HIV-1 Vif Triggers Cell Cycle Arrest by Degrading Cellular PPP2R5 Phospho-regulators

Highlights
- HIV-1 Vif degrades PP2A phospho-regulators to induce G2 cell cycle arrest
- Vif recognizes PP2A regulators through a network of electrostatic interactions
- Combinatorial knockdown studies implicated PPP2R5A, 5C, and 5D in arrest
- Vif-mediated PPP2R5 degradation is conserved in the world’s most common subtypes

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In Brief
Salamango et al. discovered that the HIV-1 accessory protein Vif degrades several PP2A phospho-regulators to induce G2 cell cycle arrest. This activity is prevalent among diverse HIV-1 subtypes and global viral populations, suggesting that virus-induced G2 arrest is advantageous for pathogenesis.
HIV-1 Vif Triggers Cell Cycle Arrest by Degrading Cellular PPP2R5 Phospho-regulators

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SUMMARY

HIV-1 Vif hijacks a cellular ubiquitin ligase complex to degrade antiviral APOBEC3 enzymes and PP2A phospho-regulators (PPP2R5A–E). APOBEC3 counteraction is essential for viral pathogenesis. However, Vif also functions through an unknown mechanism to induce G2 cell cycle arrest. Here, deep mutagenesis is used to define the Vif surface required for PPP2R5 degradation and isolate a panel of separation-of-function mutants (PPP2R5 degradation-deficient and APOBEC3G degradation-proficient). Functional studies with Vif and PPP2R5 mutants were combined to demonstrate that PPP2R5 is, in fact, the target Vif degrades to induce G2 arrest. Pharmacologic and genetic approaches show that direct modulation of PP2A function or depletion of specific PPP2R5 proteins causes an indistinguishable arrest phenotype. Vif function in the cell cycle checkpoint is present in common HIV-1 subtypes worldwide and likely advantageous for viral pathogenesis.

INTRODUCTION

HIV-1 encodes several accessory proteins that are critical for efficient virus replication in vivo (reviewed by Harris et al., 2012; Malim and Bieniasz, 2012; Sauter and Kirchhoff, 2018). One of these proteins, Vif, is conserved among primate lentiviruses and required for infection in primary CD4+ T cells, macrophages, and many different immortalized cell lines (Gabuzda et al., 1994; Kawamura et al., 1994; von Schwedler et al., 1993). The best-characterized function of Vif is counteracting the restrictive potential of the APOBEC3 family of DNA cytosine deaminases (reviewed by Desimmie et al., 2014; Harris and Dudley, 2015; Malim and Emerman, 2008; Simon et al., 2015). Vif accomplishes this job by heterodimerizing with core binding factor β (CBF-β) and then recruiting Elongin B and Elongin C (ELOB/C), CUL5, and RBX2 to form an E3 ubiquitin ligase complex that polyubiquitinates and degrades restrictive APOBEC3s prior to virus assembly and release (Guo et al., 2014; Jäger et al., 2011). However, in the absence of Vif, APOBEC3G, APOBEC3F, APOBEC3D, and APOBEC3H are capable of packaging into nascent HIV-1 virions and, following entry into a susceptible target cell, physically impede reverse transcription and catalyze C-to-U mutations in viral cDNA, which can result in G-to-A mutations that render integrated proviral DNA non-infectious.

The only other widely accepted function for HIV-1 Vif is G2 cell cycle arrest (DeHart et al., 2008; Izumi et al., 2010; Sakai et al., 2006; Wang et al., 2007; Zhao et al., 2015). Vif-induced arrest is postulated to involve degradation of an unknown cellular factor because the genetic requirements resemble those for APOBEC3 degradation. For instance, genetic depletion of CBF-β or overexpression of dominant-negative CUL5 both prevent Vif from inducing arrest (Du et al., 2019). Likewise, Vif mutants defective in binding ELOB/C or CUL5 also fail to induce arrest (DeHart et al., 2008). However, the protein surfaces used by Vif for substrate recognition are largely distinct. For instance, comparative analysis between arrest-proficient and -deficient Vif variants revealed several positions essential for inducing G2 arrest that are either fully (APOBEC3G and APOBEC3F) or partially (APOBEC3H) dispensable for APOBEC3 degradation (e.g., positions 31, 33, 36, 48, and 50; Izumi et al., 2010; Zhao et al., 2015). Despite this progress in linking HIV-1 Vif to G2 cell cycle arrest, the precise cellular substrate(s) and overall molecular mechanism(s) have remained elusive.

Quantitative proteomics studies of HIV-1-infected T cells recently revealed Vif-dependent remodeling of the host phosphoproteome and another clue to the G2 arrest mechanism (Greenwood et al., 2016; Naamati et al., 2019). New substrates for Vif degradation have been reported, including members of the PPP2R5 family of protein phosphatase 2A (PP2A) regulators. PP2As function as heterotrimeric complexes that account for a large majority of phosphatase activity in eukaryotic cells (reviewed by Nilsson, 2019; Thompson and Williams, 2018). PP2A trimers are comprised of structural, catalytic, and regulatory (B) subunits. The B subunit can be 1 of 3 distinct protein families (B55, PPP2R5/B56, or PR72/130) that regulate subcellular localization and substrate recognition of holoenzyme complexes (e.g., McCright et al., 1996; Wang et al., 2016). Prior work has linked PPP2R5 regulatory proteins to cell cycle regulation,
including the G2-to-M phase transition (reviewed by Moura and Conde, 2019; Nilsson, 2019). However, direct cause-and-effect relationships between Vif, these phospho-regulatory proteins, and G2 cell cycle arrest have yet to be established.

Here a large-scale mutant Vif library is deployed to define the surface used to target PPP2R5 proteins for degradation. A panel of separation-of-function mutants demonstrated that the Vif-PPP2R5 interaction surface is mediated by a conserved network of electrostatic interactions. This comprehensive mutation analysis revealed an inseparable relationship between PPP2R5 degradation and Vif-induced G2 arrest mechanisms. Chemical inhibition of PPP2A activity or knockdown of specific combinations of PPP2R5 transcripts also resulted in similar G2 arrest phenotypes. Functional studies and global bioinformatic analyses both indicated that PPP2R5 degradation and G2 arrest activities are prevalent among diverse HIV-1 subtypes and global viral populations.

**RESULTS**

**Library Construction and Selection Using PPP2R5A as a Vif Substrate**

To determine the surface Vif utilizes to degrade the PPP2R5 family of proteins, we generated an ultra-deep mutant library targeting 170 contiguous amino acid positions. The vif gene of HIV-1 strain IIIB (identical to NL4-3) was amplified in 6 consecutive segments using sense oligonucleotide primers with a low mutation frequency (~3%) to bias mutations toward single amino acid substitutions (Figure S1A). Individual amplicon libraries were combined by PCR shuffling to generate recombinant products with mutations distributed across all 6 regions. Recombinant amplicons were cloned into a lentiviral transduction vector and subjected to next-generation sequencing. Viral populations.

NGS Analysis and Validation of Screening Results

Following FACS, vif gene segments were recovered by high-fidelity PCR and subjected to NGS. The sampling frequencies (represented as reads per million [RPM]) were compared between EGFP-positive (non-functional mutant) and EGFP-negative (functional mutant and wild-type) populations to estimate the relative fitness of each Vif mutant (Figure 1D). Three criteria were required for inclusion of a mutant sequence in the final analysis: (1) representation by 15 RPM or more in at least one population, (2) enrichment or depletion value of 2-fold or more, and (3) presence of 3 or more amino acid substitutions. These criteria resulted in 730 Vif mutants with relative fitness ranging from +12 (functional against PPP2R5A) to +10 (non-functional against PPP2R5A) (Figure 1D). Stop codons were enriched at the 5’ end of vif, and amino acid substitution mutations occurred throughout.

