing MACS buffer for 30-60 min at 4°C in the dark. To detect cytokine production, cells were stimulated with the indicated cytokines for 16 hrs, or with PMA and ionomycin (cell stimulation cocktail 00-4970-03, ebioscience) for 3-6 hrs. In both cases, protein transport inhibitor (00-4980-03, ebioscience) was present during the stimulation. For intracellular staining of transcription factors or cytokines, cells were fixed and permeabilized using Foxp3 staining buffer kit (ebioscience) and target intracellular molecules were stained as for surface staining.

Instrument

FACSCelesta flow cytometer, BD

Software

FlowJo, LLC, https://www.flowjo.com/
BD FACS Diva

Cell population abundance

The abundance of enrichment of sorted cells were checked after sorting before performing further experiment, the enrichment of sorted cells were above 99%.
### Magnetic resonance imaging

#### Experimental design

| Design type | Indicate task or resting state; event-related or block design. |
|-------------|---------------------------------------------------------------|
| Design specifications | Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials. |
| Behavioral performance measures | State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects). |

#### Acquisition

| Imaging type(s) | Specify: functional, structural, diffusion, perfusion. |
|----------------|-------------------------------------------------------|
| Field strength | Specify in Tesla |
| Sequence & Imaging parameters | Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle. |
| Area of acquisition | State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined. |

#### Preprocessing

| Preprocessing software | Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.). |
|------------------------|--------------------------------------------------------------------------------------------------|
| Normalization | If data were normalized/standardized, describe the approach(es); specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization. |
| Normalization template | Describe the template used for normalization/ transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized. |
| Noise and artifact removal | Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration). |
| Volume censoring | Define your software and/or method and criteria for volume censoring, and state the extent of such censoring. |

#### Statistical modeling & inference

| Model type and settings | Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects, drift or auto-correlation). |
|-------------------------|--------------------------------------------------------------------------------------------------|
| Effect(s) tested | Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used. |

**Specify type of analysis:**

- [ ] Whole brain
- [ ] ROI-based
- [ ] Both

**Statistic type for inference**

(See [Kund et al., 2016](#))

- Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

**Correction**

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

#### Models & analysis

| n/a | Involved in the study |
|-----|-----------------------|
|     | Functional and/or effective connectivity |
|     | Graph analysis |
|     | Multivariate modeling or predictive analysis |
| Section                          | Description                                                                 |
|---------------------------------|-----------------------------------------------------------------------------|
| Functional and/or effective connectivity | Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information). |
| Graph analysis                  | Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.). |
| Multivariate modeling and predictive analysis | Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics. |