TASOR epigenetic repressor cooperates with a CNOT1 RNA degradation pathway to repress HIV

Roy Matkovic, Marina Morel, Sophie Lanciano, Pauline Larrous, Benjamin Martin, Fabienne Bejjani, Virginie Vauthier, Maike M. K. Hansen, Stéphane Emiliani, Gael Cristofari, Sarah Gallois-Montbrun & Florence Margottin-Goguet

The Human Silencing Hub (HUSH) complex constituted of TASOR, MPP8 and Periphilin recruits the histone methyl-transferase SETDB1 to spread H3K9me3 repressive marks across genes and transgenes in an integration site-dependent manner. The deposition of these repressive marks leads to heterochromatin formation and inhibits gene expression, but the underlying mechanism is not fully understood. Here, we show that TASOR silencing or HIV-2 Vpx expression, which induces TASOR degradation, increases the accumulation of transcripts derived from the HIV-1 LTR promoter at a post-transcriptional level. Furthermore, using a yeast 2-hybrid screen, we identify new TASOR partners involved in RNA metabolism including the RNA deadenylase CCR4-NOT complex scaffold CNOT1. TASOR and CNOT1 synergistically repress HIV expression from its LTR. Similar to the RNA-induced transcriptional silencing complex found in fission yeast, we show that TASOR interacts with the RNA exosome and RNA Polymerase II, predominantly under its elongating state. Finally, we show that TASOR facilitates the association of RNA degradation proteins with RNA polymerase II and is detected at transcriptional centers. Altogether, we propose that HUSH operates at the transcriptional and post-transcriptional levels to repress HIV proviral expression.
As of today, highly active antiretroviral therapy is very efficient in inhibiting the replication of human immunodeficiency virus (HIV) in CD4+–infected T cells, thanks to the combination of several molecules targeting different stages of the lentivirus cycle. As long as the treatment is properly followed, the viremia of the infected individual will become and remain undetectable. However, if the treatment is randomly taken off or is interrupted, a viral rebound will cause new cellular infections, increasing the number of reservoir cells in which the virus remains latent. At the proviral level, latency can be explained by an inhibition of viral transcription or by a defect in the post-transcriptional steps leading to a lack of production of new lentiviral particles. After integration, HIV-1 transcription involves transcriptional steps leading to a lack of production of new lentiviral transcripts5. This increase is amplified by the histone methyltransferase SETDB1 and resulting in position-effect variegation in an HIV-1 model of latency5,6. HUSH also contributes to silencing retrotransposons such as the Long Interspersed Elements—Class I (L1s), preferentially those belonging to the youngest families (<5 million-year-old (myo)), both in mouse embryonic stem cells (ESCs)7,8 and in human cells9,10. In addition, this complex represses hundreds of cellular genes, many of which are close to or contain retrotransposons in their introns, in particular with the help of KAP1/Trim289,9. TASOR-dependent H3K9me3 deposition was also shown to occur on ribosomal DNA, ZNF encoding genes, and on repetitive or rapidly evolving genes6,9,11,12. Altogether, HUSH appears to play a central role in maintaining genome integrity, whether the threat comes from within with L1 elements or from without with viral infections, like HIV.

We and others have previously shown that the lentiviral proteins Vpr and Vpx, and namely, Vpx from HIV-2, could counteract HUSH by preferentially inducing the degradation of TASOR and MPP8, consistent with a critical role for HUSH in antiretroviral innate immunity5,13,14. HUSH degradation leads to a decrease of H3K9me3 repressive marks on HIV-1 internal sequence and to the subsequent accumulation of LTR-initiated viral transcripts9. This increase is amplified by tumor necrosis factor-α (TNFα) treatment, a well-known inducer of transcription activation from the HIV-1 promoter15, which led us to question a possible co- or post-transcriptional repressive effect of TASOR. In support of this hypothesis, HUSH targets were found enriched in transcriptionally permissive euchromatin8,10. Hence, HUSH-mediated L1 silencing often occurs in introns of transcriptionally active genes, leading to the downregulation of host gene expression10. In addition, TASOR and Periphrin-1 were found associated with messenger RNAs16,17 or with the XIST-long non-coding RNA (lncRNA)18. Here we address the question of a possible co- or post-transcriptional repressive effect of TASOR toward HIV. Our results led us to propose a model in which HUSH cooperates with CNOT1 (CC4R-NOT transcription complex subunit 1) to control viral and host gene expression through a feedback loop mechanism spanning RNA synthesis, RNA stability, and deposition of repressive epigenetic marks.

Results
TASOR destabilizes HIV-1 LTR-driven transcripts. To address the question of the molecular mechanism employed by TASOR to repress HIV-1 expression, we chose a cellular system in which the LTR-driven active transcription could be studied independently of RNA splicing. Therefore, we used HeLa cells harboring one unique and monoclonal copy of an integrated LTR-Luciferase construct with a deleted TAR sequence (ΔTAR) to model a fully active transcription process19 (Fig. 1a). Indeed, removing the TAR RNA structure, which blocks RNA polymerase II (RNAPII) elongation, leads to a 100-fold increase of Luciferase (Luc) transcript level as compared to the WT LTR in the absence of Tat (Fig. 1a). Under these experimental conditions, we confirmed that TASOR acts as a repressor of HIV-1 expression by measuring the Luc activity upon overexpression of TASOR in CRISPR/Cas9-depleted cells (Fig. 1b), or in contrast, when reducing its expression by small interfering RNA (siRNA; Fig. 1c). Notably, TASOR lacking its N-terminus PARP13-like PARP domain (APARP), required for epigenetic repression according to Douse et al.5, does not repress LTR-driven expression in contrast to full-length TASOR (Fig. 1b, up to 170 vs 47% of initial luciferase activity respectively). To examine the role of TASOR in mRNA metabolism, we performed Nuclear Run On experiments to assess the effect of TASOR silencing on nascent Luc RNA transcription from the HIV-1 LTR, while quantifying this transcript at the total level. Then, by comparing nascent Luc transcripts (labeled with BrUPT) to the total Luc mRNA amounts (Fig. S1a), we can determine at which stage of RNA metabolism TASOR may negatively act. While TASOR downregulation by siRNA very slightly increases the levels of nascent transcripts (1.3-fold, Fig. 1c), as also observed on WT LTR cells (Fig. S1b), it triggers a 2.6-fold increase of steady-state Luc RNA levels, suggesting that TASOR depletion also impairs the turnover of LTR-driven transcripts (Fig. 1c). Of note, the increase of luciferase activity following TASOR depletion was higher than the increase of Luc RNA levels when quantifying total RNA levels, suggesting that TASOR might impact steps beyond RNA degradation, such as RNA export or its translation (Fig. 1c, 9.4- vs 2.6-fold).

Next, we used HIV-2 Vpx to further confirm the role of TASOR on transcripts turnover. Indeed, mimicking TASOR siRNA treatment, Vpx expression leads to TASOR depletion (Fig. 1d, left) and to an increase of Luc RNA accumulation relative to its transcription, consistent with a post-transcriptional impact of TASOR on LTR-driven transcripts stability (Fig. 1d, right). By contrast, Vpx only influences nascent transcript levels of the MORC2 gene, a known cellular target of HUSH12 (Fig. S1c). As expected, a mutated version of Vpx (R42A), unable to induce TASOR degradation (Fig. 1d, left) but still able to induce SAMHD1 degradation (Fig. S1e), has no effect on Luc or on MORC2 RNAs (Fig. 1d, right and S1c). Furthermore, HIV-1 Vpr, which presents structural similarities with Vpx but is unable to induce TASOR depletion5,20, was also unable to increase the expression of the LTR-ATAR-driven transcript at any stage (Fig. 1d), although it could specifically stabilize TNFα transcripts (Fig. S1d), in agreement with previous observations21. Altogether, these results suggest that Vpx is able to induce the stabilization of the LTR-driven transcripts through TASOR degradation. Structure–function analysis provided additional hints on the potential mechanism of TASOR post-transcriptional effect. Consistent with a recent study9, we found, using the pGenTHREADER22 and FFPRED23 bioinformatics tools, that the N-terminus of TASOR is predicted to fold into a PARP13-like PARP domain with high probable and reliable functions in mRNA binding, splicing and processing, together with the SPOC (Spn paralag and ortholog C-terminal) domain, often associated with transcription repression (Table S1). Moreover, bioinformatic structure prediction and amino-acid alignment of different SPOC domains showed that TASOR domain is evolutionary related to the SPOC domain of Arabidopsis thaliana FPA, a protein...
Fig. 1 TASOR destabilizes HIV-1 LTR-driven transcripts. 