A total of 10 Vif mutants predicted to either degrade or not degrade EGFP-PPP2R5A were selected for further analysis. Each mutant was transfected into 293T cells stably expressing EGFP-PPP2R5A or APOBEC3G-EGFP (control), and the degradation of each fluorescently tagged target was quantified by flow cytometry. Overall, the degradation phenotypes recapitulated the predictions from the NGS analysis in Figure 1D. For example, R15S, K22T, I31F, R33G, and N48T were predicted to have compromised ability to degrade EGFP-PPP2R5A, and indeed, these substitutions failed to reduce EGFP-PPP2R5A levels compared with wild-type Vif (Figure 1E). Interestingly, all but the K22T substitution could trigger APOBEC3G-EGFP degradation, indicating that the surfaces used to target each substrate are largely distinct. Likewise, L72R, G75T, Q83H, Q105R, and N140Y were predicted to have no effect on degradation, and all reduced both EGFP-PPP2R5A and APOBEC3G-EGFP to levels comparable with those caused by wild-type Vif (Figure 1E). Immunoblot confirmed that all Vif mutants expressed at wild-type levels (Figure S1C).

**Defining the Vif Surface Required for PPP2R5 Degradation**

To define the surface Vif uses to bind EGFP-PPP2R5A, dedicated degradation studies were done with amino acid substitution mutants that had a relative fitness score of ~1.5 or higher and are surface exposed (Guo et al., 2014). A total of 9 residues fulfilled these two criteria, and 7 of 9 were positively charged lysines or arginines (Figures 2A and 2B; W22 was excluded with a relative fitness score of ~3 because it is not surface exposed). Alanine substitutions at these positions reduced or abrogated Vif’s ability to degrade EGFP-PPP2R5A without altering Vif protein expression (Figures 2C and 2D). Moreover, all of these single amino acid substitution mutants could efficiently decrease APOBEC3G-EGFP levels, except for K22A and K26A, which are known to be required for APOBEC3G degradation (Albin et al., 2010; Russell and Pathak, 2007).
Taken together, these results strongly indicated that the Vif surface used to bind PPP2R5A is electropositive and genetically separable from the surface used to bind APOBEC3G (i.e., largely-non-overlapping).

**PPP2R5 Degradation Is Required for Vif-Mediated Cell Cycle Arrest**

Previous studies have suggested that Vif induces G2 arrest by degrading an unknown cellular factor because this process...
requires the same E3-ubiquitin ligase machinery that is utilized for APOBEC3 degradation (DeHart et al., 2008; Du et al., 2019). We therefore hypothesized that PPP2R5 degradation may, in fact, be the mechanism for Vif-induced G2 arrest. In direct support of this idea, all Vif mutants that failed to degrade eGFP-PPP2R5A were similarly compromised for cell cycle arrest (Figure 2C). Moreover, at least 2 mutants, R23A and K36A, were partially functional for both activities (but not for A3G degradation), further indicating a cause-and-effect relationship. Representative Vif mutants were similarly defective for G2 arrest following expression in the T cell line SupT11 (Figure S2). These data combined support a model in which Vif degrades the PPP2R5 phospho-regulators to induce G2 arrest.

**Vif Binds the PPP2R5 Family through Clustered Electrostatic Interactions**

We next asked whether Vif uses the same surface to recognize and degrade all 5 PPP2R5 proteins. Previous studies have indicated that Vif can degrade endogenous PPP2R5A/5D and hemagglutinin (HA)-tagged versions of PPP2R5A–E, but the surfaces and amino acids used to mediate these interactions were not defined (Evans et al., 2018; Greenwood et al., 2016; Naamati et al., 2019). Therefore, a subset of Vif mutants defective in PPP2R5A degradation (above) were transiently expressed in 293T cells stably expressing EGFP-tagged versions of each PPP2R5 family member and analyzed at the single-cell level by flow cytometry. Wild-type Vif degraded EGFP-tagged PPP2R5A–E proteins with variable efficiencies, with 5A being degraded the most and 5E the least (Figure 3A). Several Vif mutants, including K22A, K26A, R33A, and K36A, uniformly failed to reduce the levels of several of the PPP2R5 family members. Importantly, R33A and K36A cannot degrade PPP2R5 proteins but are still capable of promoting wild-type levels of APOBEC3G degradation, making these true separation-of-function mutants (compare PPP2R5 data in Figure 3A and APOBEC3G data in Figure 2C). These results strongly indicated that Vif uses the same surface to recognize and degrade the different PPP2R5 proteins.

Because most amino acids required for PPP2R5 degradation are positively charged, we wondered whether the interface between Vif and the PPP2R5s would be electrostatic in nature. The crystal structure of PPP2R5C supported this idea, with an extensive solvent-exposed surface being overwhelmingly electro-negative (PDB: 2IAE; Wang et al., 2016; Figure S3). Moreover, the other PPP2R5 proteins are homologous (80%–90% similarity) and the predicted structures superimpose with similar electro-negative surfaces (see the PPP2R5A structural model in Figure 3B and comparisons with PPP2R5C in Figure S3). This high degree of structural conservation suggests that this negatively charged surface of the PPP2R5 proteins might be recognized by the positively charged surface of Vif. To directly test this idea, conserved glutamate and aspartate residues were changed to lysine and analyzed by flow cytometry for Vif susceptibility (Figure 3C). This structure-guided mutation analysis revealed PPP2R5A residues that confer complete or partial resistance to Vif-mediated degradation (full resistance: E251, E301, E335, and D338; partial resistance: D205) and strongly implicated this electro-negative patch in binding directly to Vif.
PPP2R5A, 5C, and/or 5D Depletion Induces G2 Arrest
Independent of Vif

Although the results above unambiguously linked Vif, PPP2R5 proteins, and G2 cell cycle arrest, it was still unclear which protein (or proteins) must be degraded for this phenotype. To answer this question, we first determined which PPP2R5 proteins are expressed in cell lines that are susceptible to Vif-induced G2 arrest (i.e., 293T, HeLa, SupT11, CEM2n, H9, and THP-1 cells; Dehart et al., 2008; Du et al., 2019; Greenwood et al., 2016; Sakai et al., 2006; Wang et al., 2007; Zhao et al., 2015). qRT-PCR expression data indicated that proteins 5A, 5C, 5D, and 5E are well expressed in these lines, whereas 5B is ~10-fold lower (Figure 4A). These findings were consistent with Cancer Cell Line Encyclopedia (CCLE) RNA sequencing (RNA-seq) data (Figure 1B) and combined suggest that the most likely Vif targets are PPP2R5A, 5C, 5D, and/or 5E (with 5E least favored because of Vif resistance; Figure 3A). We predicted that short-hairpin RNA (shRNA)-mediated knockdown of the correct Vif target(s) would induce G2 arrest in the absence of Vif. However, although strong PPP2R5A, 5C, 5D, and 5E knockdowns could be achieved in 293T cells, these individual mRNA depletions had no discernable effect on the cell cycle (Figure 4B). We therefore took a combinatorial knockdown approach and found clear G2 cell cycle arrest phenotypes for 3 different combinations of PPP2R5 proteins (Figure 4C). Strong arrest phenotypes were induced by combining shPPP2R5A and shPPP2R5C, shPPP2R5A and shPPP2R5D, and shPPP2R5C and shPPP2R5D, whereas any combination involving shPPP2R5E showed no effect (Figure 4C). To independently probe whether loss of PP2A function induces G2 arrest, 293T cells were treated with a well-characterized PP2A inhibitor, okadaic acid (Favre et al., 1997; Giese et al., 1995; Ishida et al., 1992), and, interestingly, this compound induced a similarly robust G2 arrest phenotype (Figure 4D). These results demonstrated that at least 2 PPP2R5 proteins must be depleted simultaneously to induce cell cycle arrest and, taken together with the aforementioned data, strongly indicate that Vif induces G2 arrest by degrading the same combination of proteins.

