JVI Journals.ASM.org

G_2/M Cell Cycle Arrest Correlates with Primate Lentiviral Vpr Interaction with the SLX4 Complex

Gregory Berger,[a] Madeleine Lawrence,[a] Stephane Hué,[b] Stuart J. D. Neil[a]

Department of Infectious Diseases, Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom[a]; MRC Centre for Medical Molecular Virology, University College London, London, United Kingdom[b]

ABSTRACT

The accessory gene *vpr*, common to all primate lentiviruses, induces potent G_2/M arrest in cycling cells. A recent study showed that human immunodeficiency virus type 1 (HIV-1) viral protein R (Vpr) mediates this through activation of the SLX4/MUS81/EME1 exonuclease complex that forms part of the Fanconi anemia DNA repair pathway. To confirm these observations, we have examined the G_2/M arrest phenotypes of a panel of simian immunodeficiency virus (SIV) Vpr proteins. We show that SIV Vpr proteins differ in their ability to promote cell cycle arrest in human cells. While this is dependent on the DCAF1/DDB1/CUL4 ubiquitin ligase complex, interaction with human DCAF1 does not predict G_2/M arrest activity of SIV Vpr in human cells. In all cases, SIV Vpr-mediated cell cycle arrest in human cells correlated with interaction with human SLX4 (huSLX4) and could be abolished by small interfering RNA (siRNA) depletion of any member of the SLX4 complex. In contrast, all but one of the HIV/SIV Vpr proteins tested, including those that lacked activity in human cells, were competent for G_2/M arrest in grivet cells. Correspondingly, here cell cycle arrest correlated with interaction with the grivet orthologues of the SLX4 complex, suggesting a level of host adaptation in these interactions. Phylogenetic analyses strongly suggest that G_2/M arrest/SLX4 interactions are ancestral activities of primate lentiviral Vpr proteins and that the ability to dysregulate the Fanconi anemia DNA repair pathway is an essential function of Vpr *in vivo*.

IMPORTANCE

The Vpr protein of HIV-1 and related viruses is essential for the virus *in vivo*. The ability of Vpr to block the cell cycle at mitotic entry is well known, but the importance of this function for viral replication is unclear. Recent data have shown that HIV-1 Vpr targets the Fanconi anemia DNA repair pathway by interacting with and activating an endonuclease complex, SLX4/MUS81/EME1, that processes interstrand DNA cross-links. Here we show that the ability of a panel of SIV Vpr proteins to mediate cell cycle arrest correlates with species-specific interactions with the SLX4 complex in human and primate cells. The results of these studies suggest that the SLX4 complex is a conserved target of primate lentiviral Vpr proteins and that the ability to dysregulate members of the Fanconi anemia DNA repair pathway is essential for HIV/SIV replication *in vivo*.

Viral protein R (Vpr) is an accessory protein common to all primate lentiviruses whose role in viral replication remains unclear. It is packaged specifically into virions and is associated with the reverse-transcription complex during early steps of infection (1, 2). Although implicated in various virological processes, the major phenotype ascribed to Vpr is the induction of G_2/M cell cycle arrest in dividing cells (3, 4). The importance of this phenotype in human immunodeficiency virus (HIV)/simian immunodeficiency virus (SIV) replication has been notoriously difficult to define because of the minor effects of its deletion on viral replication in cell culture (5). However, reversion of inactivating mutations in both primates and humans highlights that Vpr plays an essential role for viral replication *in vivo* (6). In addition to cell cycle arrest, several SIV Vpr alleles are capable of targeting the host restriction factor SAMHD1 and that function became separated in the sooty mangabey SIV (SIVsm) lineage upon duplication to generate the *vpx* gene peculiar to this group (7). Importantly, at present HIV-1 Vpr is not known to retain any SAMHD1 counteractivity (7–10).

Vpr-mediated cell cycle arrest depends on its localization to nuclear compartments and the ability to hijack a Cullin-4/DDB1/Roc1 E3 ubiquitin ligase complex through the WD-containing DCAF-1 adaptor (11, 12). For HIV-1 Vpr, this leads ultimately to the activation of the *ataxia telangiectasia M and Rad3-related* (ATR) and Chk1 kinases to prevent cell cycle progression into mitosis (13). Although several putative Vpr targets have been described, the identity of that which triggers this event has until recently remained elusive. The requirement for the ubiquitin-proteasome system and, in particular, K48-ubiquitin linkages supported a notion that the Vpr target is degraded (14). However, Laguette and colleagues recently found that Vpr interacts with the SLX4 complex, members of the Fanconi anemia DNA repair pathway (15). SLX4, also known as Fanconi anemia complementation group P (FANCP), is a large adaptor protein that acts as a scaffold for a heterodimeric structure-specific endonuclease comprised of MUS81 and EME1. This interaction directs this endonuclease and

Received 8 August 2014 **Accepted** 6 October 2014

Accepted manuscript posted online 15 October 2014

Citation Berger G, Lawrence M, Hué S, Neil SJD. 2015. G_2/M cell cycle arrest correlates with primate lentiviral Vpr interaction with the SLX4 complex. J Virol 89:230–240. doi:10.1128/JVI.02307-14.

Editor: F. Kirchhoff

Address correspondence to Stuart J. D. Neil, stuart.neil@kcl.ac.uk.

doi:10.1128/JVI.02307-14

others to resolve interstrand cross-links (ICLs) during DNA replication and delays cell cycle progression until repair is completed through homologous recombination (HR) (16, 17). In *in vitro* interaction assays, recombinant Vpr interacts with the C terminus of human SLX4. Surprisingly, instead of mediating the degradation of members of the SLX4 complex in human cells, Vpr activates the MUS81-EME1 nuclease activity via polyubiquitination of MUS81 by the DCAF1/DDB1/CUL4 E3 ligase. RNA interference (RNAi)-mediated depletion of any member of the SLX4 complex blocked Vpr-mediated cell cycle arrest. During viral infection of cultured cells, SLX4 is recruited to proviral HIV-1 DNA only in the presence of Vpr. Interestingly, the SLX4 complex was also shown to repress interferon-stimulated gene expression, suggesting a potential link between DNA repair pathways and innate immune sensing in HIV-1 target cells (15). However, the virological reason for SLX4 complex activation by HIV-1 is still unclear.

The G_2/M arrest activity has been previously reported as a feature of several SIV Vpr proteins (3, 4). In this study, we sought to confirm that the SLX4 complex is a target of HIV-1 Vpr and to determine whether it was a common target of primate SIV Vpr alleles.

MATERIALS AND METHODS

Cell culture and antibodies. HeLa and HEK293T (293T) cells (obtained from the ATCC) and grivet COS-1 cells (kindly provided by Greg Towers) were maintained in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum and gentamicin. Mouse anti-hemagglutinin (anti-HA) and anti-FLAG monoclonal antibodies were obtained from Covance and Sigma-Aldrich, respectively. Mouse anti-human SLX4, MUS81, and EME1 antibodies were all obtained from Abcam.

Plasmids. HIV-1 Vpr was cloned from the molecular clones NL4.3 and YU-2, and site-directed mutagenesis was performed using standard QuikChange methodology to generate Q65A and R80A mutations. SIVdebCM5 Vpr and SIVmus1 Vpr were previously described (7). Vpr alleles from SIVs from African green monkey (AGM; SIVagm.Gri677 [GenBank accession no. NC_001549], SIVagm.Ver9063 [GenBank accession no. L40990], and SIVagm.Sab92018 [GenBank accession no. HQ378594]), gorilla (SIVgorCP2139_2; GenBank accession no. FJ424865), greater spot-nosed monkey (SIVgsn CN71; GenBank accession no. AF468658), Mona monkey (SIVmon L1_99CML1; GenBank accession no. AY340701), olive colobus monkey (SIVolc; GenBank accession no. FM165200), Sykes monkey (SIVsyk173; GenBank accession no. L06042), and Talapoin monkey (SIVtal00CM266; GenBank accession no. AF478595) were synthesized as codon-optimized N-terminally FLAG-tagged constructs and subcloned into pCR3.1 or the murine leukemia virus (MLV)-based retroviral vector pCMS28iresGFP. The HA-tagged SAMHD1 plasmid pCIG-HA-SAMHD1 has been described previously (18), and the HA-SLX4 expression plasmid was a kind gift from Wade Harper (Harvard University, Cambridge, MA) (19).

Cell cycle analysis. HeLa or COS-1 cells were transduced with the indicated MLV-based FLAG-Vpr internal ribosome entry site (IRES) green fluorescent protein (GFP) construct pseudotyped with vesicular stomatitis virus G (VSV-G). At 36 h posttransfection, cells were treated with 5 mM caffeine (Sigma-Aldrich) or a 10 μM concentration of the ATM inhibitor KU55933 (Abcam) when indicated (20, 21) or were left untreated. At 48 h postransduction, cells were harvested in phosphate-buffered saline (PBS)–5 mM EDTA and washed once in PBS before being fixed in 1% paraformaldehyde for 15 min. After two washes in PBS, cells were resuspended in 50 μg/ml propidium iodide (Life Technologies)–100 μg/ml RNase A (Sigma)–0.1% Triton X-100 and incubated for 1 h at room temperature. Cell cycle distribution was analyzed on a FACSCanto II cell analyzer (BD).