a HeLa HIV-1 LTR-ΔTAR-Luc cell model. HeLa HIV-1 LTR-Luc and HeLa HIV-1 LTRΔTAR-Luc cells were lysed for Luc RNA quantification (n = 3; each replicate is presented along with the mean values and the SEM. A two-sided unpaired t test was used. ****p < 0.0001). 

b TASOR overexpression decreases LTR-driven Luc expression. HeLa HIV-1 LTR-ΔTAR-Luc were TASOR-depleted by transient transfection of the pLenti-CRISPR-V2 and a sgRNA guide targeting the first exon of TASOR. These TASOR-depleted cells were then transfected with increasing amounts of HA-TASOR WT or HA-TASORΔPARP encoding pAS1B vectors. Luc activity was measured and proteins were analyzed by western blot 48 h post-transfection (n = 3; each independent replicate is presented along with the mean values and the SEM. A two-sided unpaired t test was used. **p = 0.0029; *p = 0.0262. 

c TASOR negatively impacts LTR-driven Luc transcript at a post-transcriptional step. HeLa HIV-1 LTRΔTAR-Luc were transfected for 72 h with siCtrl or siTASOR. The luciferase activity was measured and Nuclear Run On experiments were performed (n = 3, each independent replicate is presented along with the mean values and the SEM). 

d HIV-2 Vpx mimics siRNA-mediated TASOR silencing in increasing LTR-driven transcript stability. Nuclear Run On performed in HeLa HIV-1 LTRΔTAR-Luc after 48 h of pAS1B-HA, pAS1B-HA-Vpx WT or R42A HIV-2 Ghana, and pAS1B-HA-Vpr HIV-1 transfection. The HIV-1 LTR-driven luciferase RNA expression was measured by RT-qPCR (n = 4, each independent replicate is presented along with the mean values and the SEM) and a western blot analysis monitored the levels of expression of the lentiviral proteins, TASOR, and HLTF (as an HIV-1 Vpr target[74]). Source data are provided as a Source data file.
involved in epigenetic repression of retrotransposons and RNA-dependent suppression of intergenic transcription\textsuperscript{24,25} (Fig. S2a, b).

**TASOR interacts and cooperates with the CCR4-NOT complex scaffold CNOT1.** To obtain insights into the pathways contributing to TASOR post-transcriptional functions, we performed a yeast two-hybrid (Y2H) screen using the first 900 aa of the 1670 aa-long TASOR protein as bait, and the proteome from human macrophages as prey, since this cell type represents a natural target for HIV. As expected, we found that the TASOR N-terminal region binds its known HUSH partner, MPP8. Other candidate binding-proteins are factors involved in gene transcription modulation, LXR\textsubscript{α}, ARID1A (also known as BAF250), EP300, SUPT6H, KDM5C, as well as proteins involved in mRNA metabolism, CYFIP1, CNOT1, DHX29, DHX30, EIF4G1, ZFC3H1, and MATR3 (Fig. S2c). The latter has already been discovered as a TASOR interacting protein in previous large-scale interactome studies\textsuperscript{26,27}. Among these candidate partners of TASOR, some are negative regulatory factors of gene expression directly connected to RNA pol II (RNAPII) subunit RPB1 (Fig. 2a), which may indicate a role of TASOR and partners in
inhibiting gene expression during the transcription process per se. We chose to focus on CNOT1, which is the scaffold protein of the most important and conserved deadenylate complex from Yeast to Human, the Carbon catabolite repression 4-negative on TATA-less complex or CCR4-NOT28,29. Interestingly, CNOT1 was found in a siRNA screen for cellular factors involved in MMLV-Gfp reporter silencing in ESCs, along with MPP8 or ATF7IP, a protein essential for heterochromatin formation by HUSE30,31. We confirmed the interaction between epitope-tagged TASOR (TASOR-DDK) and endogenous CNOT1, in addition to already known TASOR-binding proteins such as MPP8, Periphilin, and MORC2 (Fig. 2b), and between endogenous TASOR and endogenous CNOT1 (Figs. 2c and S2d). While Dnase I-treatment does not prevent TASOR and CNOT1 interaction, RNase A-mediated RNA digestion reduces the amounts of co-immunoprecipitated CNOT1 in HeLa cells (Fig. 2d), suggesting that RNA favors the interaction between the two partners. Importantly, the inactive TASORAPARP mutant does not interact with CNOT1, which is consistent with the hypothesis that TASOR-CNOT1 association is involved in TASOR-mediated repression (Fig. 2e). CNOT1 being a scaffold protein, we checked whether TASOR could interact with other components of the CCR4-NOT complex. By immunoprecipitating DDK-tagged CNOT7, the deadenylase partner of CNOT1, we revealed an interaction with endogenous TASOR, along with the other CCR4-NOT complex CNOT9 and CNOT1 components (Fig. S2e).

Since TASOR and CNOT1 interact with each other, we tested whether they collaborate to repress LTR-driven expression at the level of the transcription per se and/or at the level of RNA stability by performing Nuclear Run On experiments as in Fig. 1. Silencing of TASOR and/or CNOT1 was checked by western blot (Fig. S2f). The main effect of CNOT1 was detected at the total level of LTR-driven transcripts, whose RNA amounts were increased by 4-fold upon CNOT1 silencing, in agreement with its known role on RNA stability (Fig. 2f). Interestingly, when silencing both TASOR and CNOT1, a strong synergistic effect was observed at the total RNA level, suggesting that the two proteins act in different pathways but cooperate to repress the expression of the transcript particularly at a post-transcriptional level (Fig. 2f: 27.2- to 2.5-fold increase on total vs nascent RNA). As discussed above, siRNA or Vpx-mediated TASOR depletion only increased the level of MORC2 mRNAs at the transcriptional stage (no difference between total and nascent RNA—Figs. 2g and S1c). In contrast, CNOT1 depletion did not increase nascent MORC2 mRNA levels but led to its accumulation (Fig. 2g, 0.9- vs 3.0-fold, respectively). On the other hand, CNOT1 has been shown to interact with TNFα mRNA and to induce its deadenylation with CNOT732,33. Accordingly, we confirmed that CNOT1 silencing increased by 8.8-fold the total TNFα mRNA level without influencing TNFα transcription (Fig. 2h). Of note, combining siCNOT7 with siCNOT1 does not further increase Luc expression as compared to siCNOT1 alone, in agreement with CNOT1 and CNOT7 belonging to the same pathway (Fig. S2g). However, the inhibition of both CNOT7 and TASOR expression suggests a collaboration between CCR4-CNOT and TASOR (Fig. S2g). Altogether, our results show that TASOR and CCR4-NOT interact both physically and functionally and can strongly cooperate at a post-transcriptional level to decrease LTR-driven transcript accumulation.

**TASOR-CNOT1 complex represses LTR-driven expression in two models of HIV-1 latency.** Next, we questioned the relevance of the TASOR-CNOT1 cooperation in more relevant HIV models. First, we confirmed that endogenous TASOR and CNOT1 interact in a Jurkat-derived latency model for HIV-1 (J-Lat A1, Fig. 3a). This T-cell line contains an HIV-1 LTR-ΔTAR-Luc vector expressing a doxycycline-inducible miRNA directed against CNOT1 mRNA or, as control, a miRNA against luciferase (Fig. 3b). The addition of doxycycline represses MORC2 mRNA levels but led to its accumulation (Fig. 2g, 0.9- vs 3.0-fold, respectively). On the other hand, CNOT1 has been shown to interact with TNFα mRNA and to induce its deadenylation with CNOT732,33. Accordingly, we confirmed that CNOT1 silencing increased by 8.8-fold the total TNFα mRNA level without influencing TNFα transcription (Fig. 2h). Of note, combining siCNOT7 with siCNOT1 does not further increase Luc expression as compared to siCNOT1 alone, in agreement with CNOT1 and CNOT7 belonging to the same pathway (Fig. S2g). However, the inhibition of both CNOT7 and TASOR expression suggests a collaboration between CCR4-CNOT and TASOR (Fig. S2g). Altogether, our results show that TASOR and CCR4-NOT interact both physically and functionally and can strongly cooperate at a post-transcriptional level to decrease LTR-driven transcript accumulation.
MORC2 mRNA expression levels are solely dependent on TASOR expression under these experimental conditions, whereas TNFα mRNAs remain very lowly expressed under all conditions (Fig. 3g, h).

