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Pathway Analysis in HEK 293T Cells Overexpressing HIV-1 Tat and Nucleocapsid

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The human immunodeficiency virus (HIV)-1 protein Tat acts as a transcription transactivator that stimulates expression of the infected viral genome. It is released from infected cells and can similarly affect neighboring cells. The nucleocapsid is an important protein that has a related significant role in early mRNA expression, and which contributes to the rapid viral replication that occurs during HIV-1 infection. To investigate the interaction between the Tat and nucleocapsid proteins, we utilized cDNA microarrays using pTat and flag NC cotransfection in HEK 293T cells and reverse transcription–polymerase chain reaction to validate the microarray data. Four upregulated genes and nine downregulated genes were selected as candidate genes. Gene ontology analysis was conducted to define the biological process of the input genes. A proteomic approach using PathwayStudio determined the relationship between Tat and nucleocapsid; two automatically built pathways represented the interactions between the upregulated and downregulated genes. The results indicate that the up- and downregulated genes regulate HIV-1 replication and proliferation, and viral entry.

Keywords: HIV, microarray, nucleocapsid, pathway analysis, Tat

Acquired immunodeficiency syndrome (AIDS) is a human immune system disease caused by human immunodeficiency virus type 1 (HIV-1), which attacks and destroys white blood cells that are essential to the immune system. Investigation of the replication mechanism of HIV at the gene and protein levels is very important to the development of treatments and, ultimately, cures for HIV infection. The cell cycle of HIV-1 continues through gene regulation of the host protein. However, the gene regulation mechanism of HIV-1 has not yet been completely elucidated.

The HIV-1 genome is encoded by a long strand of RNA. When HIV-1 RNA integrates into the host DNA genome, it synthesizes DNA using reverse transcriptase. The HIV-1 genome consists of several genes including *tat*, *gag*, *pol*, *env*, *rev*, *vif*, *vpr*, and *vpu*. It also consists of long-term repeats (LTR) at each end that are composed of three regions: U3 (3' end), R (repeated), and U5 (5' end). The integrase protein uses the LTR sticky end to insert into host DNA, while the acts HIV genome being transcripted as a primer or regulator [15]. HIV-1 LTR serves as the site of transcriptional initiation and harbors a *cis*-acting element that is required for RNA synthesis [15, 16].

The transactivator (Tat) of HIV-1 is a small cationic polypeptide that is a powerful activator of viral gene expression, and which also regulates cellular gene expression [3, 23, 25]. When Tat is present, RNA synthesis is greatly increased [11, 14]. Tat also increases the activity of heterologous viral promoters through cytokine expression and downstream signaling activation induced by HIV-1 [31]. The Tat protein is localized in the nucleus, but can be released from infected cells. Released Tat proteins are endocytosed by neighboring uninfected cells, and soon translocate to the nucleus in an active form [13, 18]. Tat protein accumulates in the nuclei of HIV-infected cells, but can also act as a pleiotropic exogenous factor because of its ability to induce various biological effects and varying degrees of cell maturation in different cell types [7, 23].

The nucleocapsid (NC) is a structural protein that acts as packaging for viral genomic RNA. The HIV-1 RNA is tightly bound to NC. NC is encoded by the 3' region of *gag* and is well conserved among retroviruses. Both NCp15 and NCp7 are highly basic proteins that contain two zinc fingers in the form of Cys-X2-cyc-X4-His-X4-Cys (CCHC) flanked by regions rich in basic residues [29].

After the Human Genome Project completion, researchers paid more attention to mapping the networks of biomolecular

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interaction than categorization of genes and proteins. Because proteins or genes do not function alone but rather interact with DNA, RNA, or small molecules to constitute molecular machines, understanding this network is important [19]. However, too much time and exertions are required for experimental validation of the many possible candidates in a wet-lab environment. Therefore, comparative genomic tools that can predict biological molecular networks have to help scientists to pathway predictions [8]. One of the methods to predict molecular interaction is text mining, the use of automated methods for exploiting the enormous amount of knowledge available in the biomedical literature [10]. PathwayStudio (Ariadne Genomics, Rockville, MD, U.S.A.) is a text mining-based pathway analysis tool that operates with MedScan in the automatic curation of Biological Association Networks (BANs). The software was derived by a natural language processing technology that contains sufficient information for the construction of thousands of mammalian signaling pathways for multiple tissues [32].

Presently, we monitored Tat and nucleocapsid gene expression by taking advantage of a microarray technology and using PathwayStudio 5.0 to clarify the interrelationships and contributory cellular factors between the Tat and nucleocapsid proteins. Our aim was to clarify the main controlling genetic factor(s) in the early life cycle of HIV-1.

Materials and Methods

Microarray Analysis

The microarray was hybridized using total RNA from pCMV(-HA) flag NC and pTat co-transfected HEK 293T cells. Slides were dried by centrifugation at low speed prior to scanning. Images were obtained by scanning the arrays in an arrayWoRx scanner (Applied Precision, Seattle, WA, U.S.A.). Signal intensities for Cy3- and Cy5-labeled probes were extracted with the ImaGene software package, version 5.0 (BioDiscovery, Marina Del Rey, CA, U.S.A.) using default settings and auto image segmentation. Mean and median intensities for signal and background as well as quality characteristics ("empty" or "poor") of the spots were obtained at this time. The threshold for empty spots was achieved by raising the threshold to a point at which all blank spots were flagged. Semiquantitative reverse transcription–polymerase chain reaction (RT–PCR) was used to validate the data obtained by microarray analysis. Five significant clusters were selected for gene ontology (GO) analysis using the Web-based tool DAVID Bioinformatics Resources 2008 (National Institute of Allergy and Infectious Disease, NIH, Bethesda, MD, U.S.A.).