IPs. For Vpr/DCAF1 interaction analyses, 5×10^5 293T cells were seeded in 6-well plates and transfected with 600 ng HA-DCAF1 and 600 ng of the indicated FLAG-Vpr construct using 1 μg/ml polyethylenimine (PEI). At 48 h posttransfection, the cells were lysed in 50 mM Tris (pH 7.4)–150 mM NaCl–0.5% NP-40 supplemented with protease inhibitors (Roche) on ice for 15 min. Cell lysates were precleared by centrifugation at 1,000 × *g* for 5 min before immunoprecipitations (IPs) using anti-FLAG antibodies and protein G agarose (Invitrogen) were performed for 2 to 4 h at 4°C. Beads were washed extensively in lysis buffer and resuspended in loading buffer prior Western blot analysis.

For SLX4 complex/Vpr interaction analyses, 10-cm-diameter dishes of subconfluent 293T cells were transfected with 5 μg of the indicated FLAG-Vpr plasmid using PEI. At 48 h posttransfection, cells were cross-linked using 0.05% formaldehyde. The cells were lysed in 20 mM Tris (pH 7.4)–150 mM NaCl– 0.1% deoxycholate–10% glycerol–200 μM *N*-ethylmaleimide (NEM)–200 μM orthovanadate–protease inhibitors. Lysates of cleared cells were immunoprecipitated with an anti-mouse FLAG antibody and protein G beads. After precipitation, the cross-links were reversed using 10 mM EDTA, 5 mM dithiothreitol (DTT), and 1% SDS. Lysate and immunoprecipitates were analyzed by Western blotting using the indicated antibody.

SAMHD1 degradation assays. SAMHD1 degradation was performed in 24-well plates. Briefly, 1×10^5 293T cells were seeded 24 h prior being transfected with 50 ng of HA-tagged SAMHD1 plasmid and 200 ng of the indicated Vpr construct. At 36 h after transfections, cells were treated with 10 μM MG132 (Sigma) or dimethyl sulfoxide (DMSO), and 12 h later, the cells were lysed in Laemmli loading buffer prior Western blot analysis.

siRNA and shRNA. Knockdown of DCAF-1 was achieved upon transduction of target cells with lentiviral vectors encoding control or DCAF-1 hairpins (22). Briefly, lentivectors were produced by cotransfection of HIV-1 Gag-Pol, VSV-G, and the indicated short hairpin RNA (shRNA) constructs as described previously using the following target sequences: for ShDCAF-1, GCGGACTGGAGGTGATCAT; and for ShControl, AGC TCCCGTGAATTGGAATCC.

For depletion of the SLX4 complex members by siRNA, 1.5×10^5 HeLa or COS-1 cells were seeded in a 12-well plate. After 3 h, cells were transfected with 50 pmol of the indicated siRNA using DharmaFECT (Dharmacon) according to the manufacturer's instructions. At 24 h posttransfection, the cells were reseeded in 12-well plates and were cotransfected with 50 pmol of siRNA and the indicated FLAG-Vpr plasmid using Lipofectamine 2000 (Invitrogen). The following siRNAs used in this study were synthesized by MWG Operon: siEME1 (CCUCAGAGCCUGACUG UAA), siMus81 (AUGGUCACCACUUCUUAAC [or CAGCCCUGGUG GAUCGAUA in African green monkey Cos cells]), and siSLX4 (UGUUC ACUAGCGGAUUCCA). The siCtrl used was the Dharmacon On-Target control silencing pool.

Phylogenetic analysis. Vpr sequences obtained from the Los Alamos database were manually aligned using the Se-Al sequence editor (http://tree.bio.ed.ac.uk/software/seal/). A maximum-likelihood phylogeny structure was reconstructed by using the General Time-Reversible model of nucleotide substitutions and adjusting evolution rates across sites (GTR+CAT) and FastTree v2.1.5 software (23). Branch support was calculated by Shimodaira-Hasegawa-like local branch support (SH-like test), as implemented in FastTree. The phylogeny was edited with the program FigTree (http://tree.bio.ed.ac.uk/software/seal/).

RESULTS

Variable induction of ATR-dependent G_2/M arrest in human cells by SIV Vpr proteins. While G_2/M arrest has been reported to be a conserved feature of HIV-1, HIV-2, and SIVmac Vpr proteins in human cells, some African green monkey SIV (SIVagm) proteins are functional only in African green monkey cells (3, 4). We first examined a panel of FLAG-tagged SIV Vpr proteins to induce G_2/M arrest as assessed by propidium iodide staining for DNA content in transiently transfected HeLa cells (Fig. 1A and B). Vpr proteins from the guenon lineage, Mona monkey SIV (SIVmon), SIVgsn, and SIVmus, all induced G_2/M arrest equivalently to a

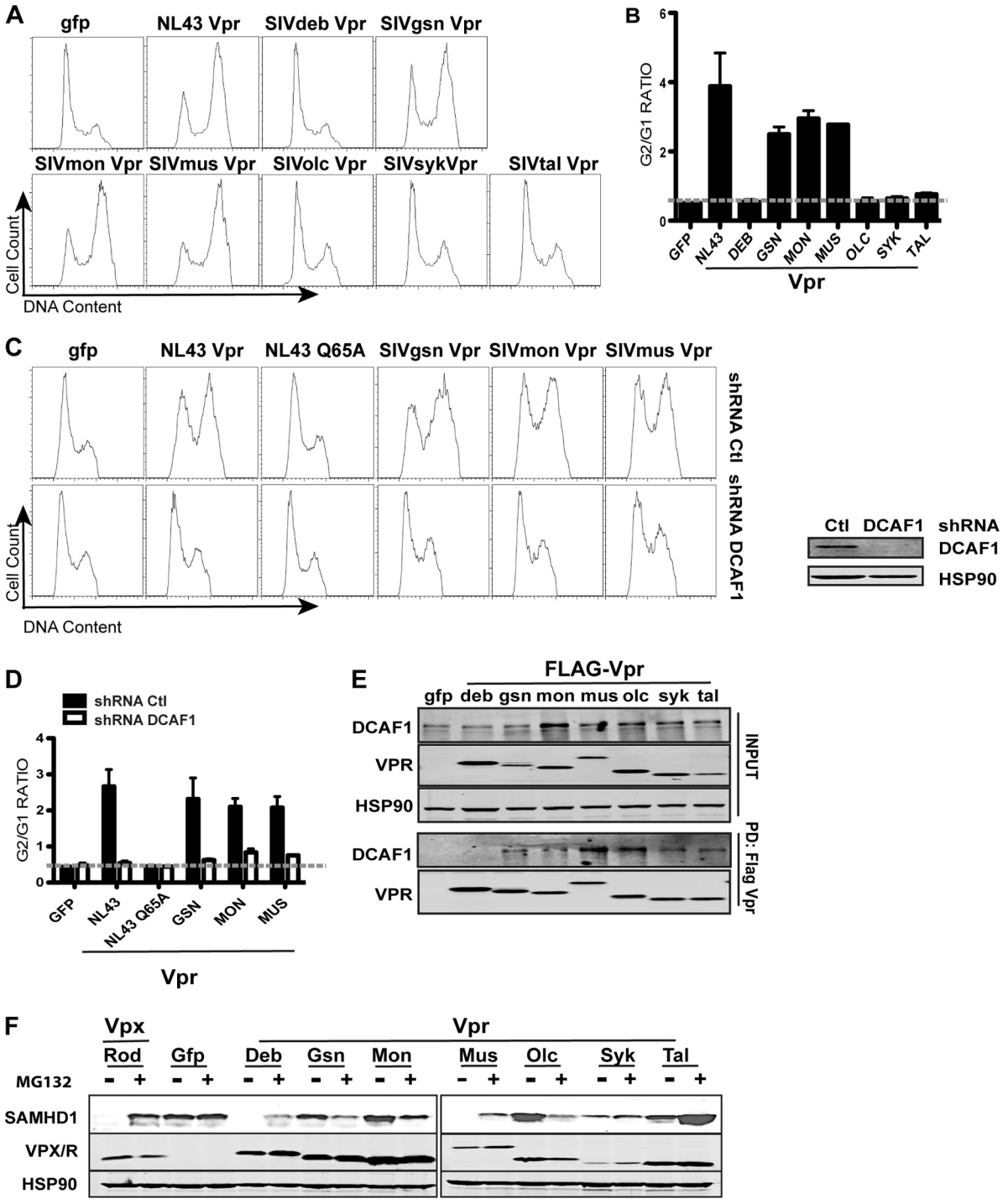

FIG 1 Induction of G_2/M arrest in human cells by SIV Vpr proteins. (A) Representative examples of G_2/M arrest by various SIV Vpr proteins. HeLa cells were transfected with pCS-IRES-GFP encoding the indicated HIV-1/SIV Vpr protein-bearing N-terminal FLAG epitope tags. At 72 h after transfection and replating, the fixed and permeabilized cells were stained for DNA content with propidium iodide and the GFP-positive (GFP^+) cell populations were analyzed by flow cytometry. (B) Ratio of the area under the curve for the G_2 and G_1 peaks for the cells whose results are shown in panel A. Error bars represent means ± standard deviations (SD) of the results of 3 independent experiments. (C) Analysis of the cell cycle distribution of Vpr-expressing cells upon knockdown of DCAF1 using shRNA lentiviral vector transduction (inset). (D) Quantification of the G_2/G_1 ratio for the data shown in panel C for three independent experiments ± SD. (E) 293T cells were cotransfected with HA-tagged DCAF1 (pCR3.1) and FLAG-Vpr (pCS-IRES-GFP) expression vectors or an empty GFP control. At 48 h later, the Vpr was immunoprecipitated using anti-FLAG monoclonal antibodies and Western blot analyses were performed on the cell lysates and pulldowns with anti-FLAG, anti-HA, and, for loading, anti-HSP90. (F) 293T cells were cotransfected with pAIP1-HA-SAMHD1 and the indicated FLAG-tagged Vpr or Vpx. At 48 h after transfection, cells were treated for 16 h with DMSO or 5 μM MG132. Cell lysates were then analyzed by Western blotting with anti-FLAG, anti-HA, and, for loading, anti-HSP90.