Conservation of the Vif-Mediated PPP2R5 Degradation and G2 Arrest Mechanism

To investigate the mechanistic conservation of this host-pathogen interaction, a panel of viruses from groups M, N, and O was analyzed for G2 arrest, PPP2R5A degradation, and APOBEC3G degradation (see Vif alignments in Figure 5A). As expected from prior studies, all variants could efficiently decrease APOBEC3G-EGFP levels, comparable with HIV-1 IIIB Vif (Figure 5B). In comparison, only group M subtypes B (IIIB), C, and D displayed an ability to degrade EGFP-PPP2R5A and induce G2 arrest, and, in all instances, these two phenotypes were linked. To further confirm this direct relationship, we converted the non-G2-arresting LAI Vif into a G2-arresting derivative by swapping five amino acids in the electro-positive surface region described above to match those in HIV-1 IIIB Vif (Figure 5C). To further investigate LAI residues involved in PPP2R5 degradation/G2 arrest, the LAI_{G2-arrest} Variant was used to generate a series of reversion mutants (i.e., to systematically exchange IIIB residues back LAI to look for loss of PPP2R5 degradation activity). Surprisingly, reversion of I31V alone nearly abolished EGFP-PPP2R5A degradation (with no effect on APOBEC3G-EGFP degradation), whereas no other single mutants had any effect. Because I31V did not completely abolish EGFP-PPP2R5A degradation, this mutant was combined with additional amino acid substitutions (Figure S4A). Only one double reversion mutant, I31V plus R33G, was able to completely abolish EGFP-PPP2R5A degradation. We further confirmed the importance of positions 31 and 33 by exchanging LAI Vif
residues with those of IIIB Vif. Interestingly, exchanging V31I and G33R in LAI Vif together, but not separately, was sufficient to confer EGFP-PPP2R5A degradation activity (Figure S4B). Similar results were obtained with V31I/G33K, demonstrating a requirement for a positive charge at position 33 (Figure S4B and S4C).

We next estimated the proportion of Vif variants in global circulation with the potential to degrade PPP2R5s and induce G2 arrest. Full-length HIV-1 sequences (>8,000 bp) from the Los Alamos database were analyzed when corresponding information was available for subtype and country of sampling (n ≈ 2,500). Consistent with previous reports (Buonaguro et al., 2007; Hemelaar et al., 2006), our analyses showed that group M subtype B is the most abundant, circulating in the Americas, Europe, and Australia, whereas group M subtype C is the most abundant in Africa (together, approximately 60% of HIV-1 isolates circulating globally; Figure 5D). These analyses also indicated that Vif amino acids required for G2 arrest (I31 and R/K33) occur at high frequencies worldwide in subtype B and C HIV-1 isolates (Figure 5D). Taken together with the deep mutation and surface-guided mutation studies above, indicating inextricable roles for these residues in PPP2R5 degradation and G2 arrest, Vif function in PPP2R5 degradation and G2 arrest is likely a contributing factor in the global spread of HIV-1.

### DISCUSSION

Although HIV-1 Vif has been intensively studied for its ability to target and degrade the APOBEC3 family of virus restriction factors, its function in G2 cell cycle arrest has been less characterized. Here we show that Vif degrades PP2A phosphoregulators to induce G2 cell cycle arrest. Using deep and structure-guided mutagenesis approaches, the Vif-PPP2R5 interface was mapped to distinct surface regions that are positively and negatively charged, respectively, and likely to interact directly through a network of favorable electrostatics. Vif mutants that were partly or fully defective in PPP2R5 degradation were similarly defective in G2 arrest, suggesting a direct cause-and-effect relationship. This likely molecular mechanism was confirmed by inducing a similar G2 arrest phenotype with pharmacologic inhibition of PP2A function as well as with pairwise genetic depletion of candidate PPP2R5 proteins (independent of Vif). Functional studies and phylogenetic analyses combined indicate mechanistic conservation in roughly half of the global virus population (with HIV-1 subtypes B and C predominating).

Our findings here support a model in which Vif degrades several PPP2R5 family members to activate the G2 checkpoint and create an environment that favors virus replication (Figure S5). PP2A phosphatases have been shown to play at least 3 important roles in the regulation of cell cycle progression from G2 to M phase, and Vif is likely to affect these processes. The first and arguably most critical event for mitotic entry is CDC25-mediated dephosphorylation of CDK1, which activates CDK1 and allows nuclear translocation of the CDK1-CyclinB complex (Lee and Kirschner, 1996; Timofeev et al., 2010). This process is initiated by PP2A/PPP2R5D-catalyzed activation of CDC25 through dephosphorylation, which triggers the release of inhibitory 14-3-3 proteins (Margolis et al., 2006; Figure S5). Thus, Vif-mediated degradation of PPP2R5D would prevent
activation of CDC25 and prevent the CDK1-Cyclin B complex from propelling cells into mitosis. In support of this first mechanism, infection of Jurkat T cells with Vif-proficient HIV-1 results in constitutive phosphorylation of CDK1 and concomitant inhibition of CDK1-CyclinB nuclear translocation (Sakai et al., 2011).

Second, PP2A/PPP2R5 complexes also control G2-to-M progression through regulation of the APC-ubiquitin-proteasome complex, which promotes mitotic exit through degradation of specific cellular targets, including Aurora kinases A and B and CDK1-CyclinB (Figure S5). Aurora kinases regulate numerous G2-to-M checkpoints, including spindle assembly, microtubule-kinetochore attachment, and cytokinesis (Fu et al., 2007; Goldenson and Crispino, 2015). In support of this second mechanism, genetic depletion of PP2A/PPP2R5A, 5C, and/or 5D complexes has been shown to result in increased Aurora kinase activity and inhibition of mitotic exit (Foley et al., 2011; Jeong and Yang, 2013). Recent results also showed that transduction of CEM T4 cells with a Vif-proficient, but not Vif-deficient, virus results in activation of Aurora kinases A/B (Greenwood et al., 2016).

Moreover, a third plausible mechanism is that Vif-mediated degradation of PP2A/PPP2R5A and 5D complexes may also prevent sister chromatid segregation and impede mitotic exit (Figure S5). Loss of PP2A/PPP2R5A/5D results in mislocalization of the centromeric cohesion protector Sgo1 and causes premature chromosomal mis-segregation (Shintomi and Hirano, 2009; Tang et al., 2006). Chromosomal segregation is also negatively affected by constitutive activation of the Aurora kinases (Foley et al., 2011). Taken together, prior studies strongly support a model in which Vif degrades multiple PPP2R5 family members to destabilize multiple independent G2/M checkpoints.