Second, we tested the TASOR-CNOT1 synergy under conditions where infected cells represent a population with diverse integration sites as previously achieved13. Jurkat T cells were infected with a genetically marked HIV-1 virus13 (Fig. 4a). This vector retains the complete LTRs, tat, and rev, but has a frameshift mutation in env, ngfr in place of nef and egfp in place of gag, pol, vif, and vpr. Finally, the splicing sites are conserved. We sorted infected cells by cytometry (EGFP-positive cells), kept the sorted cells in culture for another two weeks until EGFP expression was silenced, and finally sorted the population of
Fig. 3 TASOR and CNOT1 cooperate to repress HIV-1 expression in the J-Lat A1 model of HIV-1 latency. a TASOR interacts with CNOT1 in the J-Lat A1 T cell line. b Generation of Dox-inducible miR-RNA CNOT1 J-Lat A1 cells. J-Lat A1 cells, harboring one copy of integrated and latent LTR-tat-jRES-GFP-LTR construct, were transfected with a pINDUCER10 vector containing a doxycycline-inducible miRNA targeting CNOT1 or Luciferase (Mock) transcripts. c Expression of the miRNA targeting CNOT1 reacts to GFP expression from the latent HIV-1 minigene. J-Lat A1 miR Luc or miR CNOT1 were treated or not with doxycycline 1 μg/mL for 72 h and GFP expression was analyzed by flow cytometry. The proportion of cells that became GFP positive is indicated in green. d Dox-inducible miR-CNOT1, but not miR-Luc, decreases CNOT1 expression while delivery of VLPs containing HIV-2 Vpx WT induces TASOR depletion in contrast to Vpx R42A in the J-Lat A1 cells. J-Lat A1 miR Luc and miR CNOT1 were treated or not for 72 h with doxycycline 1 μg/mL and VLPs containing HA-Vpx WT or R42A or mock were delivered for 24 h prior to protein analysis. e-f Co-silencing of CNOT1 and TASOR synergistically increases LTR-driven GFP expression at the protein level with the proportion of cells that became GFP positive indicated in green and the median of GFP expression in the whole cell population in italic (e), at the RNA level (f), but not MORC2 RNA (g) or TNF RNA (h) in the J-Lat A1 model. J-Lat A1 miR Luc and miR CNOT1 were treated or not for 72 h with doxycycline 1 μg/mL and VLPs containing HA-Vpx WT or R42A or mock were delivered for 24 h and TNF (1 ng/mL) 16 h prior to flow cytometric analysis and RT-qPCR analyses (from d to h, n = 3 for miR Luc; n = 6 for miR CNOT1; each independent replicate, mean and SEM are shown; two-sided unpaired t test was applied). Source data are provided as a Source data file.

A set of host genes are cooperatively regulated by TASOR and CNOT1. To assess whether TASOR and CNOT1 can cooperate beyond HIV repression, we conducted quantitative polymerase chain reaction (RT-qPCR), suggesting that a co- or post-transcriptional repressive mechanism could contribute to the latent state (Fig. 4b, right). To assess the potential cooperation between TASOR and CNOT1 in this model of HIV-1 latency, we depleted TASOR and CNOT1 by Vpx and miR-CNOT1, respectively, as previously (Fig. 4c). Analysis of EGFP expression by cytometry or quantification of unspliced RNA transcribed from the HIV-1 5′LTR demonstrate the synergy between TASOR and CNOT1 in repressing HIV-1 LTR-driven expression in T cells (Fig. 4d, e, 16.1× fold increase when silencing both TASOR and CNOT1 and expression in comparison to 3.0× and 3.6× upon TASOR and CNOT1 silencing respectively). Only a twofold increase in MORC2 transcript levels (corresponding to TASOR silencing) and no effect on the TNFa transcripts were observed under these experimental conditions (Fig. 4f, g).

TASOR interacts and cooperates with nuclear RNA degradation factors. To gain insight into the mechanism of TASOR-CNOT1-mediated repression mechanism, we questioned the involvement of MTR4 present in the human TRAMP, NEXT or PAXT complexes, but also human EXOSC10 (RRP6 in yeast) from the exosome and the m6A reader YTHDF2. Indeed, in yeast, Not1, the homolog of human CNOT1, has been shown to recruit Mtr4 and the exosome, resulting in the degradation of nuclear defective and nascent RNAs38,39. In addition, MTR4 and EXOSC10/RRP6 are members of an RNA surveillance complex that inhibits LTR-driven expression in the HeLa HIV-1 WT LTR-Luc model40. Finally, YTHDF2 was reported on the one hand to interact with CNOT1, which leads to the destabilization of nuclear defective and nascent RNAs41 and on the other hand to be recruited onto HIV-1 5′LTR, Nef, and 3′LTR genomic RNA sequences42,43. By cell fractionation, we show that TASOR protein is predominantly detected in the nucleolus, while CNOT1 is a shuttling protein, present both in the cytoplasm and nucleolus, in agreement with previous studies44–46 (Fig. 6a). As controls, the MATR3 and GAPDH proteins fractionated in the nucleus and the cytoplasm, respectively, as expected (Fig. 6a). Then, in an endogenous TASOR immunoprecipitate from nuclear extracts, we could retrieve EXOSC10, MTR4, and YTHDF2, in addition to CNOT1 and CNOT7 (Fig. 6b) and, in a CNOT1 immunoprecipitate, we could retrieve CNOT7 and YTHDF2 as expected, as well as MTR4, EXOSC10 and TASOR (Fig. 6c). The same components were pulled-down in an MTR4 immunoprecipitate except for CNOT7 (Fig. 6c). Next, we overexpressed TASOR-DDK in cells and performed a DDK immunoprecipitate to recover TASOR and its bound partners in the absence or in presence of RNAse A (Figs. 6d and S3a). MATR3 is the only protein along with MPP8, our positive control, to be efficiently co-immunoprecipitated with TASOR under RNAse A treatment, which confirms our Y2H results and that the interactions between TASOR and MATR3, MPP8 are RNA independent (Fig. 6d). However, the interactions between TASOR-DDK, CNOT1, and its known partners MTR4, YTHDF2 seem to rely on the presence of RNAs since TASOR affinity for these proteins is decreased upon RNAse A treatment. (Fig. 6d).

To verify the involvement of these new factors in the TASOR repression of LTR expression, we silenced these proteins expressions alone or in combination with TASOR and measured Luciferase activity (Fig. 6e). The strongest synergistic effect was always obtained under TASOR and CNOT1 co-silencing (Fig. 6e). Nonetheless, we observed that TASOR also cooperates with

latently infected cells (EGFP-negative cells; Fig. 4b, left). These cells do not express EGFP at the protein level whereas HIV-1-unspliced RNA could be detected by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR), suggesting that a co- or post-transcriptional repressive mechanism could contribute to the latent state (Fig. 4b, right). To assess the potential cooperation between TASOR and CNOT1 in this model of HIV-1 latency, we depleted TASOR and CNOT1 by Vpx and miR-CNOT1, respectively, as previously (Fig. 4c). Analysis of EGFP expression by cytometry or quantification of unspliced RNA transcribed from the HIV-1 5′LTR demonstrate the synergy between TASOR and CNOT1 in repressing HIV-1 LTR-driven expression in T cells (Fig. 4d, e, 16.1× fold increase when silencing both TASOR and CNOT1 and expression in comparison to 3.0× and 3.6× upon TASOR and CNOT1 silencing respectively). Only a twofold increase in MORC2 transcript levels (corresponding to TASOR silencing) and no effect on the TNFa transcripts were observed under these experimental conditions (Fig. 4f, g).