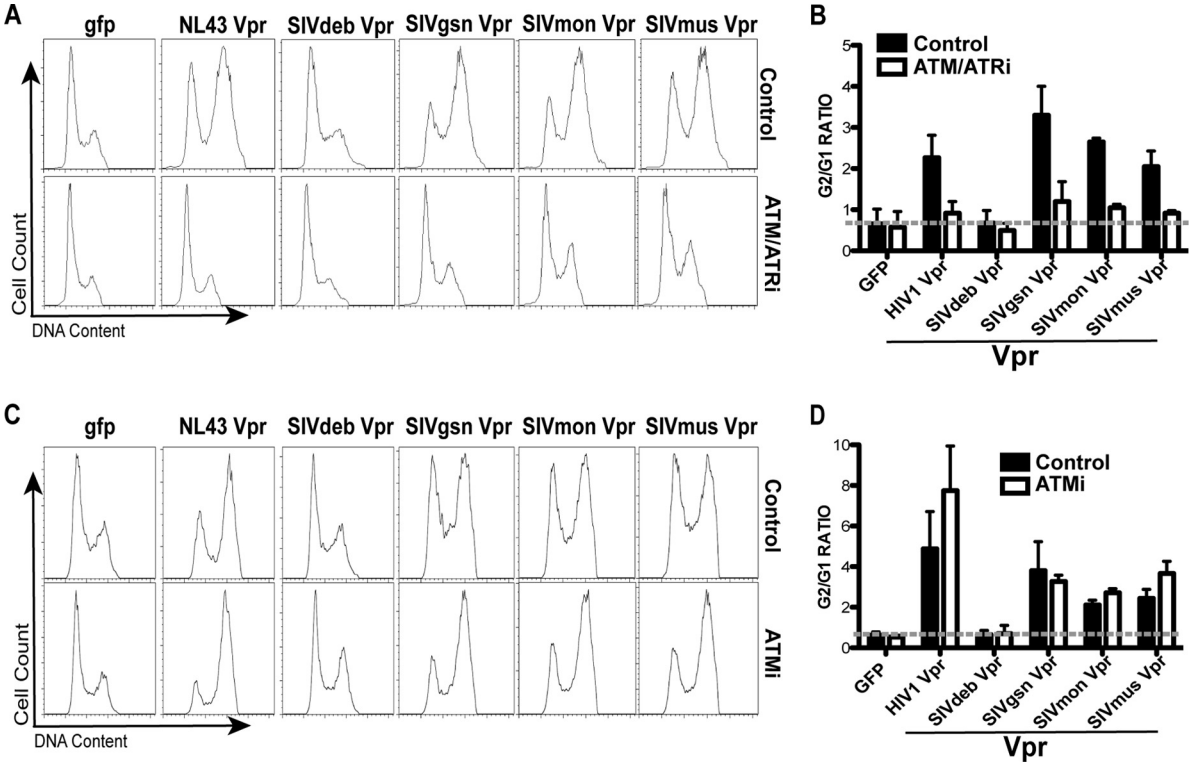

FIG 2 SIV Vprs require ATR to induce a G_2/M block. (A) Representative experiment performed with the G_2/M block induced in HeLa cells upon transfection with different SIV Vpr alleles followed by overnight treatment with or without the ATR inhibitor caffeine (ATR/ATMi) (5 mM). (B) Quantitation of the G_2:G_1 ratio for the cells whose results are shown in panel A. (C and D) Cells treated with a 10 μM concentration of the ATM inhibitor KU55933 were analyzed as described for panels A and B. Error bars represent means ± standard deviations of the results of 3 independent experiments.

prototypic HIV-1 Vpr (NL4.3), whereas those from SIVdeb, SIVolc, SIVsyk, and SIVtal did not. G_2/M arrest by guenon SIV Vpr proteins in HeLa cells was completely reversed by shRNA depletion of DCAF1 (Fig. 1C and D), suggesting that they all hijack the DCAF1/DDB1/CUL4 ubiquitin ligase complex. However, aside from SIVdeb, all the SIV Vpr proteins tested were able to coimmunoprecipitate human HA-tagged DCAF1 when cotransfected into 293T cells. Thus, the interaction with human DCAF1 was not predictive of cell cycle arrest in human cells by different SIV Vprs (Fig. 1E). Moreover, both SIVdeb and SIVmus Vpr were able to mediate an MG132-sensitive degradation of human SAMHD1 under the same conditions (Fig. 1F). Thus, SIVdeb Vpr is able to mediate ubiquitin-dependent degradation of a target molecule in human cells and, similarly to the findings of others (7), this mediation appears to be DCAF1 independent.

Cell cycle arrest by HIV-1 Vpr is mediated through the activation of the kinase ATR and induction of the Chk1-dependent G_2/M checkpoint. Both ATR and its close relative ATM can be inhibited by caffeine treatment. Overnight incubation with caffeine fully reversed G_2/M arrest in HeLa cells triggered by SIVgsn, SIVmon, and SIVmus Vpr proteins (Fig. 2A and B). To rule out a role for ATM, we also used KU55933, which inhibits ATM without affecting ATR, and found that, unlike the results seen with caffeine, this did not prevent SIV Vpr proteins from mediating G_2/M arrest in HeLa cells (Fig. 2C and D). Together, these data indicate that SIV Vpr proteins that mediate cell cycle arrest do so through activation of a common ATR-dependent DNA damage pathway that requires the DCAF1/DDB1/CUL4 complex.

G_2/M arrest by SIV Vpr proteins in human cells correlates with interaction with SLX4. To address whether the SLX4 complex was a common target for lentiviral Vpr proteins, we knocked down SLX4, MUS81, and EME1 in Vpr-transfected HeLa cells by siRNA. Under these conditions, the proportion of cells arrested in G_2 by HIV-1 Vpr transfection was reduced, as was the arrest triggered by SIVgsn, SIVmon, and SIVmus Vpr (Fig. 3). However, only minor effects of these siRNAs were observed in cells transfected with SIVdeb Vpr or the DCAF1-binding-deficient mutant of HIV-1 Vpr (Q65A). In contrast, treatment of the cells with nocodazole to destabilize the mitotic spindle was still capable of inducing G_2/M arrest in the presence of SLX4 complex siRNA treatment. Therefore, these data indicate that, in similarity to the results described for HIV-1, SIV Vpr proteins that are capable of inducing G_2/M arrest in human cells do so by a mechanism sensitive to SLX4 complex depletion.

To confirm that the data presented above correlate with interaction of SIV Vpr proteins with the SLX4 complex itself, we performed coimmunoprecipitation of HIV-1 and SIV Vpr proteins with endogenous SLX4. First, 293T cells were transfected with FLAG-tagged HIV-1 YU-2 Vpr proteins, a DCAF1-binding mutant (Q65A), or a G_2/M arrest-defective mutant (R80A) in combination with HA-DCAF1 as an internal control. At 48 h later, cells were treated for 10 min with 0.05% paraformaldehyde to cross-link proximal proteins and were subjected to anti-FLAG immunoprecipitation followed by cross-link reversal. As expected, DCAF1 coprecipitated with wild-type YU-2 Vpr and Vpr R80A but not with the Q65A mutant (Fig. 4A). In agreement with