The vif gene is conserved in all primate lentiviral lineages and assumed to function predominantly in preservation of viral genomic integrity by degrading APOBEC3 enzymes. However, our results indicate that nearly half of the global viral population may be able to cause PPP2R5 degradation and G2 arrest (Figure 5). Interestingly, HIV-1 Vpr also induces G2 arrest, further suggesting that cell cycle regulation is important for HIV-1 pathogenesis. However, the Vif and Vpr G2 arrest mechanisms are clearly distinct because Vpr requires a different E3-ubiquitin ligase complex (Hrecka et al., 2007). Interestingly, it has been suggested that Vif can delay Vpr-induced G2 arrest by targeting Vpr for proteasomal degradation (Wang et al., 2008). These authors speculated that this mechanism may provide a selective advantage for the virus by delaying G2 arrest and, therefore, the impending cytotoxic phenotype associated with Vpr expression. This model is intriguing because it would result in a longer half-life of an infected T cell, which could lead to an increase in the amount of progeny virions generated during each round of infection. It is also possible that HIV-1 deploys functionally redundant Vif and Vpr mechanisms to...
achieve greater evolutionary flexibility. For instance, it has been suggested that certain Vif variants have adapted to target APOBEC3H for degradation at the cost of losing G2 arrest activities (Zhao et al., 2015). In this scenario, the virus could still induce G2 arrest through Vpr while simultaneously allowing Vif adaptation.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

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**SUPPLEMENTAL INFORMATION**

Supplemental Information can be found online at https://doi.org/10.1016/j.cell.2019.09.057.

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**AUTHOR CONTRIBUTIONS**

D.J.S. and R.S.H. led the project. D.J.S. made all DNA constructs and performed most of the experiments. J.L.M. contributed immunobLOTS, A.A.S. and S.A.M. to knockdown studies, T.I. and J.W. to cell-based studies, D.E. and M.C.J. to sequence comparisons, and W.L.B. to project logistics. D.J.S. and R.S.H. drafted the manuscript, and all authors contributed to revisions.

**DECLARATION OF INTERESTS**

R.S.H. is a co-founder, shareholder, and consultant of ApoGen Biotechnologies Inc.

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# STAR METHODS

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Vif                  | NIH Reagent Program | #6459 |
| Actin               | Cell Signaling | #4970; RRID: AB_2223172 |
| rabbit IRdye 800CW  | LICOR  | #827-08365; RRID: AB_10796098 |
| mouse IRdye 680LT   | LICOR  | #925-68020; RRID: AB_2687826 |
| rabbit HRP          | Cell Signaling | #7074P2; RRID: AB_2099233 |
| mouse HRP           | Cell Signaling | #7076P2; RRID: AB_330924 |
| **Bacterial and Virus Strains** | | |
| E. coli DH10B       | Harris Lab Collection | N/A |
| E. coli 5-alpha electrocompetent | NEB | C2989K |
| HIVIII Vif/VprXX provirus plasmid | Salamango et al., 2018a | N/A |
| **Critical Commercial Assays** | | |
| FxCycle Pi/RNase Staining | Invitrogen | F10797 |
| **Deposited Data** | | |
| Illumina MiSeq Data | ArrayExpress | E-MTAB-8357 |
| **Experimental Models: Cell Lines** | | |
| HEK293T             | ATCC   | #CRL-3216 |
| HeLa                | NIH Reagent Program | #153 |
| Cem2n               | Refslan et al., 2012 | N/A |
| SupT11              | Refslan et al., 2010 | N/A |
| THP-1               | Berger et al., 2011 | N/A |
| H9                  | NIH Reagent Program | #87 |
| **Oligonucleotides** | | |
| All oligonucleotide sequences used in this study are provided in Table S1 | This paper | Table S1 |
| **Recombinant DNA** | | |
| All recombinant DNA used in this study are listed in Table S2 | This paper | Table S2 |
| **Software and Algorithms** | | |
| UCSF Chimera        | Pettersen et al., 2004 | http://www.cgl.ucsf.edu/chimera/ |
| Phyre2 Protein Fold Recognition Server | Kelley et al., 2015 | http://www.sbg.bio.ic.ac.uk/html/page.cgi?id=index |
| FASTX-Toolkit       | N/A    | N/A |
| EMBASS-TRANSEQ      | Rice et al., 2000 | http://emboss.sourceforge.net/ |
| FASTAptamer         | Alam et al., 2015 | https://www.burkelab.missouri.edu/fasta.html |
| Statistical analysis software R and RStudio | N/A | http://www.r-project.org |
| FlowJo 8.8.6        | N/A    | https://www.flowjo.com/ |
| GraphPad Prism 6    | N/A    | https://www.graphpad.com/ |
| **Other**           |        |            |
| CellBind 96 well microplate | Corning | #CLS3340 |
| TransIT LTI transfection reagent | Mirus Bio | #MIR2306 |
| EVOS FL Color microscope | Thermo Fisher Scientific | #AMEFC4300 |
| BD FACSCanto II Flow Cytometer | BD Biosystems | N/A |
LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Reuben S. Harris (rsh@umn.edu). All constructs generated in this study are all freely available upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cell Lines

HEK293T cells were obtained from ATCC (#CRL-3216) and were maintained in DMEM (Hyclone, South Logan, UT) supplemented with 10% FBS (GIBCO, Gaithersburg, MD) and 0.5% pen/strep (50 units). HeLa (#153) and H9 (#87) cells were obtained from the NIH Reagent Program and were maintained in RPMI (Hyclone) supplemented with 10% FBS (GIBCO) and 0.5% pen/strep (50 units). CEM2n (Refsland et al., 2012) and SupT11 (Refsland et al., 2010) cells were generated in the Harris Lab and were maintained in RPMI (Hyclone) supplemented with 10% FBS (GIBCO) and 0.5% pen/strep (50 units). THP-1 cells were provided by Dr. Andrea Cimarelli (INSERM, France) (Berger et al., 2011) and were maintained in RPMI (Hyclone) supplemented with 10% FBS (GIBCO) and 0.5% pen/strep (50 units).

METHOD DETAILS

Plasmids and Cloning

All expression plasmids used in this study were cloned into the pQCXIH retroviral expression vector (Salamango and Johnson, 2015). The cDNA sequences for all PPP2R5 genes were ordered as gblocks from Integrated DNA Technologies (IDT) and cloned in-frame with an eGFP coding sequence using AgeI and BsiWI restriction sites. The pQCXIH APOBEC3G-eGFP expression vector has been described (Salamango et al., 2018b). PPP2R5A point mutants were generated by PCR amplification using Phusion high fidelity DNA polymerase (NEB, Ipswich, MA) and overlapping PCR to introduce the indicated mutations. All constructs were confirmed by restriction digestion and Sanger sequencing. All oligonucleotide sequences used to generate the constructed used in this study are listed in the Key Resources Table.

Cell Lines and Culture Conditions

293T and HeLa cells were maintained in DMEM (Hyclone, South Logan, UT) supplemented with 10% FBS (GIBCO, Gaithersburg, MD) and 0.5% pen/strep (50 units). SupT11, CEM2n, H9 and THP-1 cells were maintained in RPMI (Hyclone) supplemented with 10% FBS (GIBCO) and 0.5% pen/strep (50 units). The creation and characterization of the SupT11 T cell line has been reported (Refsland et al., 2010). 293T cells were transfected with TransIT LTI (Mirus, Madison, WI) according to the manufacture’s protocol. For okadaic acid treatments (Santa Cruz), the indicated concentration was added to the culture medium for 20 hours and then the cells were harvested and subjected to PI staining and flow cytometry to determine DNA content.