TASOR interacts and cooperates with nuclear RNA degradation factors. To gain insight into the mechanism of TASOR-CNOT1-mediated repression mechanism, we questioned the involvement of MTR4 present in the human TRAMP, NEXT or PAXT complexes, but also human EXOSC10 (RRP6 in yeast) from the exosome and the m6A reader YTHDF2. Indeed, in yeast, Not1, the homolog of human CNOT1, has been shown to recruit Mtr4 and the exosome, resulting in the degradation of nuclear defective and nascent RNAs38,39. In addition, MTR4 and EXOSC10/RRP6 are members of an RNA surveillance complex that inhibits LTR-driven expression in the HeLa HIV-1 WT LTR-Luc model40. Finally, YTHDF2 was reported on the one hand to interact with CNOT1, which leads to the destabilization of nuclear defective and nascent RNAs41 and on the other hand to be recruited onto HIV-1 5′LTR, Nef, and 3′LTR genomic RNA sequences42,43. By cell fractionation, we show that TASOR protein is predominantly detected in the nucleolus, while CNOT1 is a shuttling protein, present both in the cytoplasm and nucleolus, in agreement with previous studies44–46 (Fig. 6a). As controls, the MATR3 and GAPDH proteins fractionated in the nucleus and the cytoplasm, respectively, as expected (Fig. 6a). Then, in an endogenous TASOR immunoprecipitate from nuclear extracts, we could retrieve EXOSC10, MTR4, and YTHDF2, in addition to CNOT1 and CNOT7 (Fig. 6b) and, in a CNOT1 immunoprecipitate, we could retrieve CNOT7 and YTHDF2 as expected, as well as MTR4, EXOSC10 and TASOR (Fig. 6c). The same components were pulled-down in an MTR4 immunoprecipitate except for CNOT7 (Fig. 6c). Next, we overexpressed TASOR-DDK in cells and performed a DDK immunoprecipitate to recover TASOR and its bound partners in the absence or in presence of RNAse A (Figs. 6d and S3a). MATR3 is the only protein along with MPP8, our positive control, to be efficiently co-immunoprecipitated with TASOR under RNAse A treatment, which confirms our Y2H results and that the interactions between TASOR and MATR3, MPP8 are RNA independent (Fig. 6d). However, the interactions between TASOR-DDK, CNOT1, and its known partners MTR4, YTHDF2 seem to rely on the presence of RNAs since TASOR affinity for these proteins is decreased upon RNAse A treatment. (Fig. 6d).

To verify the involvement of these new factors in the TASOR repression of LTR expression, we silenced these proteins expressions alone or in combination with TASOR and measured Luciferase activity (Fig. 6e). The strongest synergistic effect was always obtained under TASOR and CNOT1 co-silencing (Fig. 6e). Nonetheless, we observed that TASOR also cooperates with
MATR3, YTHDF2, MTR4 (Fig. 6e), or with EXOSC10 (Fig. S3b). In humans, MTR4 is found in at least three different complexes that use the exosome complex to eventually degrade the targeted RNA: the nucleolar TRAMP complex, the nucleoplasmic TRAMP-like NEXT and PAXT complexes (reviewed in ref. 48). Of note, in our Y2H screen, we also identified ZFC3H1, a member of the PAXT complex, as a partner of TASOR (Fig. S2c). We confirmed, in nuclear immunoprecipitates, that TASOR interacts with the two main members of the TRAMP-like PAXT complex MTR4 and ZFC3H1 (Fig. S3c). Interestingly, ZFC3H1 has already been discovered as one of Periphilin’s partners47,49. In conclusion, our results suggest that TASOR and CNOT1 together form a platform recruiting different factors involved in RNA degradation pathways (Fig. 6f).
TASOR recruits RNA-degradation factors onto elongating RNAPII. The ability of TASOR to propagate H3K9me3 marks and its association with CNOT1, with subunits of a TRAMP-like/PAXT complex, and with the exosome led us to envision that HUSH might function similarly to the fission yeast RNA-induced silencing complex (RITS). Indeed, RITS induces transcriptional silencing by adding H3K9me3 marks concomitantly to the synthesis of a transcript and its processing by a TRAMP complex and the exosome.\(^{50}\) Alternatively, in fission yeast, silencing can depend on recognition of the transcript by small interfering RNAs produced by Dicer dsRNA-cleavage activity\(^{51,52}\). The inhibition of DICER by siRNAs does not increase LTR-driven Luc expression in our experimental model (Fig. S3d) suggesting inhibition of DICER by siRNAs does not increase LTR-driven genes covered by both RNAPII and TASOR-dependent and its association with CNOT1, with subunits of a TRAMP-like/PAXT complex, and with the exosome\(^{50}\). Alternatively, in fission yeast, silencing can depend on recognition of the transcript by small interfering RNAs produced by Dicer dsRNA-cleavage activity\(^{51,52}\). The inhibition of DICER by siRNAs does not increase LTR-driven Luc expression in our experimental model (Fig. S3d) suggesting inhibition of DICER by siRNAs does not increase LTR-driven genes covered by both RNAPII and TASOR-dependent genes; two-sided unpaired t test was applied; p values are indicated in the graph). Source data are provided as a Source data file.

### Discussion
Overall, our results support a model in which TASOR, in association with CNOT1, provides a platform along transcription to destabilize nascent transcripts. This conclusion is supported by: (i) the effect of TASOR beyond transcription per se at a post-transcriptional level; (ii) the interaction of TASOR with CNOT1 and their synergistic repressive effect on HIV-1 LTR-driven expression; (iii) the interaction of TASOR with members of the TRAMP-like/PAXT complex, ZFC3H1 and MTR4, and with the RNA exosome component EXOSC10, as well as its functional cooperation with them; (iv) the fact that the interactions between TASOR and RNA degradation factors are stabilized in the presence of RNA; (v) the decoupling of the coding sequence under TASOR silencing; (vi) the interaction of TASOR with elongating RNAPII; (vii) the recruitment of RNA degradation factors to RNAPII when TASOR is overexpressed, and (viii) the accumulation of TASOR in transcription centers and the interaction of TASOR and CNOT1 with nascent transcripts. While indirect effects cannot totally be ruled out from siRNA, miRNA or Vpx-mediated deletion experiments, this latter point suggests a direct effect of TASOR and CNOT1 on LTR-derived RNA metabolism.

Consistent with our observations, RPRD2, a regulator of RNAPII, together with RNA metabolism proteins were also identified as TASOR partners in a BioID assay\(^9\). Keeping in mind...
the role of TASOR at the epigenetic level, we propose a feedback control mechanism in which TASOR and CNOT1 would follow RNAPII during HIV-1 provirus transcription by association with the nascent transcript and recruit RNA metabolism proteins, leading in turn to the degradation of the transcript and the deposition of H3K9me3 marks (Fig. S5b).

This molecular defense mechanism was not already described in mammals although it seems to be quite well conserved throughout the evolution of eukaryotes in unicellular organisms such as Schizosaccharomyces pombe or more complex organisms such as Drosophila melanogaster or A. thaliana to preserve genome integrity. Indeed, our model parallels the gene expression repression mechanism proposed for the RITS complex in fission yeast or for the piRNA-guided transcriptional silencing Piwi-Asterix-Panoramix-Eggless complex model in drosophila. In each case, transcriptional epigenetic repression is coupled to the
Fig. 5 TASOR cooperates with CNOT1 to repress a subset of genes in HeLa cells. a HeLa cells were transfected for 72 h with siRNA targeting TASOR or CNOT1 or both transcripts. A western blot assessed the downregulation of TASOR and CNOT1 prior to RNAseq analyses. (western blot of the three different replicates is presented). b Number of genes that are significantly upregulated (by at least 20%), upon TASOR + CNOT1 co-silencing as compared to control, TASOR, or CNOT1 individual silencing in HeLa cells (log2 fold change > 0.2630344; p adjusted values < 0.05 after Benjamini–Hochberg multiple testing correction of Wald test p value). c Cooperativity of TASOR and CNOT1 regulation on host genes. The interaction term of the model used for differential gene expression analysis (ΔACostrast, DESeq2). d Cooperative effects affecting genes upregulated in one or the other of the single siRNA conditions (siTASOR or siCNOT1) and not downregulated in the second condition (log2 fold change > 0; p adjusted values < 0.05 after Benjamini–Hochberg multiple testing correction of Wald test p value). Fold changes (FC1 and FC2, as defined above) for individual genes were plotted showing the potential interaction modes of TASOR and CNOT1. e MA plot representing the differential expression of transposable element (TE) families in HeLa cells, upon co-silencing of TASOR and CNOT1 as compared to cells transfected with control siRNA. Significantly upregulated and downregulated TE families are highlighted in red and blue, respectively (log2(FC) > 0.2630344; p adjusted values < 0.05, Wald test with Benjamini–Hochberg multiple testing correction). ns not significant. f Number of TE families that are significantly upregulated (by at least 20%), upon TASOR + CNOT1 co-silencing as compared to control, TASOR, or CNOT1 individual silencing in HeLa cells (log2 fold change > 0.2630344; p adjusted values < 0.05 after Benjamini–Hochberg multiple testing correction of Wald test p value). More information is available in Source data file.