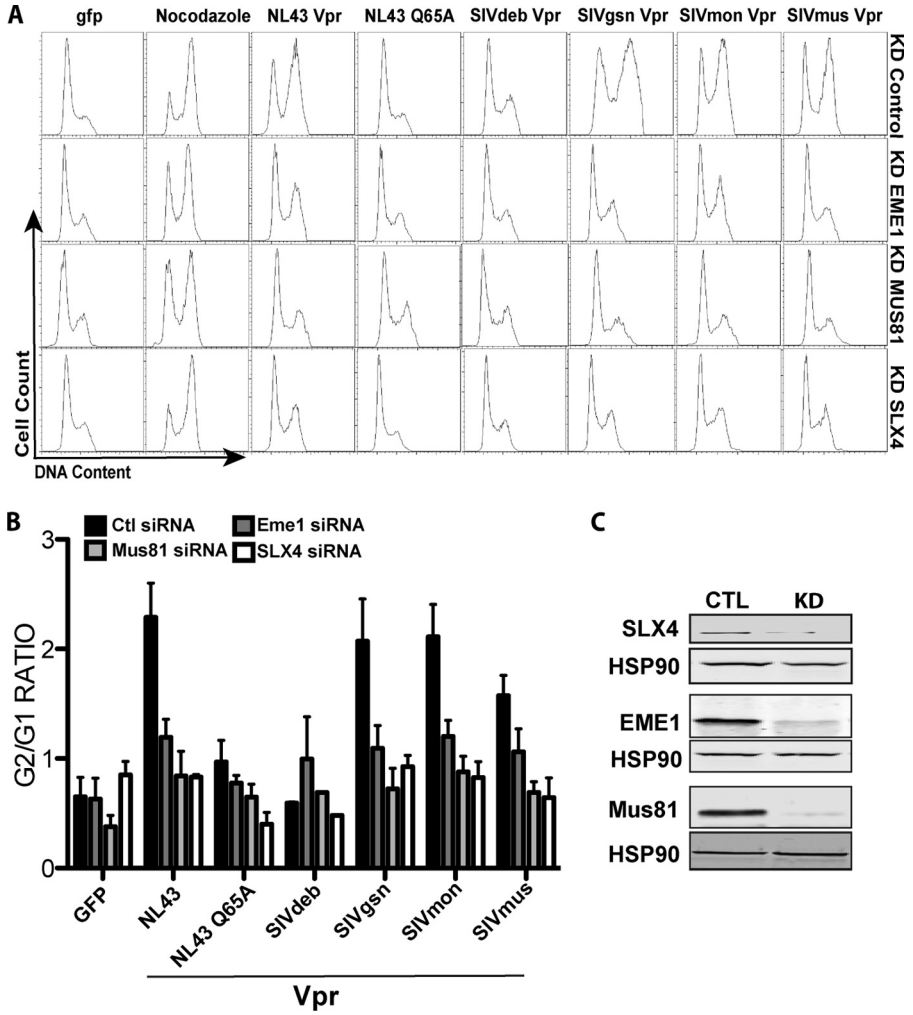

FIG 3 siRNA depletion of SLX4 complex components blocks G_2/M arrest by SIV Vpr proteins in human cells. (A) HeLa cells treated twice with control, SLX4, MUS81, or EME1-specific siRNA oligonucleotides. After 48 h, the cells were transfected with the indicated HIV-1 or SIV Vpr or with a GFP control and analyzed for DNA content as described for Fig. 1. (B) Quantification of G_2:G_1 ratios of the results shown in panel A. Data and error bars represent means ± standard deviations of the results of 3 independent experiments. (C) The level of knockdown of endogenous SLX4 complex components of the cells whose results are presented in panel A was analyzed by Western blotting of cell lysates with the following antibodies: anti-human SLX4 and anti-human MUS81 and/or anti-human EME1.

Laguette et al., the endogenous SLX4 protein was also present in wild-type and R80A mutant Vpr immunoprecipitates but not with the Q65A mutant (15). In parallel, the lack of a cell cycle arrest phenotype for these mutants was confirmed in HeLa cells (Fig. 4B). Performing the same experiment with SIVgsn, SIVmon, and SIVmus Vpr proteins that mediate G_2/M arrest in HeLa cells, and with the SIVdeb Vpr protein, which does not (Fig. 4C and D), revealed that cell cycle arrest mediated by the former Vpr proteins correlated with the ability to coprecipitate the SLX4.

Since the Q65A mutant of Vpr lacks both DCAF1 and SLX4 interactions, it remained possible that the observed co-IP of SLX4 with SIV Vpr proteins might occur indirectly through their binding to DCAF1. To rule out this possibility, we took advantage of the lack of DCAF1 interaction shown by SIVdeb Vpr by the use of its closest relative in our panel, SIVmus, which interacts with both DCAF1 and the SLX4 complex. In comparison to SIVmus Vpr, SIVdeb Vpr contains an in-frame deletion of 21 amino acids from position 108, which could explain the lack of DCAF1 binding (Fig. 5A). Therefore, we created a SIVdeb C-terminal chimeric protein fused at position 108. This chimera failed to confer a HeLa G_2/M arrest phenotype to SIVdeb (Fig. 5B and C) but did permit DCAF1 binding in co-IPs (Fig. 5D). Unlike SIVmus Vpr, the chimera did not acquire the ability to co-IP with either SLX4 or another member of the complex, MUS81 (Fig. 5E), indicating that determinants of interactions of DCAF1 and the SLX4 complex can be genetically separated. Together, these data indicate that Vpr proteins from the guenon SIV lineage are capable of interacting with the SLX4 complex and suggest that they activate its nuclease activity similarly to HIV-1, leading to downstream G_2/M arrest.

Cell cycle arrest in AGM grivet cells mediated by HIV-1 and SIV Vpr proteins indicates differences in SIV Vpr interactions with primate SLX4com. The lack of a G_2/M arrest phenotype of several SIV Vpr proteins in human cells prompted us to ask whether this might reflect differences in interactions with SLX4 complex components of different primate species rather than a lack of activity *per se*. While tractable cell lines from the host spe-

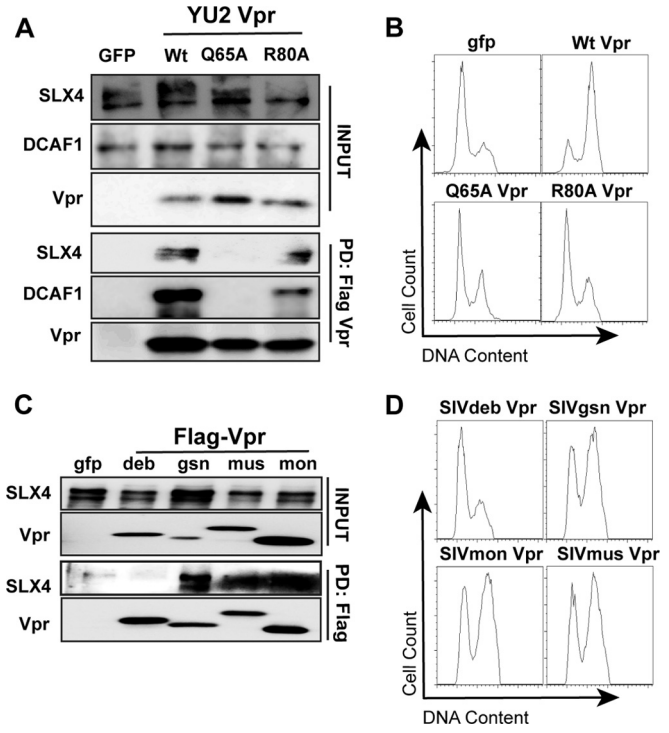

FIG 4 Interaction with SLX4 correlates with cell cycle arrest mediated by SIV Vpr proteins in human cells. (A) 293T cells were transfected with the indicated FLAG-tagged wild type or with mutants of the HIV-1 YU-2 Vpr in combination with an HA-DCAF1 expression vector. At 48 h after transfection, cell lysates were immunoprecipitated with anti-FLAG antibodies and IPs and lysates analyzed by Western blotting with anti-SLX4, anti-HA, and anti-FLAG. (B) DNA content was analyzed for parallel HeLa cell transfectants of Vpr expression vectors used as described for panel A. (C) 293T cells transfected with the indicated SIV Vpr and cell lysates immunoprecipitated as described for panel A and analyzed for FLAG-Vpr and endogenous SLX4. (D) DNA content was analyzed for parallel HeLa cell transfectants of Vpr expression vectors used as described for panel C.

cies of most of these viruses are not available, previous studies had indicated that Vpr alleles from HIV-1 and different strains were capable of G_2/M arrest in COS1 cells from grivets (*Chlorocebus aethiops*). In keeping with this, transduction of retroviral vectors encoding HIV-1 YU-2 Vpr, but not the Q65A mutant, induced G_2/M arrest in COS1 (Fig. 6A and B). Furthermore, immunoprecipitation of flag-tagged YU-2 Vpr from COS1 cells revealed an interaction with the grivet orthologue of MUS81, indicating that HIV-1 Vpr retains the ability to associate with the grivet SLX4 complex (Fig. 6C). We then examined the panel of SIV Vpr proteins in COS1 cells and found that several that had been inactive in human cells, including SIVolc, SIVdeb, SIVsyk, and alleles from SIVagm SIVagmGri isolates (for which *C. aethiops* is the natural host), were able to induce G_2/M arrest in this setting, whereas other examples of SIVagm such as SIVagmVer and SIVagmSab induced a less potent block. In some cases, G_2/M arrest appeared more potent than the activity of HIV-1 Vpr itself (Fig. 6D and E). Furthermore, unlike the results seen in human cells, FLAG-tagged SIVdeb, SIVolc, and SIVsyk Vprs could coimmunoprecipitate MUS81, which is consistent with their G_2/M phenotype (Fig. 6F). While sequence information on old-world primate SLX4, MUS81, and EME1 is limited, we designed siRNAs against the *Chlorocebus sabeus* MUS81 (GenBank accession no. XM_007981794.1). We then transfected these oligonucleotides in combination with transduction of the panel of SIV Vpr constructs. In all cases where a given SIV Vpr caused G_2/M arrest in COS-1 cells, this phenotype was abolished by agm-MUS81 siRNA treatment (Fig. 6G and H). Thus, SIV Vpr-mediated G_2/M arrest correlates with SLX4com interaction, indicating that differences in activity or potency likely represent host adaptation of Vpr proteins to interact with primate SLX4 components.