To generate stable PPP2R5 and APOBEC3G cell lines, viruses were produced from 293T cells transfected with the pQCXIH retroviral expression vectors described above, an MLV GagPol packaging vector, and a VSV-G vector. Media was harvested 48 hours post-transfection and frozen at −80°C for 4-6 hours, thawed and centrifuged at 1500 x g, and combined with fresh 293T cells. To generate pure cell populations, samples were treated with hygromycin B (Sigma, 200 μg/ml) 48 hours post-transduction.

Library Generation and NGS Analysis

Library synthesis was modified from previous methods (Salamango et al., 2016) and is depicted in Figure S1. Mutagenic oligonucleotide primers were generated by IDT with a mutation frequency of 3% per nucleotide position. These primers were used to amplify IIIIB vif to produce 6 consecutive mutagenic gene segments. Amplicons were run on an agarose gel and purified (Thermo Fisher gel extraction kit) to ensure each fragment contained only mutagenic segments. Purified DNA fragments were combined in a pseudo-PCR reaction without oligonucleotide primers and cycled for 35 reactions to induce recombination. The reaction product was then diluted 1:40 and added to a PCR reaction containing flanking oligonucleotide primers and cycled for 30 reactions to generate the final library product. The library PCR was run on an agarose gel, purified, digested with Clai and EcoRI, and cloned into the indicated lentiviral expression vector. Bacterial clones were scraped from agar plates and pooled into one culture mix that was grown at 37°C for 3 hours and then DNA plasmid was extracted. Plasmid DNA was PCR amplified with Illumina NGS adaptor primers and subjected to a 2 × 300 bp MiSeq run to quantify the number of mutations in the library.

For NGS analysis following library selection, FACs sorted samples were lysed and genomic DNA was extracted and amplified using Illumina NGS adaptor primers. Samples were pooled and subjected to a 2 × 300 bp MiSeq run. Resulting sequence reads were paired, trimmed, and filtered for quality using the FASTx toolkit. Processed reads were translated using EMBOSS and redundant sequences were collapsed and counted into one unique sequence using FASTAptamer (Alam et al., 2015). The functional and non-functional sequence files were compared and the resulting enrichment and depletion scores were used to generate the plot in Figure 1D (see text for sequence cutoff criteria).
Cancer Cell Line Expression and HIV-1 Subtype Analysis

Gene expression information for relevant cell lines and tissue types was obtained from the CCLE (https://portals.broadinstitute.org/ccle). Reads per Kilobase per Million (RPKM) values were obtained for available hematopoietic and lymphoid cells lines from the latest CCLE preprocessing pipeline (n = 178). To quality control reads, align/assemble reads to a human reference genome, and quantify individual human gene expression RPKM values (the file CCLE_DepMap_18Q1_RNaseq_RPKM_20180214.gct was used those as analyses). PPP2R5 gene family mRNA expression was quantified relative to a housekeeping gene, TBP. Data were formatted using the statistical analysis software R and RStudio and visualized using the R package ggplot2. For global subtype distributions, full-length HIV-1 sequences were downloaded from http://www.hiv.lanl.gov/content/index (length > 8000bp) with information of HIV-1 subtype and country of sampling. Using one sequence per patient (n=2,500), the distribution of HIV-1 subtypes across the globe was estimated as depicted by region in Figure 5. To identify allelic frequency of specific Vif residues such as positions 31 and 33, sequences were compared to the reference sequence for LAI (accession number K03455).

Quantitative PCR

To determine expression levels of PPP2R5 transcripts in the panel of cell lines depicted in Figure 4, approximately 250,000 cells were collected and subjected to qPCR analysis. RNA was extracted from the indicated cells using a High Pure RNA Isolation Kit (Roche). For generating stable shRNA knockdown/vector control lines, 293T cells were transfected with the shRNA vector, an HIV-1 Gag/Pol packaging construct, and a VSV-G expression vector. Media was changed to complete medium and the manufacturer recommended protocol on a LightCycler 480 (Roche). For generating stable shRNA knockdown/vector control lines, 293T cells (listed in the Key Resources Table) and the housekeeping gene TBP (Burns et al., 2013). All qPCR was done according to the manufacturer recommended protocol on a LightCycler 480 (Roche). For generating stable shRNA knockdown/vector control lines, 293T cells were transfected with the shRNA vector, an HIV-1 Gag/Pol packaging construct, and a VSV-G expression vector. Media was harvested 48 h post-transfection and frozen at -80 °C for 4-6 hours, thawed and centrifuged at 1500 x g, and combined with fresh 293T cells. Stable cells either expressed Turbo GFP, and purity was quantified by flow cytometry, or, expressed puromycin resistance and were drug treated for 48 hours to produce a pure population (Sigma, 1 μg/ml).

Cell Cycle Arrest and PPP2R5 Degradation Assays

For cell cycle arrest assays, approximately 250,000 293T cells were plated into a 12 well culture plate and allowed to adhere overnight. The next day, cells were transfected with 1 μg of the indicated construct and allowed to incubate overnight. After 24 hours, cells were split into 6 well culture plates and allowed to adhere overnight. At 48 hours post-transfection, cells were detatched using PBS/EDTA and centrifuged at 500 x g for 10 minutes. Cell pellets were resuspended in 50 μL PBS, mixed with 500 μL cold 70% ethanol, and fixed on ice for 30 minutes. After fixing, cells were centrifuged at 500 x g for 10 minutes and washed twice with PBS. Cell pellets were re-suspended in FixCycle PI/RNase staining solution (Invitrogen) for 45 minutes and then analyzed for cell cycle profiling using flow cytometry.

For PPP2R5 degradation assays, cell lines stably expressing either eGFP-PPP2R5A or APOBEC3G-eGFP were plated into 12 well plates at a seeding density of approximately 250,000 cells per well. The next day, cells were transfected with 250 ng of the indicated Vif construct and allowed to incubate for 48 h. After 48 hours post-transfection, cells were harvested using PBS/EDTA and collected for both flow cytometry and immunoblotting to confirm Vif expression. For PPP2R5 degradation assays, cell lines stably expressing either eGFP-PPP2R5A or APOBEC3G-eGFP were plated into 12 well plates at a seeding density of approximately 250,000 cells per well. The next day, cells were transfected with 250 ng of the indicated Vif construct and allowed to incubate for 48 h. After 48 hours post-transfection, cells were harvested using PBS/EDTA and collected for both flow cytometry and immunoblotting to confirm Vif expression. To determine relative eGFP loss, samples were subjected to analysis via flow cytometry. Sample populations were first separated by forward and side scatter to isolate live singular events. After the live/singlet event population was isolated, the mCherry positive population was isolated and the eGFP fluorescence intensity was assessed. The resulting histograms depicted throughout the manuscript display the eGFP fluorescence profile of the isolated mCherry positive population.

Fluorescence Microscopy Experiments

Approximately 10,000 293T cells stably expressing eGFP-PPP2R5A were plated into a 96-well CellBIND microplate (Corning) and allowed to adhere overnight. The next day, cells were transduced with virus containing the indicated mCh-T2A-Vif variant as indicated in appropriate figure panels and imaged 48 h post transduction. Images were collected at 40x magnification using an EVOS FL Color microscope (ThermoFisher). All images expressing mCherry have been pseudo-colored with magenta instead of red to accommodate readers that may be affected by colorblindness.