synthesis of a transcript and sometimes to its degradation. The fission yeast RITS complex seems to match well our HUSH model, with yeast Chp1 and Tas3 sharing structural features with TASOR, MPP8 and PPHLN-1, as also noticed by Douze et al.9,33 and with the ability of RITS to interact with Not1/CNOT159, the exosome and MTR4 alike HUSH (Fig. S5a, b). In addition, Skalska et al. recently showed that HUSH is recruited to chromatin upon RNAPII inhibition or RNase treatment, suggesting that RNA degradation of the nascent transcript could modify HUSH binding to chromatin, perhaps due to binding competition between nascent RNA and methylated histones, in agreement with our model60. Then, the role of HUSH in transcript destabilization could also be disconnected from its ability to epigenetically repress HUSH target genes. Indeed, in pluripotent stem cells, MPP8 lacking its chromodomain, which permits its binding to H3K9me3 marked chromatin, can still repress LINE-1 elements61, suggesting that in this configuration, HUSH could repress its targets independently from chromatin binding and maintenance of H3K9me3. It is not yet known whether the reverse, i.e. repression at the epigenetic level unrelated to RNA degradation mechanisms, is also possible. Therefore, a fundamental unanswered question is whether HUSH-mediated epigenetic repression could be effective by itself or whether HUSH must always rely on RNA degradation factors recruitment to enforce HIV silencing.

Inhibition of DICER has no effect on LTR-driven expression in our experimental system, suggesting that DICER-produced small interfering RNAs are not involved in HUSH-mediated repression of HIV-1, while small RNAs are known to mediate chromatin silencing in fission yeast or D. melanogaster for instance62. However, we cannot rule out the possible involvement of small interfering RNAs in the silencing of other HUSH gene targets.

To extend our observations beyond HIV transcription, we performed a transcriptomic analysis of cells silenced for TASOR, CNOT1, or both. Our data highlight that TASOR and CNOT1 cooperate to regulate a number of cellular genes, suggesting that a RITS-like mechanism could also apply to endogenous cellular targets. To our surprise, we do not detect upregulation of L1 RNAs following TASOR silencing, as expected from previous observations in other cell types7–10,37, suggesting that additional and predominant repressive mechanisms may operate in HeLa cells to silence L1 elements, such as DNA methylation63. Alternatively, long-term shRNA silencing or full knock-out by CRISPR may be required to fully reveal the entire landscape of HUSH-regulated transcripts10,37.

Regarding HIV, our results with two models of HIV-1 latency may suggest HUSH to be involved in the maintenance of HIV latency. Nonetheless, the post-transcriptional activity of HUSH along with RNA synthesis suggests that HUSH activity needs the production of the viral RNA. However, we were unable to reveal a repressive role for HUSH in biological systems of high sustained viral RNA transcription, either due to proviral integration into transcriptionally active chromatin sites or due to high levels of the Tat protein. Since HUSH repression is dependent on the integration site6, it is conceivable that HUSH triggers H3K9 tri-methylation—or its maintenance—only on provirus sequences integrated into poorly, but still, transcribing regions with signals of heterochromatinization. In this spatial window HUSH could counteract stochastic bursts of expression from the lowly expressed viral promoter64.

Our study also opens questions concerning the role of HIV-2 Vpx. By inducing HUSH degradation, Vpx is able to increase the proviral transcription from the HIV LTR and the stability of the LTR-driven transcript, thus Vpx hinders the silencing of the provirus. One could reasonably think at first that Vpx confers an advantage in HIV-2 replication. Though HIV-2 is able to counter several restriction factors, such as SAMHD1, APOBEC3G and BST2, HIV-2 replication is still very weak as compared to HIV-1 that does not counteract SAMHD1 or HUSH. As a repressor of retroelements expression, HUSH serves as a guardian of the integrity of the host genome. Then, by counteracting HUSH to increase HIV proviral expression, Vpx could, at the same time and as collateral damage, induce genomic deregulation and establish an unfavorable environment that subsequently limits HIV-2 replication. Nonetheless, directly linking clinical presentations to restriction factors activity may overlook other biological processes, including the immune response triggered by other components of the virus and the replication cycle itself. Overall, our study provides new insights into how HUSH represses retroelements such as the HIV provirus. We propose that TASOR acts as a hub linking the elongating RNAPII, the epigenetic repressor HUSH, and RNA degradation factors to repress the transgene both at the transcriptional level by the deposition of H3K9me3 marks and at the post-transcriptional level to degrade the synthesized target RNA. As a result, the host invader remains poorly expressed. Excitingly, the study by Jiang et al.65, shows that, in elite controllers, intact HIV-1 proviruses are present in H3K9me3-rich heterochromatin such as centromeric regions and in KRAB-Zinc finger genes, which are targeted by HUSH65. Understanding how HUSH is precisely recruited to provirus sequences or retroviral RNA could help decipher its role in the establishment of latent HIV reservoirs in infected patients.

Methods

Plasmids. TASOR expression vectors pLenti-myc-DDK, pLenti-TASOR-myc-DDK were purchased from Origene. pLenti-TASOR-myc-DDK expresses
a TASOR isoform of 1512 amino acids (NCBI Reference Sequence: NP_001106207.1). TASOR-ΔPARP construct was obtained by deleting the DNA sequence corresponding to the 106-319aa using the CloneAmp™ HiFi PCR Premix (639298-Takarabio) and the following 5′-3′-oriented primers: F-CCAGGAAGTATGCAGTTGTGTCTTTTACTTACA, R-CTGCATACTTCCTGGGGATCTGAAACTCC. pLentiCRISPRV2-sgTASOR-Cas9 was obtained by subcloning the following 5′-3′-oriented annealed primers: F-CACCGCTTTCCCAACTCGCATC CGT, R-AAACACGGATGCGAGTTGGGAAAGC, containing the sgRNAs targeting the first exon of TASOR, with the enzyme BsmBI. For complementation assays, pLenti-TASOR-DDK was made resistant to the guide by mutating the sgRNA-targeted sequence with 5′-CCAACAAGGTGCTGGGATCTGCA-3′. The TASOR ORF was then subcloned into the pAS1B-HA vector. DDK-CNOT7 plasmid is a kind gift from Nancy Standart. The Dox-inducible miR CNOT1 and miR Luc pINDUCER10 plasmids were kind gifts from Alfonso Rodriguez-Gil. The R42A mutant of Vpx HIV-2 Ghana was produced by site-directed mutagenesis using the pAS1B-HA-Vpx HIV-2 Ghana construct as a template.
**Fig. 6 TASOR interacts and cooperates with nuclear RNA degradation factors.**