DISCUSSION

In this study, we examined whether the G_2/M arrest mediated by a variety of SIV Vpr alleles correlates with interaction with the SLX4 complex. We found that all SIV Vpr alleles competent for G_2/M arrest of human cells do so in a manner dependent on the SLX4 complex and interact with it in immunoprecipitations. Furthermore, we found that several of those SIV Vprs tested that failed to arrest cell cycle in human cells did so in COS1 cells from the grivet African green monkey species. This correlates with the ability to interact with the grivet SLX4 complex. Thus, the SLX4 complex is a common target of SIV Vpr proteins and the data suggest that limited species specificity in this interaction accounts for variations in G_2/M arrest potency in human and primate cells.

The apparent species specificity in G_2/M arrest in SIV Vpr proteins initially seems to be more broad than the interactions that have been documented for lentiviral accessory genes and the restriction factors APOBEC3G, tetherin, and SAMHD1 (24). Guenon Vpr proteins can interact with the human SLX4 complex, whereas several SIVagm alleles, as well as those of SIVdeb, SIVolc, and SIVsyk, co-IP the complex from grivet cells. Laguette et al. suggested the presence of a direct interaction between HIV-1 Vpr and the C-terminal SLX-binding domain of SLX4 using bacterially expressed proteins (15). We have so far been unable to confirm that this is also true for the SIV Vpr proteins or whether the SBD determines the differences in SIV Vpr activity in human and grivet cells that we have observed (not shown). Whether this means that Vpr interacts additionally with other determinants in the SLX4/MUS81/EME1 complex in a species-specific manner remains to be investigated. An added complication for studying this is that the DCAF1-binding mutant of HIV-1 Vpr, Q65A, also fails to interact with the SLX4 complex. Consistent with the requirement for DCAF1/DDB1/CUL4 ligase activity, G_2/M arrest by SIV Vpr proteins is impaired upon their knockdown. However, conferring DCAF1 binding to SIVdeb Vpr by grafting the C-terminal region of SIVmus Vpr was not sufficient to induce SLX4 binding. This indicates that Vpr/SLX4 interactions are not indirectly mediated through DCAF1 but rather may be consistent with the SLX4 complex forming a ternary complex with Vpr and the ubiquitin ligase dependent on DCAF1. Such a model would also require direct interaction of Vpr with at least one member of the complex, but at present it remains unclear which one. Furthermore, the HIV-1 Vpr mutant resulting from a C-terminal mutation, R80A, which is defective for G_2/M arrest, retains both DCAF1 and SLX4 interaction, suggesting there may be another unidentified determinant in the complex essential for Vpr activity. This is reminiscent of the interactions of SIV Vpr and Vpx with primate orthologues of SAMHD1, where determinants are spread between the N and C termini, and the requirements differ among different viral proteins (25, 26).

At present, sequence information on primate SLX4 complex subunits is limited. Given the apparent species differences in in-

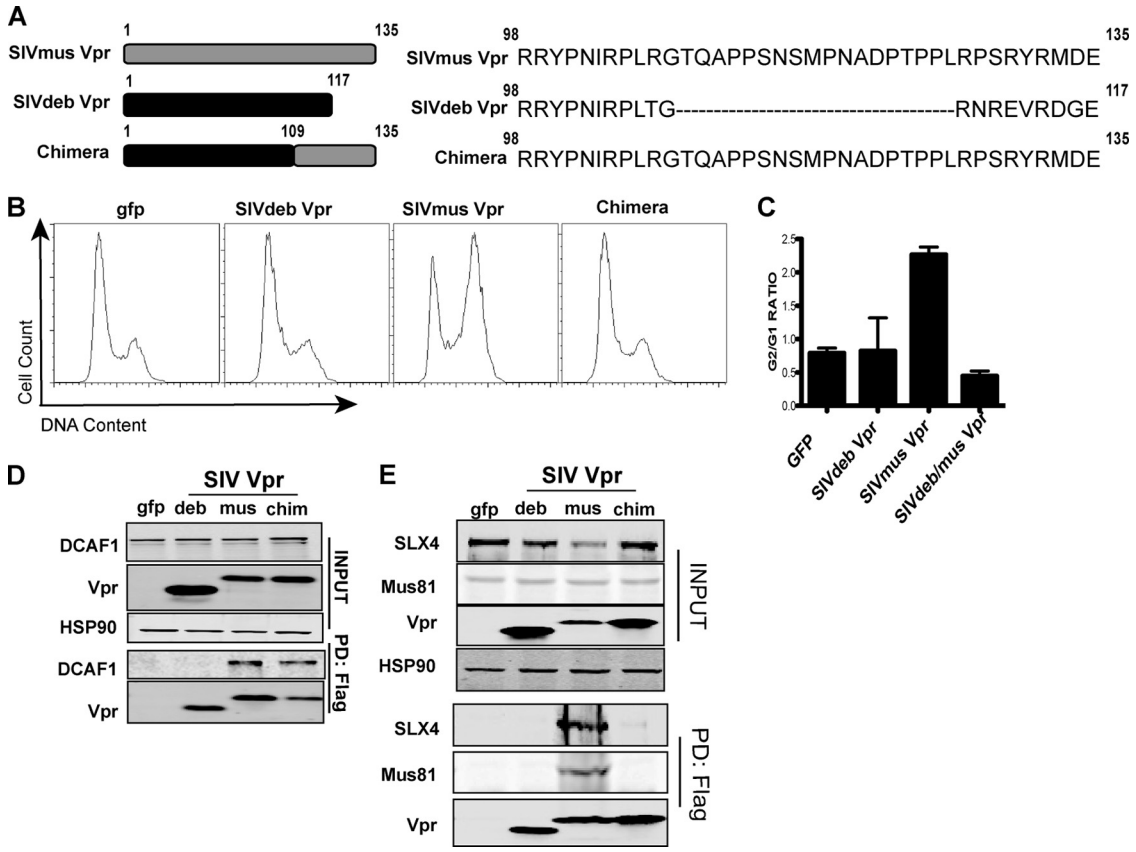

FIG 5 Conferring DCAF1 interaction to SIVdeb Vpr does not lead to G_2/M arrest or human SLX4 complex interaction. (A) Alignment of SIVdeb and SIVmus and chimeric Vpr proteins. (B) DNA content analysis of HeLa cells transiently transfected with the constructs as described for panel A and Fig. 1. (C) Quantification of G_2:G_1 ratios of the data presented in panel B. Columns and error bars represent means ± standard deviations of the results of 3 independent experiments. (D) 293T cells transfected with FLAG-tagged SIVdeb, SIVmus, or chimeric Vpr expression vectors in combination with HA-DCAF1. At 48 h later, anti-FLAG immunoprecipitates and cell lysates were subjected to Western blotting for anti-HA and anti-FLAG. (E) The immunoprecipitates and lysates described in the panel D legend were further analyzed for endogenous SLX4 and MUS81 coprecipitation.

teraction that we have observed, a detailed analysis should yield an answer regarding which member of the SLX4 directly interacts with Vpr *in vivo* as well as whether members of the complex are under positive selection in primates that is consistent with SIV selective pressure. However, we also note a further caveat that should be borne in mind. Most of the data published on the mechanism of Vpr-mediated cell cycle arrest, as well as the data presented here, have been obtained by transient transfection or ectopic expression of Vpr (3, 4, 11, 13, 14, 27, 28). The levels of HIV-1 Vpr incorporated into incoming particles have been shown to be sufficient to induce some G_2/M arrest (27), and of course *de novo* expression in the infected cells does this robustly. If Vpr interaction with SLX4com to recruit it to incoming proviral DNA in the nucleus is important, as previously suggested (15), the former phenotype is likely to be the more relevant. While we have attempted to use inputs of FLAG-tagged Vpr constructs to ensure similar expression levels, how representative they are of viral expression levels from the different SIVs is unknown. Therefore, the limited species specificity that we have observed here may overestimate the potency (and hence the host adaptation) of a given SIV Vpr protein against SLX4 complex components from outside its host species. Such studies require Vpr-competent and -defective isogenic clones representative of different SIV lineages.