Immunoblot Analysis

For immunoblotting assays, the indicated cell line was plated at a seeding density of approximately 250,000 cells per well in a 12 well plate. The next day, cells were either transfected with 250 ng of the indicated Vif construct, or, transduced with the indicated virus and allowed to incubate for 48 h. After 48 hours post-transfection/transduction, cells were collected into 1.5 mL tubes and centrifuged at 500 x g for 10 minutes. Cell pellets were resuspended in 100 μL RIPA buffer (10 mM Tric-Cl [pH 8.0], 1 mM EDTA, 0.1 mM EGTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, and 140 mM NaCl) and 10% of the total cell lysate samples were combined with 2.5x SDS-PAGE loading buffer. Samples were separated by a 4%–20% gradient SDS-PAGE gel and transferred to PVDF-FL membranes (Millipore). Membranes were blocked in blocking solution (5% milk + PBS supplemented with 0.1% Tween20) and then incubated with primary antibody diluted in blocking solution. Secondary antibodies were diluted in blocking solution + 0.01% SDS. Membranes were imaged with a LICOR Odyssey instrument or film. Primary antibodies used in these experiments were αActin (Cell Signaling #4970) and αVif (NIH Reagent Program #6459). Secondary antibodies used were αrabbit IRdye
800CW (LICOR 827-08365), αmouse IRdye 680LT (LICOR 925-68020), αrabbit HRP (Cell Signaling 7074P2), and αmouse HRP (Cell Signaling 7076P2).

QUANTIFICATION AND STATISTICAL ANALYSIS

All flow cytometry data were analyzed using FlowJo 8.8.6 software. The associated histogram profiles of the flow cytometry data in Figures 1, 2, 3, 4, and 5, S2, and S3 were generated using FlowJo 8.8.6 software. The data shown are representative from one of three independent experiments. Bar graphs in Figure 4 and pie charts in Figure 5 were generated using GraphPad Prism 6 software. The “n” values depicted in Figure 5 represent patient Vif sequence data obtained from the Los Alamos database (described above). Protein structures depicted in Figures 2, 3, and S3 were generated using Chimera protein modeling software. Error bars generated for qPCR data in Figure 4 were calculated based on SEM of the data obtained from three independent sample sets.

DATA AND CODE AVAILABILITY

The unique deep sequencing data generated from the Vif library selections used for this study are available at ArrayExpress (E-MTAB-8357). All other materials are available upon request and will be fulfilled by the Lead Contact, Reuben S. Harris (rsh@umn.edu).
Supplemental Information

HIV-1 Vif Triggers Cell Cycle Arrest
by Degrading Cellular PPP2R5 Phospho-regulators