**a** TASOR is a nuclear protein. Cytoplasmic and nuclear protein extracts from HeLa HIV-1 LTR-ΔTAR-Luc cells were loaded on a SDS-PAGE gel. GAPDH and MATR3 are markers of the cytoplasmic and nuclear fractions respectively (n > 3). **b** Endogenous TASOR interacts with the endogenous, nuclear CNOT1, and its partners CNOT7, YTHDF2, the endogenous TRAMP-like/NEXT/PAXT component MTR4, and the endogenous RNA exosome factor EXOSC10. HeLa HIV-1 LTR-ΔTAR-Luc cells were fractionated and endogenous CNOT1 and MTR4 immunoprecipitation was performed in the nuclear fraction. Lamin B1 is negative control (n > 3). **c** Reverse immunoprecipitation confirmed the interaction between endogenous CNOT1 and MTR4 proteins with endogenous TASOR in the nucleus. HeLa HIV-1 LTR-ΔTAR-Luc cells were fractionated and endogenous CNOT1 and MTR4 immunoprecipitation was performed in the nuclear fraction. The asterisk shows the SPU67 band (n = 3). **d** TASOR interacts with the known CNOT1 partners in an RNA-dependent manner. DKD and TASOR-DKD vectors were transfected in HeLa HIV-1 LTR-ΔTAR-Luc cells. After 48 h, lysates were treated or not with RNase A for 30 min at room temperature. Anti-DDK immunoprecipitation was then performed. All lanes are from the same gel (n > 3). **e** TASOR cooperates with the nuclear RNA destabilization/degradation factors. After 72 h of siRNA transfections in HeLa HIV-1 LTR-ΔTAR-Luc, cells were lysed and luciferase activity was measured and normalized on protein concentration (n = 5 for the siRNA TASOR and siRNA CNOT1 conditions; n = 3 for other siRNA transfections; each color represents one different independent experiment, mean and SEM are shown). **f** Schematic representation of RNA-mediated interactions between TASOR and CNOT1 and its partners: the TRAMP-like/NEXT/PAXT component MTR4, the Nuclear Exosome, and the m6A reader YTHDF2. Source data are provided as a Source data file.

**Virus and VLP production.** VSV-G pseudo-typed viruses and VLPs were produced in 293 T by the calcium-phosphate co-precipitation method. SV3 + ΔVprΔVpx packaging vectors were a gift from N. Landau and is described in 60. VLPs (Vpx expressed from pAS1B and incorporated into VLPs) were obtained by co-transfection of VSV-G plasmid, SV3 + ΔVprΔVpx packaging vector, and pAS1B-HA-Vpx HIV-2 Ghana (WT or R42A) or pAS1B-HA (empty). The HIV-1 LTR-EGFP virus presented in Fig. 4a was a gift from Jeremy Luban (Addgene plasmid # 115809) and was described in ref. 13. Tissue cultures were maintained for all conditions. Analysis was performed on the whole GFP-positive population when possible or arbitrary (as for J-Lat cells), and the same gate was maintained for all conditions. Analysis was performed on the whole GFP-positive population.

**Nuclear Run On (NRO).** HeLa LTR-ΔTAR-Luc cells were grown in 10 cm dishes and transfected with siRNA ctrl, TASOR + ctrl, CNOT1 + ctrl, and TASOR + CNOT1 at a final concentration of 100 nM each. NRO was performed as precisely described by Roberts and colleagues 61, except for these specific points: At step 14, RNA extractions with DNase treatment were performed with NuclearSpin RNA, Mini Kit (749052.250, Macherey-Nagel). At Step 44, Reverse transcription was performed with Maxima First Strand cDNA Synthesis Kit with dNTPase (K1672, ThermoFisher). qPCR was finally performed as described in the RT-qPCR section.

**RT-qPCR.** Except for step 14 in the Nuclear Run On experiments, all RNA extractions and purifications were based on a classical TRI Reagent (T9424-200ML, Merck) protocol. Reverse transcription steps were performed with Maxima First Strand cDNA Synthesis Kit with dNTPase. PCRs were performed through the LightCycler®480 (Roche) using a mix of 1x LightCycler®480 SYBR Green 1 Master (Roche) and 0.5 µM primers which sequences are described in Supplementary Table 2.

**FAIRE-qPCR.** Around 6x10^6 HeLa HIV-1 LTR-Luc cells were transfected with siRNA Ctrl or siRNA TASOR for 72 h. TNFα (10 ng/mL) was added or not 48 h before cell recovery. The FAIRE-qPCR protocol from 61 was then followed. At step 3.2, the chromatin of each sample was sonicated with a Diagenode BIORUPTOR Pico with 10 rounds of sonication 30 s on/30 s off. qPCR was performed using the primers listed in Supplementary Table 2.

**Cell fractionation.** HeLa cells grown in 10 cm dishes were washed with cold Dulbecco’s PBS 1× (ThermoFisher). After trypsinization (25200056, Thermo-Fisher), cells were recovered in 1.5 mL tubes and washed once with ice-cold PBS. After addition of 1 mL of Cytoplasmic Lysis Buffer and re-centrifugation for 4 min at 300 × g for 4 min at 4 °C. The supernatant was saved for cytoplasmic fraction. The pellet was resuspended with 1 mL of Cytoplasmic Lysis Buffer and re-centrifuged at 300 × g for 4 min at 4 °C twice. Finally, the nuclear pellet was lysed with 200 µL of RIPA Buffer.

**Immunoprecipitation, western blot protocols, and antibodies.** For TASOR-DKD or DDK-CNOT7 immunoprecipitations: HeLa/293T cells grown in 10 cm dishes were transfected with pLenti-DDK or with pLenti-TASOR-DDK or Flag-CNOT7 plasmids with CaCl2. 48 h after transfection, cells were lysed in 500 µL of Cytoplasmic Lysis Buffer (10 mM TRIS-HCl pH 7.5, 10 mM NaCl, 1 mM MgCl2, and 0.5% IGEA® CA-630 (18896-100ML-Merck)) and added to the cell pellet was resuspended in ice for 5 min. Cells were then centrifuged at 300 × g × 4 min at 4 °C. The supernatant was saved for cytoplasmic fraction. The pellet was resuspended with 1 mL of Cytoplasmic Lysis Buffer and re-centrifuged at 300 × g for 4 min at 4 °C twice. Finally, the nuclear pellet was lysed with 200 µL of RIPA Buffer.
1-AP, Proteintech 1/500; anti-CNOT9 (22503-1-AP, Proteintech) 1/500; anti-DHX9 (17721-1-AP, Proteintech) 1/1000; anti-EXOSC10 (11178-1-AP, Proteintech) 1/1000; anti-IF281 (22803-1-AP, Proteintech) 1/1000; anti-KPNB1 (ab17984, Abcam) 1/1000; anti-IGF2BP1 (22803-1-AP, Proteintech) 1/1000; anti-KPNB1 (HPA029878-100ul, lot D114771, Merck) 1/1000; anti-MATR3 (12202-2-AP, Proteintech) 1/1000; anti-MORC2 (PA5-51172, ThermoFisher) 1/1000; anti-MTR4 (For WB and IP: 12719-2-AP, Proteintech) 1/1000; anti-PPHLN-1 (HPA038902, Lot A104626, Merck) 1/1000; anti-RNAPII (For IP and WB: F12, sc-55492, Santa Cruz Biotechnology) 1/1000; anti-Ser2P-RNAPII (13499S, Cell Signaling technology) 1/1000 in 2.5% BSA-TBS-Tween 0.1%; anti-Ser5P-RNAPII (13523S, Cell Signaling technology) 1/1000 in 2.5% BSA-TBS-Tween 0.1%; anti-SUPT6H (For WB and IP: 23073-1-AP, Proteintech) 1/1000; anti-U1snRNP70 (sc-390899 (C3), Santa Cruz Biotechnology) 1/500; anti-YTHDF2 (24744-1-AP, Proteintech) 1/1000; anti-ZFC3H1 (HPA007151, Merck) 1/1000, anti-β-Actin (AC40, A3853, Merck) 1/1000; anti-α-Tubulin (T9026-.2mL, lot 081M4861, Merck) 1/1000; anti-GAPDH (6C5, SC-32233, Santa Cruz) 1/1000. All secondary antibodies anti-mouse (31430, lot VF297958, ThermoFisher) and anti-rabbit (31431, lot VF297959, ThermoFisher).
anti-rabbit (31460, lots VC297287, UK293475 ThermoFisher) were used at a 1/10,000 dilution before reaction with Immobilon Western HRP substrate (WBLU01001, Merck Millipore).