The identification of the SLX4 complex as the Vpr target that triggers G_2/M arrest raises important questions about why the virus is specifically targeting this DNA repair pathway. Since Vpr is not required for lentiviral replication *per se*, activation of SLX4/MUS81/EME1 is unlikely to be essential for the physical steps of the viral life cycle in the nucleus. Although it has been suggested that ATR-mediated G_2/M arrest may enhance HIV-1 gene expression at the level of translation (6, 29), this is not reflected in the replicative capacity of Vpr-defective viruses in activated CD4-positive ($CD4^+$) T cells. In contrast, Vpr-dependent growth phenotypes have been demonstrated in nondividing macrophages, although these are often weak and donor variable (30). Interestingly, in cells from FANCP patients or HeLa cells depleted of either DCAF1 or SLX4, there is activation of type 1 interferon and interferon-induced gene expression at the RNA level (15). Furthermore, FANCP cells are less permissive for HIV-1 vector transduction than the isogenic line reexpressing SLX4. This suggests a potential interplay between this nuclear DNA repair pathway and the suppression of innate immune sensing of "dangerous" nuclear DNA. Interestingly, a recent study has demonstrated that late replication events of herpes simplex virus 1 (HSV-1) require an intact Fanconi anemia repair pathway and that, in its absence, viral replication is restricted in part by the nonhomologous end-joining

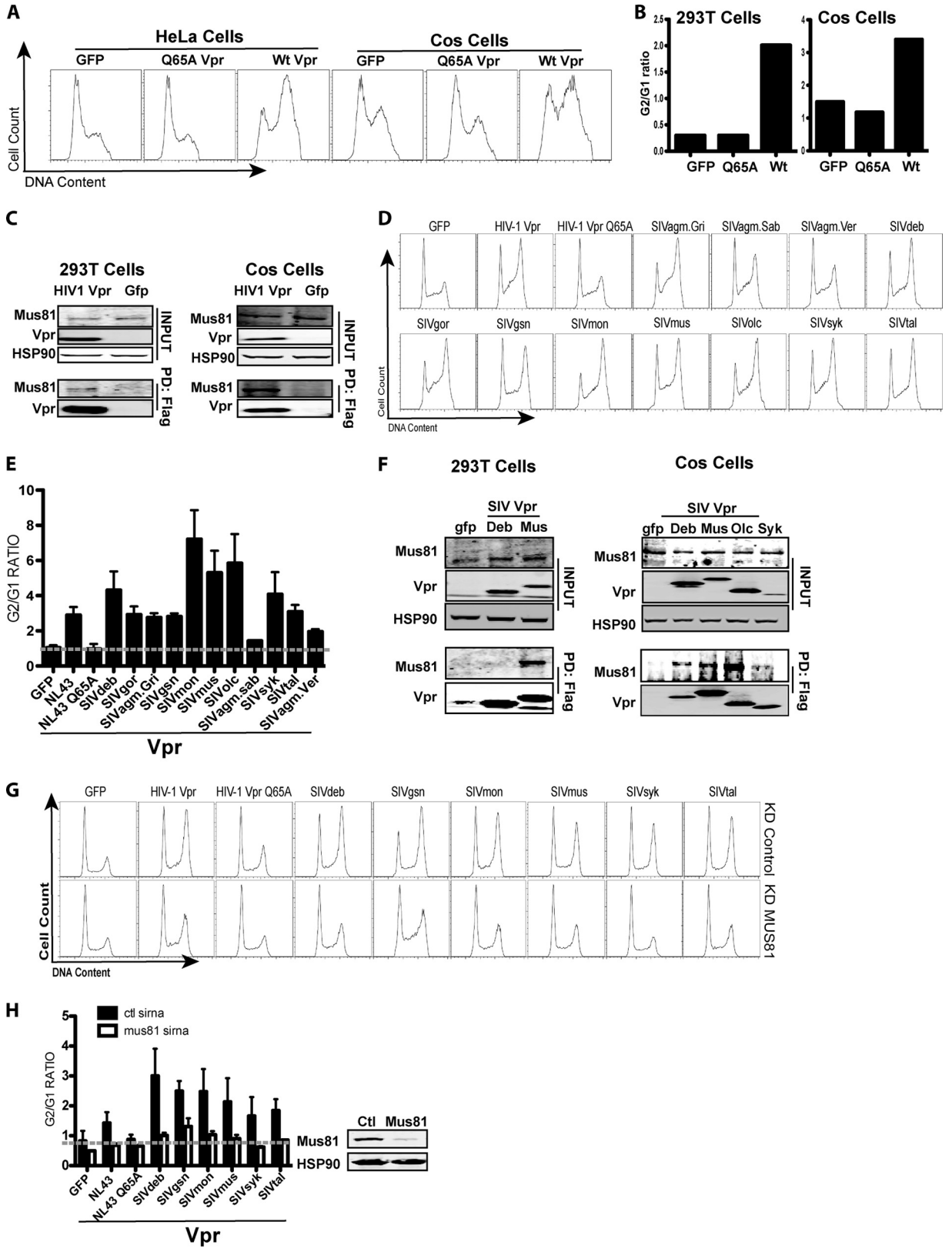

FIG 6 Species specificity of SIV Vpr-mediated G_2/M arrest correlates with SLX4 complex interaction in African green monkey cells. (A) 293T and COS-1 were transduced with an MLV-based IRES-GFP vector encoding the indicated FLAG-Vpr. At 72 h postinfection, cells were harvested, stained, and analyzed for their cell cycle distributions. (B) Quantification of the G_2/G1 ratio of the results shown in panel A. (C) Immunoprecipitates and cell lysates of either 293T cells or COS-1 cells transduced by retroviral vectors encoding HIV-1 YU-2 Vpr were analyzed for MUS81 coimmunoprecipitation. (D) Representative example of G_2/M arrest in COS-1 cells upon transduction with an IRES-GFP MLV vector encoding the indicated FLAG-Vpr. At 72 h postinfection, cells were harvested and stained

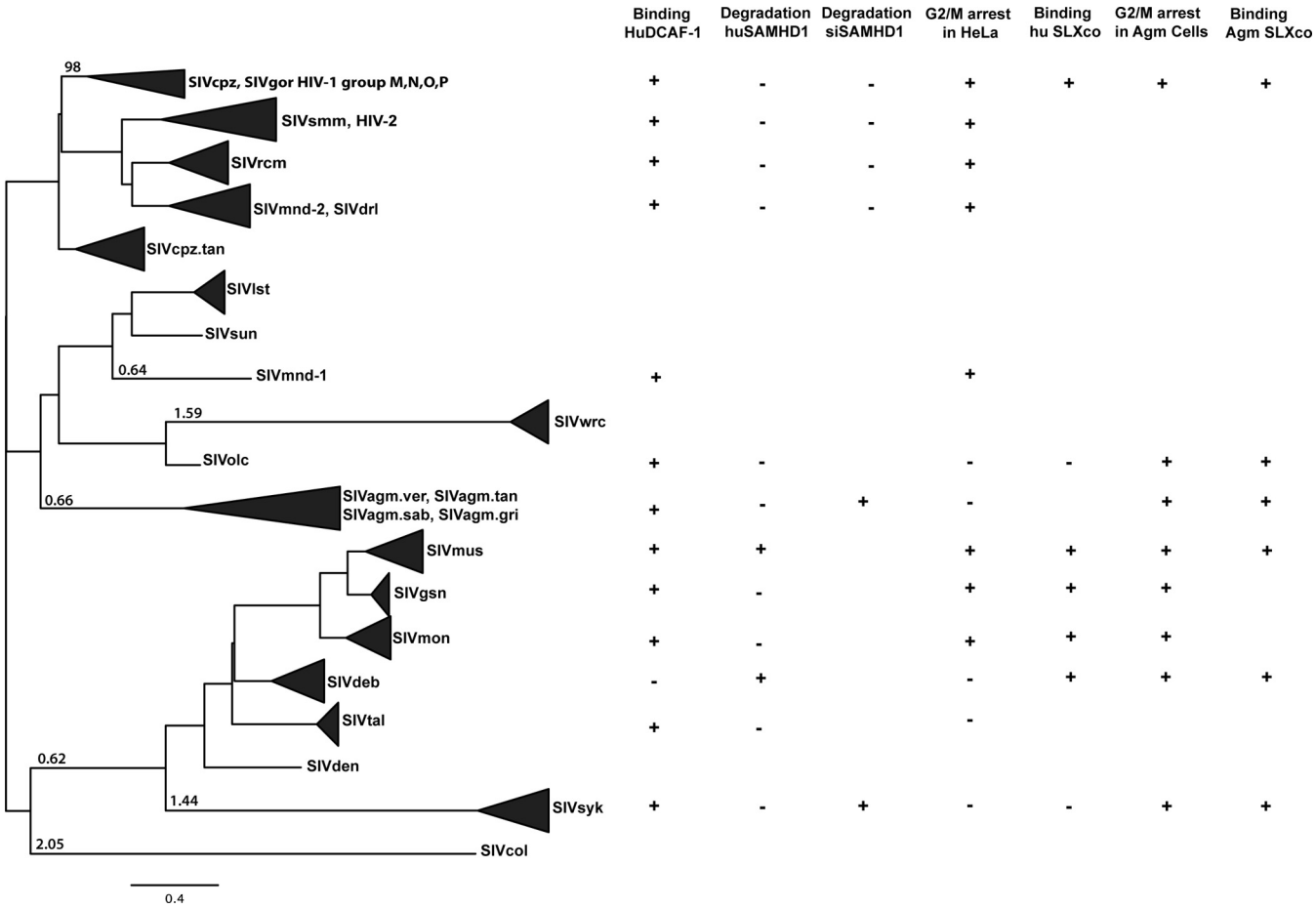

FIG 7 Cell cycle arrest and SAMHD1 counteraction phenotypes of primate lentiviral Vpr proteins. Maximum-likelihood phylogeny of 160 full-length Vpr nucleotide sequences derived from 24 HIV/SIV strains in the Los Alamos HIV Sequence Database (http://www.hiv.lanl.gov). Branch support values of ≥50% are shown on the branches. Branch lengths indicate the number of nucleotide substitutions per site. The tree is rooted by midpoint rooting. Functional activities of SIV Vpr alleles are based on this study and data obtained online (http://tree.bio.ed.ac.uk/software/seal/).

pathway (NHEJ) that deals with double-stranded breaks (DSBs) in nuclear DNA (31). Vpr expression has been shown to promote H2AXγ foci in the nucleus (28), a marker of DSBs, as well as the FANCD2 foci that are hallmarks of ICL repair (15). Unintegrated forms of proviral DNA are circularized by the NHEJ pathway into forms widely thought to be dead-end byproducts of failed integration reactions (32). Furthermore, there is evidence that DNA-PK, a key component of the NHEJ pathway, can act as a DNA pattern recognition sensor (33) and, in one report, that DNA-PK may be activated by HIV-1 integration intermediates to cause $CD4^+$ T cell death (34). It is becoming appreciated that, in certain circumstances, cytoplasmic DNA sensors can recognize the proviral DNA form of retroviral genomes (35–37). Therefore, one possible role of Vpr is to protect the proviral genome during either entry into the nucleus or the subsequent steps of integration by recruiting the SLX4 complex to prevent some form of innate nuclear DNA sensing. While it has been suggested that Vpr might inhibit IFN induction (38), it is not clear whether these observations are physiologically relevant, and Vpr-defective viruses have not been shown to induce robust type 1 IFN responses in primary target cells. Therefore, much more work needs to be done to understand the relevance of Vpr/SLX4com interactions to lentiviral replication and pathogenesis.