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Figure S1. Construction and Validation of a Large-Scale Mutant Vif Library. Related to Figure 1. (A) Schematic depicting the method used to generate full-length mutant vif sequences. Red-colored segments represent mutant oligonucleotide DNA segments. (B) Illumina NGS results for the original HIV-1 Vif library (pre-screening). Black boxes indicate non-synonymous mutations with previously characterized interaction surfaces labeled and highlighted in red. Each mutagenic segment is indicated by solid lines shown above the plot, with the numbers below the plot indicating Vif residue numbers. (C) Immunoblots of Vif proteins transiently expressed in 293T cells stably expressing eGFP-PPP2R5A. Actin is shown as a loading control.
Figure S2. Transduction of SupT11 Cells with Vif Variants Defective for PPP2R5-Degradation. Related to Figure 2. (A) Schematic of the proviral construct used to assess Vif-induced G2 arrest following transduction of SupT11 cells. (B) SupT11 cells were transduced at a high MOI HIV-1 with virus that has wild-type IIIB Vif or the indicated single amino acid substitution mutants. DNA content was determined with the left peak indicating G0/G1 and the right peak indicating G2. The G2 peak is higher in point mutant and SLQ-to-AAA due to transduction conditions (i.e. Vif-independent). Histogram profiles are as described in Figure 1A. (C) Immunoblots of transduced SupT11 cells expressing the indicated Vif protein. Actin is shown as a loading control.
Figure S3. Amino Acid and Structural Conservation of the Human PPP2R5 Family. Related to Figure 3. (A) Amino acid alignment of PPP2R5A-E with red asterisks/boxes highlighting negatively charged residues required for Vif degradation and black asterisks/boxes highlighting residues that are dispensable. The numbers above the alignment correspond to PPP2R5A and mutant constructs used in this study. (B) Ribbon schematic comparing the crystal structure of PPP2R5C (cyan; PDB: 2IAE) and the predicted structure of PPP2R5A (grey). Amino acid substitution of the residues colored red disrupt the interaction with HIV-1 Vif.
Figure S4. Vif Residues 31 and 33 are Critical for Inducing PPP2R5A Degradation. Related to Figure 5. (A-C) The top and bottom panels report eGFP fluorescence of 293T cells stably expressing either eGFP-PPP2R5A or APOBEC3G-eGFP and transiently expressing the indicated Vif construct. Histogram profiles are as described in Figure 1A. See main text for details.
Figure S5. Regulation of G2/M Cell Cycle Checkpoint by PPP2R5 Phospho-Regulation. Related to Figures 2 and 4. Each box depicts a PPP2R5-mediated dephosphorylation event required for transitioning through the G2-to-M phase of the cell cycle. Vif-mediated degradation of the indicated PPP2R5 proteins causes a G2 checkpoint arrest (not depicted and see text for additional details).
| Oligonucleotide name | Sequence |
|---------------------|----------|
| IIIB Vif wild-type Forward | CAGGGATTATGGAAAAtcGATGGCAGGTGATGATTG |
| IIIB Vif wild-type Reverse | NNNNGAATTCCTAGTGTGCATTCTATTTGATG |
| IIIB Vif SLQ-AAA Forward | ctaacacagtgaagctgactteggcaactagca |
| IIIB Vif SLQ-AAA Reverse | tgcattgcacagtcgactctcattctgtagttatg |
| IIIB Vif R15S Forward | GTTGCGCAAGTAGACTGATGAGGATTAAACACA |
| IIIB Vif R15S Reverse | TGTGTATACTCCTATGAGTCTACTTGCAC |
| IIIB Vif K22T Forward | AGGATACAACATGGAcAAGATTAGTAAAACAC |
| IIIB Vif K22T Reverse | GTGTTTTAATCTGTAATGCTCATATTTC |
| IIIB Vif I31F Forward | AAAACACATAGTTATTTTCAAGGAAAGCTAAG |
| IIIB Vif I31F Reverse | CTTAGCTTTCCTGAAAATACATACTTGGGTGTTT |
| IIIB Vif R33G Forward | CATATGTAATTCAgGAAAGCTAAG |
| IIIB Vif R33G Reverse | CCAGTCCTTAGCTTTCCcTGAAATATACATATGG |
| IIIB Vif N48T Forward | CACTATGAAAGTACTAcTCCAAAAATAAGTTCA |
| IIIB Vif N48T Reverse | TGAACTTATTTTTGGAgTAGTACTTTCATAGTG |
| IIIB Vif L72R Forward | ACAACATATTGGGTCAcGGAGTCTCCATAGAA |
| IIIB Vif L72R Reverse | TCTTTCCTCATGATCcGCACAAAATATGTTGT |
| IIIB Vif G75T Forward | CATATGAAAGTACTAcTCCAAAAATAAGTTCA |
| IIIB Vif G75T Reverse | GTGCCAGTATAGTgcGAGCCCATATGTTGT |
| IIIB Vif Q83H Forward | GACTGCGATTCCTAGGTGAGGAGTTAATCCTCA |
| IIIB Vif Q83H Reverse | TTCATATGGAGCTGCTAGTACCAGGAAAGCTAAG |
| IIIB Vif Q105R Forward | CCTGACCTAGCAGACCgACTAATTCATCTGCAC |
| IIIB Vif Q105R Reverse | GTGCAGTATAGTgcGAGCCCATATGTTGT |
| IIIB Vif N140Y Forward | TATCAAGCAGGACATTAAGGTTAGATCTCTA |
| IIIB Vif N140Y Reverse | TAGAGATCCTACCTTGTATAGTCTACTTGG |
| IIIB Vif R15A Forward | GTTGCGCAAGTAGACTGATGAGGATTAAACAC |
| IIIB Vif R15A Reverse | TTTAATCTCCTTCATGCTACTCCTTGGG |
| IIIB Vif K22A Forward | agacaggtgagttacatacagggcaagattagtaaaacaccatatgtat |
| IIIB Vif K22A Reverse | atatacatggtttttttaatcttcacctgtctcagctcgtct |
| IIIB Vif R23A Forward | gacaggtgaggttaacatggaagaactagtttaaaacaccatatgg |
| IIIB Vif R23A Reverse | gaaatatatacattgtgtttttttacaatctatttcacttgg |
| IIIB Vif K26A Forward | cagaggtgaggttaacatggaagaagattagtagacaacacctagatataatc |
| IIIB Vif K26A Reverse | tgaatatatacattgtttgtctactaatcttcatcttcacttc |
| IIIB Vif I31A Forward | ctaggaaagttataagacatagtaagtttaataagatcagtacatcagttat |
| IIIB Vif I31A Reverse | gatgtctataacatatgtgtgtetaatcagattacatcatcagttat |
| IIIB Vif R33A Forward | gaaagagttgaanaacaccacatgtattttttcaacagtagctcaagtattttt |
| IIIB Vif R33A Reverse | ataatatacagttacctccttagttttttaataagatcagtacatcagttat |
| IIIB Vif K36A Forward | aaaaacaccatatgtattttttcaacagtagctcaagtacatcagttat |
| IIIB Vif K36A Reverse | gatgtctataacatatgtgtgtetaatcagattacatcatcagttat |
| IIIB Vif N48A Forward | gatggtttttagacatacattatggaagaagtttaaaacaccacatgtatttttt |
| IIIB Vif N48A Reverse | gaaagagttgaanaacaccacatgtattttttcaacagtagctcaagtacatcagttat |
| IIIB Vif K50A Forward | acaaatatacatttcctacctgtgttttttcacagagtccagtacatcagttat |
| IIIB Vif K50A Reverse | tggatatgttttaactccggtagtttttttagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagtttaagagttt
| PPP2R5A Full-length Reverse: | attaagcgtacgttaTTCGGCACTCGTATTGGAC |
|----------------------------|-------------------------------------|
| PPP2R5A E149K Forward:     | GATCCGAGAAGAGCAaAGCCAACCTTTGAAGCG |
| PPP2R5A E149K Reverse:     | CGCTTCAAGGTTGTTCCTTCTTGGAATC      |
| PPP2R5A E153K Forward:     | GACGAGAGAACAACTCTTTGAAGCG        |
| PPP2R5A E153K Reverse:     | ATGGGAGCCTTACCTTTCATGGAATC       |
| PPP2R5A E164K Forward:     | ATCCAGTTGGTTTATAAATTCTTTCTGGTTT |
| PPP2R5A E164K Reverse:     | AAACCGCAGAAAGAATTATAATCTCGGATC   |
| PPP2R5A E171K Forward:     | TTTCTGCGGTTTACTCAAAAGTCCTGACTTA |
| PPP2R5A E171K Reverse:     | ATGGGCTTTGAGAAATTTTCGTTCTCTGATC |
| PPP2R5A E248K Forward:     | TTCAATGGTGTCGCGAAAATTCTTGAGATTCT |
| PPP2R5A E248K Reverse:     | GAGAATCTCAAGCAAATCCGAGACACCATGA |
| PPP2R5A E251K Forward:     | GTCGCGGAGTTGCTTAATAATTCTCGGGTCTA |
| PPP2R5A E251K Reverse:     | AATAGACCCGAGAATTTTCGTTCTCTGATC  |
| PPP2R5A D205K Forward:     | GATCCTAGAGAACGAATTTCTCAAAACGGTT |
| PPP2R5A D205K Reverse:     | AACCGTTTTGAGAAATTTTCGTTCTCTGATC |
| LAI G2-arrest Forward:     | TGGAAAATTGATGGCAGGTGATGATTGTGGGAAGTAGACAGGRTAGAGGATTAGAACATGGAAAAGTTTAGTAAAACACCATATGTAATTTCAaGGAAAGCTAAgGGGATGGTTTTATAGACATCACTATGAAAGCCCTaATCCAAaAATAAGTTCAGAAGTACAC |
| LAI G2-arrest Reverse:     | NNNNGAATTCCTAGTGTCCATTCATTGTATG |
| LAI G2-arrest I31V Forward:| AAACACCATATGTAAGTTTCAaGGAAAGCTAaG |
| LAI G2-arrest I31V Reverse:| CTTAGCTTTCTCGAAacATACATAGGTTTCTT |
| LAI G2-arrest R33G Forward:| CATATGATATTTTCAaGGAAAGCTAaGGGATG |
| LAI G2-arrest R33G Reverse:| CACACCTCTAGCTTTCTCGAAacATACATAG |
| LAI G2-arrest K36R Forward:| aTTTCAaGGAAAGCTAaGGGATGTTTCTTCTAGA |
| LAI G2-arrest K36R Reverse:| TCTATAAACCATCCCTAGCCTTTCTCGAAat |
| LAI G2-arrest N48H Forward:| CACTAGAAAGCCCTaATCCAAaAATAGACCTA |
| LAI G2-arrest N48H Reverse:| TGAACTTATTTGAGATGAGGGCTTTCATAGTG |
| LAI G2-arrest K50R Forward:| GAAAGCCCTaATCCAAaAATAGACCTAAGTAG |
| LAI G2-arrest K50R Reverse:| TACTTCTGAACCTATTCTTTGAGATGTTTCTC |
| LAI G2-arrest I31V/R33G Forward:| AAACACCATATGTAaGGTAAGTTTCAaGGAAAGCTAaGGGATG |
| LAI G2-arrest I31V/R33G Reverse:| CACACCTCTAGCTTTCTCGAAacATACATAGGTTTCTTCTAGA |
| LAI G2-arrest I31V/K36R Forward:| gTTTCAaGGAAAGCTAaGGGATGTTTCTTCTAGA |
| LAI G2-arrest I31V/K36R Reverse:| TCTATAAACCATCCCTAGCCTTTCTCGAAat |
| LAI G2-arrest I31V/G33R Forward:| AAACACCATATGTAaGGTAAGTTTCAaGGAAAGCTAaGGGATG |
| LAI V31I Forward:          | AAACACCATATGTAaGGTAAGTTTCAaGGAAAGCTAaGGGATG |
| LAI V31I Reverse:          | CACTACCTCCTCGAAatATACATAGGTTTCTTCTAGA |
| LAI G33R Forward:          | CATATGATTTTTCAaGGAAAGCTAaGGGATG |
| LAI G33R Reverse:          | CACACCTCTAGCCTTTCTCGAAacATACATAG |
| LAI V31I/G33R Forward:     | AAACACCATATGTAaGGTAAGTTTCAaGGAAAGCTAaGGGATG |
| LAI V31I/G33R Reverse:     | CACACCTCTAGCCTTTCTCGAAacATACATAGGTTTCTTCTAGA |
| LAI V31I/G33K Forward:     | AAACACCATATGTAaGGTAAGTTTCAaGGAAAGCTAaGGGATG |
| LAI V31I/G33K Reverse:     | CACACCTCTAGCCTTTCTCGAAacATACATAGGTTTCTTCTAGA |
| Gene   | qPCR Forward       | qPCR Reverse       |
|--------|--------------------|--------------------|
| PPP2R5A | tgccaattatgttgccagt | cattagggttttcagcacttg |
| PPP2R5B | cgcaaacagtgcaaccac | acaccattgaagtgctcgaa |
| PPP2R5C | cggttgtccttctccatatic | gaggacgcaacactgacgta |
| PPP2R5D | cttggccaaacttggaga | ccaccctcagctggcata |
| PPP2R5E | tggatctttccccagaagc | ttccgagaaaatccgtctactt |