**RNA seq.** HeLa LTR-ATAR-Luc were grown in 10 cm dishes. Forty-eight hours post-transfections, the cells were recovered and labeling of nascent RNAs was undertaken with the *Nuclear Run on* protocol\(^{68}\). 10% of nuclei were put aside for purification of BrUTP incorporated Nascent RNAs (input). From the remaining nuclei, RNA-IP was then undertaken with the MagnaRIP RNA Binding Protein Immunoprecipitation Kit (17-700, Merck) from step 1.3. TASSOR, CNOT1, or anti-Rabbit IgG immunoprecipitations were performed with 4 µg of anti-TASSOR (HPA017142-Merck), anti-CNOT1 (14276-1-AP-ProteinTech), anti-Rabbit IgG (12-370) on Nuclear Lysate at 4 °C overnight. From step 3.6. of the MagnaRIP protocol, 10% of IPed lysate was recovered and washed three times with IP wash buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl) and loaded on SDS-PAGE gel to check DDK pull-down efficiency. The remaining IPed lysate was then washed from step 3.7. of the MagnaRIP protocol. Finally, purification of BrUTP incorporated IPed Nascent RNAs was undertaken from step 25 of the *Nuclear Run on* protocol.\(^{69}\) After purification, RT-qPCR of all purified RNAs was performed with primers listed in Supplementary Table 2.

Six hundred thousand HeLa cells containing an integrated copy of LTR-HIV-1-Luciferase Firefly were plated in 6-well plates. Cells were transfected with siRNAs targeting Mock, or TASSOR or CNOT1 or both TASSOR and CNOT1 using a masking level of 35, and at least 2 bp spacing between single probes. Probes (−5 orientation): #1 accacagtctttccct; #2 gacaagggcctctagc; #3 acctgcctctactccttc; #4 gtgttcaagtcgcactcg; #5 ccgttcgactgagacttt; #6 tacaagctgtttctct; #7 gttggagagatggctc; #8 tatttcgacatcagctca; #9 tcaagttgttcctctt; #10 acatttgagcgcagctc; #11 cctctgctgttgctgt; #12 tcaacctgtctgttac; #13 cttggctgcctggtgtg; #14 gacctctctgtc; #15 gctctaggtcttgc; #16 gctctaggtcttgc; #17 gctctaggtcttgc; #18 gctctaggtcttgc; #19 gctctaggtcttgc; #20 gctctaggtcttgc; #21 gctctaggtcttgc; #22 gctctaggtcttgc; #23 cagctgctctgtct; #24 gctctaggtcttgc; #25 gctctaggtcttgc.

Probes were conjugated with Quasar 670. VLPs containing Vpx WT or Vpx R42A or mock have been delivered on Jurkat cells latently infected with HIV-1 LTR-EFPG for 24 h. Cells were treated with 1 ng/ml TNFα (Sigma, catalog T0157) 16 h before fixation. The cells were washed with 10 mL of PBS solution and then immobilized on Cell-Tak coated six-well plates. Cells were then fixed with 5% formaldehyde in PBS for 10 min. Fixed cells were stored in 70% Ethanol at 4 °C for a minimum of 1 h to permeabilize the cell membranes. Probes were diluted to a final concentration of 50 nM in 1X PBS. 1% Formaldehyde and 10% Formamidase and allowed to hybridize at 37 °C overnight in a dark and humid chamber. Wash steps and DAPI staining were performed as described in Hansen et al.\(^{73}\). Then, cells were permeabilized once more with PBS-Triton 0.1% for 10 min at room temperature for TASSOR immunofluorescence staining. After PBS-mediated washes, a 1/500 anti-TASSOR (HPA006735-Merck) dilution in IF buffer (PBS-Tween 0.05%-BSA 0.2%) was incubated on the cells for 1 h at RT in a dark chamber. The cells were then washed three times with PBS and anti-rabbit Alexa 594 was added at a dilution of 1/1000 in IF buffer for 1 h at room temperature in a dark chamber. After three PBS-mediated washes, the coverslips were mounted on slides with the use of ProLong® Diamond Antifade Mountant (P36970-ThermoFisher). Imaging was performed with a Spinning-Disk IXplore Olympus confocal microscope, using a ×100 objective, a Hammatsu sCMOS Orca flash 4.0 V3 camera, and the cellSens Dimension acquisition software. GFP-positive cells were randomly captured. Analysis of images was performed with ImageJ and with the help of the Python core analysis package big-fish available at https://fish-quant.github.io/.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The RNA sequencing data generated in this study have been deposited in the GEO database under the accession code GSE184399. The CTRTag and CTRRun data of TASSOR GSM4710610 and H3K36me3 GSM4710590 and GSM4710594, respectively, were published by Douse et al.\(^{12}\) and deposited at GEO under the accession number GSE155693. ChIPseq and RNAseq were performed by Liu et al.\(^{13}\) and deposited at GEO under the accession number GSE95374. Source data and full western blots are provided with this article.
References

1. Jordan, A., Defechereux, P. & Verdin, E. The site of HIV-1 integration in the human genome determines basal transcriptional activity and response to Tat transcriptional activation. EMBO J. 20, 1726–1738 (2001).

2. Lewinski, M. K. et al. Genome-wide analysis of chromosomal features repressing human immunodeficiency virus transcription. J. Virol. 79, 6610–6619 (2005).

3. Brady, T. et al. HIV integration site distributions in resting and activated CD4+ T cells infected in culture. AIDS 23, 1461–1471 (2009).

4. Marcello, A., Dhir, S. & Dieudonne, M. Nuclear positional control of HIV-1 infection after acute infection of T cells in vitro. EMBO J. 22, 1868–1877 (2003).

5. Yi, W. et al. CRISPR-assisted detection of RNA-protein interactions in living cells. Nucleic Acids Res. 42, 643–660 (2014).

6. Contreras, X. et al. Nuclear RNA surveillance complexes silence HIV-1 transcription. PloS Pathog. 14, e1006950 (2018).

7. Du, H. et al. YTHDF2 destabilizes m(6)A-containing RNA through direct recruitment of the CCR4-NOT deadenylase complex. Nat. Commun. 7, 1266 (2016).

8. Kennedy, E. M. et al. Posttranscriptional m(6)A editing of HIV-1 mRNAs is essential for heterochromatin formation by the HUSH complex. Nucleic Acids Res. 47, 451–461 (2019).

9. Chekulaeva, M. et al. mRNA repression involves GW182-mediated recruitment of yeast TRAMP complex to cation of RNA-protein interactions. EMBO J. 30, 1218–1230 (2011).

10. Colmenares, S. U., Buker, S. M., Buhler, M., Dlakic, M. & Moazed, D. The Chp1-essential for heterochromatin formation by the HUSH complex. EMBO J. 30, 451–461 (2011).

11. Schalch, T., Job, G., Shanker, S., Partridge, J. F. & Joshua-Tor, L. The Chp1 chromodomain binds the H3K9me tail and the nucleosome core to assemble euchromatic model and pathogenic fungi-implications for fungal virulence. Front. Genet. 4, 302 (2013).

12. Polli, A. et al. The Ccr4–Not complex Silencing by HUSH complex regulates position-dependent variegation in human cells. Science 348, 1481–1485 (2015).

13. Fukuda, K., Okuda, A., Yusa, K. & Shinkai, Y. A CRISPR knockout screen identifies SETDB1-target retroelement silencing factors in embryonic stem cells. Genome Res. 28, 846–858 (2018).

14. Dousse, C. H. et al. TASSOR is a pseudo-PARP that directs HUSH complex-dependent pathways in yeast. Nat. Commun. 11, 4940 (2020).

15. Liu, N. et al. Selective silencing of euchromatic L1s revealed by genome-wide screens for L1 regulators. Nat. Commun. 11, 5387 (2020).

16. Azrouz, N. et al. A cohesin/HUSH- and LINC-dependent pathway controls ribosomal DNA double-strand break repair. Genes Dev. 33, 1175–1190 (2019).

17. Tao, G. et al. Hyperactivation of HUSH complex function by Charcot-Marie-Tooth disease mutation in MORC2. Mol. Cell 39, 108–118 (2010).

18. Yi, W. et al. CRISPR-assisted detection of RNA-protein interactions in living cells. Nucleic Acids Res. 42, 643–660 (2014).

19. Contreras, X. et al. Nuclear RNA surveillance complexes silence HIV-1 transcription. PloS Pathog. 14, e1006950 (2018).