The phylogeny of Vpr (Fig. 7) is complicated in primate lentiviruses by the recombinant nature of SIVcpz, the HIV-1 precursor (39). SIVcpz is derived from recombination between a guenon SIV and one closely related to that whose natural host is the red-capped mangabey (SIVrcm). SIVrcm is a member of the same lineage as SIVsmm and HIV-2, in which the *vpr* gene was duplicated to generate *vpx*, with the former retaining cell cycle arrest activity and the latter targeting SAMHD1 (8, 10, 40). Upon recombination with a guenon SIV, the 5′ half of the genome, includ-

for DNA content and the cell cycle distribution of the GFP^+ population was analyzed by flow cytometry. (E) Quantification of the G_2/G1 ratio of the cells shown as described for panel D. (F) Cell lysates and FLAG immunoprecipitates of 293T cells or COS-1 cells expressing the indicated SIV Vpr were analyzed using a human anti-MUS81 antibody. (G) COS-1 cells were transfected with agm-MUS81 siRNA before being transduced with the indicated Vpr-IRES-GFP MLV vector. At 48 h postransduction, cycle analysis was performed on the GFP-positive populations as described previously. (H) Quantification of the G_2/G1 ratio of the histogram shown. agm-MUS81 knockdown was confirmed using human anti-MUS81.

ing Vpr, was derived from the SIVrcm-like virus, and *vpx* was lost. A recent study has shown that some of the *vpx* coding sequence was "overprinted" into the SIVcpz *vif* open reading frame, leaving it, and, subsequently, HIV-1, without a SAMHD1 antagonist (41). The ability to target SAMHD1, however, is an ancestral trait of SIV Vpr proteins prior to the generation of *vpx* (7). Data from previous studies on SAMHD1 counteraction by SIV Vpr proteins (7, 25), and our observations of differences in SIV Vpr interactions with human and grivet SLX4, show that the most divergent Vpr that we have tested, SIVsyk, has the ability to target rhesus macaque SAMHD1 and also promotes G_2/M arrest in COS-1 cells and interacts with the grivet SLX4 complex (Fig. 6). Thus, it is likely that both SAMHD1 counteraction and SLX4 targeting are ancestral activities of primate lentiviral Vpr proteins. Although there is no known linkage between SAMHD1 and the SLX4 complex, it is interesting that, in addition to the type 1 interferonopathy Aicardi Goutières syndrome, SAMHD1 mutations are also found in chronic lymphocytic leukemias and that SAMHD1 itself is recruited to DNA damage foci (42). Thus, there is plenty of scope for convergence of pathways involving DNA damage sensing, repair processes, and the ability of the cell to defend itself against DNA-based pathogens in the nucleus (43). Study of Vpr/SLX4 interactions will therefore yield further insight into the regulation of these pathways.

ACKNOWLEDGMENTS

We thank Wade Harper for the human SLX4 expression vector, the NIH AIDS Reagent Program for reagents, and members of the laboratory of S.J.D.N. for support.

This work was funded by a European Research Council Consolidator Grant (281598) and a Wellcome Trust Senior Research Fellowship (WT098049AIA) to S.J.D.N.

G.B. and S.J.D.N. planned the study, analyzed the data, and wrote the paper. G.B. performed the experiments; M.L. established methodology. G.B. and S.H. performed the phylogenetic analysis.

REFERENCES

1. **Selig L, Pages JC, Tanchou V, Preveral S, Berlioz-Torrent C, Liu LX, Erdtmann L, Darlix J, Benarous R, Benichou S.** 1999. Interaction with the p6 domain of the gag precursor mediates incorporation into virions of Vpr and Vpx proteins from primate lentiviruses. J Virol **73:**592–600.
2. **Kondo E, Mammano F, Cohen EA, Gottlinger HG.** 1995. The p6gag domain of human immunodeficiency virus type 1 is sufficient for the incorporation of Vpr into heterologous viral particles. J Virol **69:**2759–2764.
3. **Stivahtis GL, Soares MA, Vodicka MA, Hahn BH, Emerman M.** 1997. Conservation and host specificity of Vpr-mediated cell cycle arrest suggest a fundamental role in primate lentivirus evolution and biology. J Virol **71:**4331–4338.
4. **Planelles V, Jowett JB, Li QX, Xie Y, Hahn B, Chen IS.** 1996. Vpr-induced cell cycle arrest is conserved among primate lentiviruses. J Virol **70:**2516–2524.
5. **Ayinde D, Maudet C, Transy C, Margottin-Goguet F.** 2010. Limelight on two HIV/SIV accessory proteins in macrophage infection: is Vpx overshadowing Vpr? Retrovirology **7:**35. http://dx.doi.org/10.1186/1742-4690-7-35.
6. **Goh WC, Rogel ME, Kinsey CM, Michael SF, Fultz PN, Nowak MA, Hahn BH, Emerman M.** 1998. HIV-1 Vpr increases viral expression by manipulation of the cell cycle: a mechanism for selection of Vpr in vivo. Nat Med **4:**65–71. http://dx.doi.org/10.1038/nm0198-065.
7. **Lim ES, Fregoso OI, McCoy CO, Matsen FA, Malik HS, Emerman M.** 2012. The ability of primate lentiviruses to degrade the monocyte restriction factor SAMHD1 preceded the birth of the viral accessory protein Vpx. Cell Host Microbe **11:**194–204. http://dx.doi.org/10.1016/j.chom.2012.01.004.
8. **Tristem M, Marshall C, Karpas A, Hill F.** 1992. Evolution of the primate lentiviruses: evidence from vpx and vpr. EMBO J **11:**3405–3412.
9. **Spragg CJ, Emerman M.** 2013. Antagonism of SAMHD1 is actively maintained in natural infections of simian immunodeficiency virus. Proc Natl Acad Sci U S A **110:**21136–21141. http://dx.doi.org/10.1073/pnas.1316839110.
10. **Laguette N, Sobhian B, Casartelli N, Ringeard M, Chable-Bessia C, Segeral E, Yatim A, Emiliani S, Schwartz O, Benkirane M.** 2011. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature **474:**654–657. http://dx.doi.org/10.1038/nature10117.
11. **Le Rouzic E, Belaidouni N, Estrabaud E, Morel M, Rain JC, Transy C, Margottin-Goguet F.** 2007. HIV1 Vpr arrests the cell cycle by recruiting DCAF1/VprBP, a receptor of the Cul4-DDB1 ubiquitin ligase. Cell Cycle **6:**182–188. http://dx.doi.org/10.4161/cc.6.2.3732.
12. **Schröfelbauer B, Hakata Y, Landau NR.** 2007. HIV-1 Vpr function is mediated by interaction with the damage-specific DNA-binding protein DDB1. Proc Natl Acad Sci U S A **104:**4130–4135. http://dx.doi.org/10.1073/pnas.0610167104.
13. **Roshal M, Kim B, Zhu Y, Nghiem P, Planelles V.** 2003. Activation of the ATR-mediated DNA damage response by the HIV-1 viral protein R. J Biol Chem **278:**25879–25886. http://dx.doi.org/10.1074/jbc.M303948200.
14. **Belzile JP, Richard J, Rougeau N, Xiao Y, Cohen EA.** 2010. HIV-1 Vpr induces the K48-linked polyubiquitination and proteasomal degradation of target cellular proteins to activate ATR and promote G2 arrest. J Virol **84:**3320–3330. http://dx.doi.org/10.1128/JVI.02590-09.
15. **Laguette N, Bregnard C, Hue P, Basbous J, Yatim A, Larroque M, Kirchhoff F, Constantinou A, Sobhian B, Benkirane M.** 2014. Premature activation of the SLX4 complex by Vpr promotes G_2/M arrest and escape from innate immune sensing. Cell **156:**134–145. http://dx.doi.org/10.1016/j.cell.2013.12.011.
16. **Garner E, Kim Y, Lach FP, Kottemann MC, Smogorzewska A.** 2013. Human GEN1 and the SLX4-associated nucleases MUS81 and SLX1 are essential for the resolution of replication-induced Holliday junctions. Cell Rep **5:**207–215. http://dx.doi.org/10.1016/j.celrep.2013.08.041.
17. **Wyatt HD, Sarbajna S, Matos J, West SC.** 2013. Coordinated actions of SLX1-SLX4 and MUS81-EME1 for Holliday junction resolution in human cells. Mol Cell **52:**234–247. http://dx.doi.org/10.1016/j.molcel.2013.08.035.
18. **Schaller T, Pollpeter D, Apolonia L, Goujon C, Malim MH.** 2014. Nuclear import of SAMHD1 is mediated by a classical karyopherin alpha/beta1 dependent pathway and confers sensitivity to VpxMAC induced ubiquitination and proteasomal degradation. Retrovirology **11:**29. http://dx.doi.org/10.1186/1742-4690-11-29.
19. **Svendsen JM, Smogorzewska A, Sowa ME, O'Connell BC, Gygi SP, Elledge SJ, Harper JW.** 2009. Mammalian BTBD12/SLX4 assembles a Holliday junction resolvase and is required for DNA repair. Cell **138:**63–77. http://dx.doi.org/10.1016/j.cell.2009.06.030.
20. **Ward J, Davis Z, DeHart J, Zimmerman E, Bosque A, Brunetta E, Mavilio D, Planelles V, Barker E.** 2009. HIV-1 Vpr triggers natural killer cell-mediated lysis of infected cells through activation of the ATR-mediated DNA damage response. PLoS Pathog **5:**e1000613. http://dx.doi.org/10.1371/journal.ppat.1000613.
21. **Hickson I, Zhao Y, Richardson CJ, Green SJ, Martin NM, Orr AI, Reaper PM, Jackson SP, Curtin NJ, Smith GC.** 2004. Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM. Cancer Res **64:**9152–9159. http://dx.doi.org/10.1158/0008-5472.CAN-04-2727.
22. **Goujon C, Arfi V, Pertel T, Luban J, Lienard J, Rigal D, Darlix JL, Cimarelli A.** 2008. Characterization of simian immunodeficiency virus SIVSM/human immunodeficiency virus type 2 Vpx function in human myeloid cells. J Virol **82:**12335–12345. http://dx.doi.org/10.1128/JVI.01181-08.
23. **Price MN, Dehal PS, Arkin AP.** 2010. FastTree 2–approximately maximum-likelihood trees for large alignments. PLoS One **5:**e9490. http://dx.doi.org/10.1371/journal.pone.0009490.
24. **Telenti A, Johnson WE.** 2012. Host genes important to HIV replication and evolution. Cold Spring Harb Perspect Med **2:**a007203. http://dx.doi.org/10.1101/cshperspect.a007203.
25. **Fregoso OI, Ahn J, Wang C, Mehrens J, Skowronski J, Emerman M.** 2013. Evolutionary toggling of Vpx/Vpr specificity results in divergent recognition of the restriction factor SAMHD1. PLoS Pathog **9:**e1003496. http://dx.doi.org/10.1371/journal.ppat.1003496.