Table S1. Oligonucleotide sequences used in this study. Related to Key Resources Table.
| Recombinant DNA                                                                 | Source                      |
|--------------------------------------------------------------------------------|-----------------------------|
| pQCXIH retroviral vector                                                          | Clontech                    |
| Lentiviral expression vector                                                      | (St Martin et al., 2018)   |
| HIV-1 IIIB modified proviral plasmid                                              | (Salamango et al., 2018a)  |
| Lentiviral expression vector, wild-type Vif<sub>IIIB</sub>                         | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> SLQ/AAA                         | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> R15S                            | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> K22T                            | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> I31F                            | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> R33G                            | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> R33K                            | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> N48T                            | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> L72R                            | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> G75T                            | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> Q83H                            | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> Q105R                           | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> N140Y                           | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> R15A                            | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> K22A                            | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> R23A                            | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> K26A                            | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> I31A                            | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> R33A                            | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> K36A                            | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> N48A                            | This paper                  |
| Lentiviral expression vector, Vif<sub>IIIB</sub> K50A                            | This paper                  |
| Lentiviral expression vector, wild-type Vif<sub>LAI</sub>                       | This paper                  |
| Lentiviral expression vector, Vif<sub>LAI</sub> SLQ/AAA                         | This paper                  |
| Lentiviral expression vector, Vif<sub>LAI</sub> G2-Arrest                        | This paper                  |
| Lentiviral expression vector, Vif<sub>LAI</sub> G2-Arrest I31V                   | This paper                  |
| Lentiviral expression vector, Vif<sub>LAI</sub> G2-Arrest R33G                   | This paper                  |
| Lentiviral expression vector, Vif<sub>LAI</sub> G2-Arrest K36R                   | This paper                  |
| Lentiviral expression vector, Vif<sub>LAI</sub> G2-Arrest N48H                    | This paper                  |
| Lentiviral expression vector, Vif<sub>LAI</sub> G2-Arrest K50R                    | This paper                  |
| Lentiviral expression vector, Vif<sub>LAI</sub> G2-Arrest I31V/R33G               | This paper                  |
| Lentiviral expression vector, Vif<sub>LAI</sub> G2-Arrest I31V/K36R              | This paper                  |
| Lentiviral expression vector, Vif<sub>LAI</sub> G2-Arrest I31V/N48H               | This paper                  |
| Lentiviral expression vector, Vif<sub>LAI</sub> G2-Arrest I31V/K50R               | This paper                  |
| Lentiviral expression vector, Vif<sub>LAI</sub> V31I                             | This paper                  |
| Lentiviral expression vector, Vif<sub>LAI</sub> G33R                             | This paper                  |
| Lentiviral expression vector, Vif<sub>LAI</sub> V31I/G33R                         | This paper                  |
| Lentiviral expression vector, Vif<sub>LAI</sub> V31I/G33K                         | This paper                  |
| Lentiviral expression vector, wild-type Vif<sub>A1</sub>                        | This paper                  |
| Lentiviral expression vector, wild-type Vif<sub>C</sub>                         | This paper                  |
| Lentiviral expression vector, wild-type Vif<sub>D</sub>                         | This paper                  |
| Lentiviral expression vector, wild-type Vif<sub>F</sub>                         | This paper                  |
| Recombinant DNA vectors                                      | Source                  |
|-------------------------------------------------------------|-------------------------|
| Lentiviral expression vector, wild-type VifN1                | This paper              |
| Lentiviral expression vector, wild-type VifN2                | This paper              |
| Lentiviral expression vector, wild-type Vifo1                | This paper              |
| Lentiviral expression vector, wild-type Vifo2                | This paper              |
| pQCXIH eGFP-PPP2R5A vector                                   | This paper              |
| pQCXIH eGFP-PPP2R5B vector                                   | This paper              |
| pQCXIH eGFP-PPP2R5C vector                                   | This paper              |
| pQCXIH eGFP-PPP2R5D vector                                   | This paper              |
| pQCXIH eGFP-PPP2R5E vector                                   | This paper              |
| pQCXIH eGFP-PPP2R5A E149K vector                             | This paper              |
| pQCXIH eGFP-PPP2R5A E153K vector                             | This paper              |
| pQCXIH eGFP-PPP2R5A E164K vector                             | This paper              |
| pQCXIH eGFP-PPP2R5A E171K vector                             | This paper              |
| pQCXIH eGFP-PPP2R5A D205K vector                             | This paper              |
| pQCXIH eGFP-PPP2R5A E248K vector                             | This paper              |
| pQCXIH eGFP-PPP2R5A E251K vector                             | This paper              |
| pQCXIH eGFP-PPP2R5A E301K vector                             | This paper              |
| pQCXIH eGFP-PPP2R5A E335K vector                             | This paper              |
| pQCXIH eGFP-PPP2R5A E338K vector                             | This paper              |
| pQCXIH eGFP-PPP2R5A E338K vector                              | This paper              |
| pQCXIH eGFP-PPP2R5A E338K vector                              | This paper              |
| pLKO.1 no insert                                             | UMN genomics core       |
| pLKO.1 shPPP2R5A                                              | UMN genomics core       |
| pLKO.1 shPPP2R5C                                              | UMN genomics core       |
| pLKO.1 shPPP2R5D                                              | UMN genomics core       |
| pLKO.1 shPPP2R5E                                              | UMN genomics core       |
| HIV-1 IIIB modified proviral plasmid, wild-type Vif          | This paper              |
| HIV-1 IIIB modified proviral plasmid, Vif SLQ/AAA            | This paper              |
| HIV-1 IIIB modified proviral plasmid, Vif K22A               | This paper              |
| HIV-1 IIIB modified proviral plasmid, Vif K26A               | This paper              |
| HIV-1 IIIB modified proviral plasmid, Vif R33A               | This paper              |
| HIV-1 IIIB modified proviral plasmid, Vif K36A               | This paper              |

Table S2. Recombinant DNA vectors used in this study. Related to Key Resources Table.