20. Du, H. et al. YTHDF2 destabilizes m(6)A-containing RNA through direct recruitment of the CCR4-NOT deadenylase complex. Nat. Commun. 7, 1266 (2016).

21. Kennedy, E. M. et al. Posttranscriptional m(6)A editing of HIV-1 mRNAs enhances viral gene expression. Cell Host Microbe 19, 675–685 (2016).

22. Meola, N. et al. Identification of a nuclear exosome decay pathway for cation of factors for provirus silencing in human genome determines basal transcriptional activity and response to Tat transcriptional activation. EMBO J. 20, 1726–1738 (2001).

23. Cozzetto, D., Minneci, F., Currant, H. & Jones, D. T. FFPred 3: feature-based superfamily discrimination. Nat. Rev. Mol. Cell Biol. 17, 227–239 (2016).

24. Huttlin, E. L. et al. Architecture of the human interactome defines protein communities and disease networks. Nature 545, 505–509 (2017).

25. Buhler, M., Haas, W., Gygwi, S. P. & Moazed, D. RNAi-dependent and -independent RNA turnover mechanisms contribute to heterochromatin gene silencing. Cell 129, 797–721 (2007).

26. Matsumoto, M. et al. Two RNAi complexes, RITS and RDRC, physically interact and localize to noncoding centromeric RNAs. Cell 179, 789–802 (2004).

27. Colominas, S. U., Buker, S. M., Buhler, M., Klucakova, S. & Halic, M. The Chp1 essential for heterochromatin formation by the HUSH complex. Cell Rep. 27, 469–471 (2019).

28. Chalouh, K. et al. Selective silencing of euchromatic L1s revealed by genome-wide screens for L1 regulators. Nature 553, 228–232 (2018).

29. Siomi, M. C., Sato, K., Pezic, D. & Aravin, A. A. PIWI-interacting small RNAs: the vanguard of genome defence. Nat. Rev. Mol. Cell Biol. 12, 246–258 (2011).

30. Ge, D. T. & Zamoere, P. D. Small RNA-directed silencing: the fly finds its own endogenous factors for provirus silencing in embryonic stem cells. Cell 163, 230–245 (2015).

31. Buhler, M., Haas, W., Gygwi, S. P. & Moazed, D. RNAi-dependent and -independent RNA turnover mechanisms contribute to heterochromatin gene silencing. Cell 129, 797–721 (2007).

32. Matsumoto, M. et al. Two RNAi complexes, RITS and RDRC, physically interact and localize to noncoding centromeric RNAs. Cell 179, 789–802 (2004).

33. Colominas, S. U., Buker, S. M., Buhler, M., Klucakova, S. & Halic, M. The Chp1 essential for heterochromatin formation by the HUSH complex. Cell Rep. 27, 469–471 (2019).

34. Chalouh, K. et al. Selective silencing of euchromatic L1s revealed by genome-wide screens for L1 regulators. Nature 553, 228–232 (2018).

35. Siomi, M. C., Sato, K., Pezic, D. & Aravin, A. A. PIWI-interacting small RNAs: the vanguard of genome defence. Nat. Rev. Mol. Cell Biol. 12, 246–258 (2011).

36. Ge, D. T. & Zamoere, P. D. Small RNA-directed silencing: the fly finds its own endogenous factors for provirus silencing in embryonic stem cells. Cell 163, 230–245 (2015).
58. Yu, Y. et al. Panoramix enforces piRNA-dependent cotranscriptional silencing. Science 350, 339–342 (2015).
59. Cotobal, C. et al. Role of Ccr4-Not complex in heterochromatin formation at meiotic genes and subtelomeres in fission yeast. Epigenetics Chromatin 8, 28 (2015).
60. Skalska, L. et al. Nascent RNA antagonizes the interaction of a set of regulatory proteins with chromatin. Mol. Cell, https://doi.org/10.1016/j.molcel.2021.05.026 (2021).
61. Muller, I. et al. MPP8 is essential for sustaining self-renewal of ground-state pluripotent stem cells. Nat. Commun. 12, 3034 (2021).
62. Moazed, D. Small RNAs in transcriptional gene silencing and genome defence. Nature 457, 413–420 (2009).
63. Deniz, O., Frost, J. M. & Branco, M. R. Regulation of transposable elements by DNA modifiers. Nat. Rev. Genet. 20, 417–431 (2019).
64. Razooky, B. S., Pau, A., Aull, K., Rouzine, I. M. & Weinberger, L. S. A hardwired HIV latency program. Cell 160, 990–1001 (2015).
65. Jiang, C. et al. Distinct viral reservoirs in individuals with spontaneous control of HIV-1. Nature 585, 261–267 (2020).
66. Gramberg, T., Sunseri, N. & Landau, N. R. Evidence for an activation domain at the amino terminus of simian immunodeficiency virus Vpx. J. Virol. 84, 1387–1396 (2010).
67. du Chene, I. et al. Suv39H1 and HP1gamma are responsible for chromatin-mediated HIV-1 transcriptional silencing and post-integration latency. EMBO J. 26, 424–435 (2007).
68. Roberts, T. C. et al. Quantification of nascent transcription by bromouridine immunocapture nuclear run-on RT-qPCR. Nat. Protoc. 10, 1198–1211 (2015).
69. Rodriguez-Gil, A., Riedlinger, T., Ritter, O., Saul, V. V. & Schmitz, M. L. Formaldehyde-assisted isolation of regulatory elements to measure chromatin accessibility in mammalian cells. J. Vis. Exp. https://doi.org/10.3791/57272 (2018).
70. Dobin, A. et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29, 15–21 (2013).
71. Jin, Y., Tam, O. H., Paniagua, E. & Hammell, M. T3ertranscripts: a package for including transposable elements in differential expression analysis of RNA-seq datasets. Bioinformatics 31, 3593–3599 (2015).
72. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550 (2014).
73. Hansen, M. M. K. et al. A post-transcriptional feedback mechanism for noise suppression and fate stabilization. Cell 173, 1609.e15–1621.e15 (2018).
74. Lahouassa, H. et al. HIV-1 Vpr degrades the HLTF DNA translocase in T cells and macrophages. Proc. Natl Acad. Sci. USA 113, 5311–5316 (2016).

Acknowledgements

We thank all the Retrovirus, Infection and Latency group members at Institut Cochin for fruitful discussions. We warmly thank Nancy Standart for providing the Flag-CNOT7 vectors. We thank Adham Safieddine for sharing the smRNA FISH protocol. We acknowledge the CYBIO, GENOMIC, and IMAGIC platforms of the Institut Cochin. This work was supported by grants from the “Agence Nationale de la Recherche sur le SIDA et les hépatites virales” (ANRS) and SIDACTION. R.M. was supported by ANRS and SIDACTION. B.M. is supported by ENS Paris Saclay and P.L. by Université de Paris. Work in the laboratory of G.C. is supported by grants from Fondation pour la Recherche Médicale (FRM, DESQ2018039170), the Agence Nationale de la Recherche (LABEX SIGNALIFE, ANR-11-LABX-0028-01; RetroMet, ANR-16-CE12-0020; ImpactTE, ANR-19-CE12-0032), and CNRS (GDR 3546).

Author contributions

R.M. and F.M.-G. conceived the study, designed experiments, and interpreted data. R.M., M.M., P.L., and B.M. performed experiments. R.M. and M.H. brought their expertise and tools for smRNA FISH experiments. B.M. and R.M. analyzed the smFISH data. F.B. analyzed previously published ChIPseq, CUT&Tag, and RNAseq data and with R.M. compared them with our RNAseq data. S.L. and G.C. analyzed RNAseq data, including the expression of cellular genes and retroelements. V.V. contributed to the set-up of TASOR overexpression experiments in a CRISPR-TASOR background. S.E. and S.G.-M. provided critical reagents and brought their expertise through regular discussions. S.G.-M. analyzed data and performed splicing experiments. R.M. and F.M.-G. wrote the paper. All authors reviewed and edited the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-021-27650-5.

Correspondence and requests for materials should be addressed to Roy Matkovic or Florence Margottin-Goguet.

Peer review information Nature Communications thanks the anonymous reviewers for their contribution to the peer review of this work. Peer reviewer reports are available.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022