26. **Laguette N, Rahm N, Sobhian B, Chable-Bessia C, Munch J, Snoeck J, Sauter D, Switzer WM, Heneine W, Kirchhoff F, Delsuc F, Telenti A, Benkirane M.** 2012. Evolutionary and functional analyses of the interaction between the myeloid restriction factor SAMHD1 and the lentiviral Vpx protein. Cell Host Microbe **11:**205–217. http://dx.doi.org/10.1016/j.chom.2012.01.007.
27. **Maudet C, Bertrand M, Le Rouzic E, Lahouassa H, Ayinde D, Nisole S, Goujon C, Cimarelli A, Margottin-Goguet F, Transy C.** 2011. Molecular insight into how HIV-1 Vpr protein impairs cell growth through two genetically distinct pathways. J Biol Chem **286:**23742–23752. http://dx.doi.org/10.1074/jbc.M111.220780.
28. **Zimmerman ES, Chen J, Andersen JL, Ardon O, Dehart JL, Blackett J, Choudhary SK, Camerini D, Nghiem P, Planelles V.** 2004. Human immunodeficiency virus type 1 Vpr-mediated G2 arrest requires Rad17 and Hus1 and induces nuclear BRCA1 and gamma-H2AX focus formation. Mol Cell Biol **24:**9286–9294. http://dx.doi.org/10.1128/MCB.24.21.9286-9294.2004.
29. **Sharma A, Yilmaz A, Marsh K, Cochrane A, Boris-Lawrie K.** 2012. Thriving under stress: selective translation of HIV-1 structural protein mRNA during Vpr-mediated impairment of eIF4E translation activity. PLoS Pathog **8:**e1002612. http://dx.doi.org/10.1371/journal.ppat.1002612.
30. **Fouchier RA, Meyer BE, Simon JH, Fischer U, Albright AV, Gonzalez-Scarano F, Malim MH.** 1998. Interaction of the human immunodeficiency virus type 1 Vpr protein with the nuclear pore complex. J Virol **72:**6004–6013.
31. **Karttunen H, Savas JN, McKinney C, Chen YH, Yates JR, III, Hukkanen V, Huang TT, Mohr I.** 2014. Co-opting the Fanconi anemia genomic stability pathway enables herpesvirus DNA synthesis and productive growth. Mol Cell **55:**111–122. http://dx.doi.org/10.1016/j.molcel.2014.05.020.
32. **Sloan RD, Wainberg MA.** 2011. The role of unintegrated DNA in HIV infection. Retrovirology **8:**52. http://dx.doi.org/10.1186/1742-4690-8-52.
33. **Ferguson BJ, Mansur DS, Peters NE, Ren H, Smith GL.** 2012. DNA-PK is a DNA sensor for IRF-3-dependent innate immunity. Elife **1:**e00047. http://dx.doi.org/10.7554/eLife.00047.
34. **Cooper A, Garcia M, Petrovas C, Yamamoto T, Koup RA, Nabel GJ.** 2013. HIV-1 causes CD4 cell death through DNA-dependent protein kinase during viral integration. Nature **498:**376–379. http://dx.doi.org/10.1038/nature12274.
35. **Rasaiyaah J, Tan CP, Fletcher AJ, Price AJ, Blondeau C, Hilditch L, Jacques DA, Selwood DL, James LC, Noursadeghi M, Towers GJ.** 2013. HIV-1 evades innate immune recognition through specific cofactor recruitment. Nature **503:**402–405. http://dx.doi.org/10.1038/nature12769.
36. **Jakobsen MR, Bak RO, Andersen A, Berg RK, Jensen SB, Tengchuan J, Laustsen A, Hansen K, Ostergaard L, Fitzgerald KA, Xiao TS, Mikkelsen JG, Mogensen TH, Paludan SR.** 2013. IFI16 senses DNA forms of the lentiviral replication cycle and controls HIV-1 replication. Proc Natl Acad Sci U S A **110:**E4571–E4580. http://dx.doi.org/10.1073/pnas.1311669110.
37. **Gao D, Wu J, Wu YT, Du F, Aroh C, Yan N, Sun L, Chen ZJ.** 2013. Cyclic GMP-AMP synthase is an innate immune sensor of HIV and other retroviruses. Science **341:**903–906. http://dx.doi.org/10.1126/science.1240933.
38. **Okumura A, Alce T, Lubyova B, Ezelle H, Strebel K, Pitha PM.** 2008. HIV-1 accessory proteins VPR and Vif modulate antiviral response by targeting IRF-3 for degradation. Virology **373:**85–97. http://dx.doi.org/10.1016/j.virol.2007.10.042.
39. **Sharp PM, Hahn BH.** 2011. Origins of HIV and the AIDS pandemic. Cold Spring Harb Perspect Med **1:**a006841. http://dx.doi.org/10.1101/cshperspect.a006841.
40. **Hrecka K, Hao C, Gierszewska M, Swanson SK, Kesik-Brodacka M, Srivastava S, Florens L, Washburn MP, Skowronski J.** 2011. Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. Nature **474:**658–661. http://dx.doi.org/10.1038/nature10195.
41. **Etienne L, Hahn BH, Sharp PM, Matsen FA, Emerman M.** 2013. Gene loss and adaptation to hominids underlie the ancient origin of HIV-1. Cell Host Microbe **14:**85–92. http://dx.doi.org/10.1016/j.chom.2013.06.002.
42. **Clifford R, Louis T, Robbe P, Ackroyd S, Burns A, Timbs AT, Wright Colopy G, Dreau H, Sigaux F, Judde JG, Rotger M, Telenti A, Lin YL, Pasero P, Maelfait J, Titsias M, Cohen DR, Henderson SJ, Ross MT, Bentley D, Hillmen P, Pettitt A, Rehwinkel J, Knight SJ, Taylor JC, Crow YJ, Benkirane M, Schuh A.** 2014. SAMHD1 is mutated recurrently in chronic lymphocytic leukemia and is involved in response to DNA damage. Blood **123:**1021–1031. http://dx.doi.org/10.1182/blood-2013-04-490847.
43. **Chatzinikolaou G, Karakasilioti I, Garinis GA.** 8 July 2014, posting date. DNA damage and innate immunity: links and trade-offs. Trends Immunol http://dx.doi.org/10.1016/j.it.2014.06.003.