Pathway Analysis

Pathway analysis utilized PathwayStudio version 5.0 and ResNet 5.0 (both from Adriadne Genomics). PathwayStudio builds and provides a graphic display of a pathway for a queried protein, and finds interacting molecules for the target protein or other biological entity. The ResNet database of PathwayStudio contains information concerning relationships (direct physical interaction and indirect regulation) between proteins, small molecules, protein functional classes, cell processes, and disease. The direct physical interactions include binding, protein modification, and promoter binding. The indirect regulation events include regulation, expression regulation, molecular transport regulation, and molecular synthesis regulation. Relationships in ResNet are generated from multiple literature sources including the entire PubMed database containing 13,000,000 scientific abstracts and 43 publicly available full-test journals [32]. From ResNet, we were able to add our interesting gene product onto a new pathway diagram and build a new pathway based on well-known interactions referred to in the relevant literature.

Pathway Reconstruction Algorithms

The algorithm in PathwayStudio constructs a pathway based on the interaction between proteins. Initially, it starts with a ligand–receptor pair and finds all proteins related to the receptor and ligand in the ResNet database. It also connects all found downstream proteins using physical interactions such as binding or protein modification, and removes unconnected targets. In addition, a second algorithm assigns weights to all connections in ResNet by scoring the reference count and the number of similar relationships for paralogous proteins. It then determines the optimal path in the weighted graph between the receptor and the downstream transcription factor using physical interactions in ResNet. This algorithm considers only transcription factors that are regulated by the receptor in the ResNet database [32].

Results and Discussion

Microarray Analysis

HEK 293T cells were used for pTat and flag NC gene cotransfection. We performed microarray analysis, which contained normalization, hierarchical clustering, and GO analysis. To validate the data obtained by microarray analysis, differentially expressed transcripts representing up- or downregulated genes were analyzed by RT–PCR. We selected four upregulated genes and nine downregulated

Table 1. Upregulated genes in flag NC and pTat cotransfection of HEK 293T cells.

Accession no.	Description	HIV-1 protein interaction	Fold change
NM_003029	**SHC1** SHC (Src homology 2 domain-containing) transforming protein 1	Tat	25.803
NM_002253	**KDR** kinase insert domain receptor (a type III receptor tyrosine kinase)	Tat	31.479
NM_000575	**IL-1A** interleukin 1α	Tat	17.021
NM_006200	**PCSK5** proprotein convertase subtilisin/kexin type 5		16.778

Table 2. Downregulated genes in flag NC and pTat cotransfected HEK 293T cells.

Accession no.	Description	HIV-1 protein interaction	Fold change
NM_003998	**NFKB1** nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)	Tat, Vpr, Vpu, NC	0.237
NM_006534	**NCOA3** nuclear receptor coactivator 3	Tat	0.203
NM_030662	**MAP2K2** mitogen-activated protein kinase kinase 2	Tat	0.153
NM_003478	**CUL5** cullin 5	Vif	0.233
NM_004399	**DDX11** DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 (CHL1-like helicase homolog, *S. cerevisiae*)		0.53
NM_001448	**GPC4** glypican 4		0.796
NM_002083	**GPX2** glutathione peroxidase 2 (gastrointestinal)	Tat	0.706
NM_139207	**NAP1L1** nucleosome assembly protein 1-like 1		0.777
NM_005381	**NCL** nucleolin	NC	0.779

genes in flag NC and pTat cotransfected HEK 293T cells. SHC1, KDR, and IL-1A that are three of upregulated genes are already known to interact with HIV Tat protein (Table 1). Downregulated genes NFKB1, NCOA3, MAP2K2, and GPX2 also interacted with some HIV-1 genes. NCL interacted with *gag*, and CUL5 interacted with *vif* (Table 2). GO analysis was carried out to define the biological processes of the gained genes ($p<0.05$). Upregulated genes were related to proliferation, blood vessel development, and anatomical structure formation (Table 3). Downregulated genes were also analyzed, but the results were not significant ($p>0.08$).

Building and Visualizing Pathways with PathwayStudio

PathwayStudio was used to build several pathways from the gene list given by the user and has found relationships such as binding, expression, regulation, and molecular synthesize among molecules/cell objects/processes. We described the methodology for automatic curation of BANs derived by a natural language processing technology [32]. The curated data were used for automatic pathway reconstruction. Based on the expression profiles of flag NC and pTat cotransfection in HEK293T cells, we imported our cotransfection upregulated genes, downregulated genes, and HIV-1 proteins into PathwayStudio. Therefore, we obtained each possible pathway. Fig. 1 displays the resultant pathways. Each line between the genes or proteins means connected molecules have at least one reference about their relationship. Because of this, we were able to find their relationship rapidly.

Fig. 1A shows upregulated genes in flag NC and pTat cotransfected HEK 293T cells, and HIV-1 protein. SHC (Src homology 2 domain-containing) transforming protein 1 (SHC1), kinase insert domain receptor (a type III receptor tyrosine kinase) (KDR), and interleukin-1 alpha (IL-1A) are known to be Tat interactive genes [1, 5, 12, 17]. KDR is also known to be upregulated in HIV-1 Tat-treated human epithelial cells [4]. The interaction of HIV-1 Tat with KDR/VEGFR2 is implicated in the activation of the mitogen activated protein kinase (MAPK) signaling pathways [17]. Activation of the MAPK signaling pathway leads to the phosphorylation of Shc, a protein involved in signal transduction downstream of receptor tyrosine kinase activation [20]. KDR is also involved in SHC1 phosphorylation [33]. Appropriately, we propose that the upregulation of SHC1 phosphorylation regulates mitosis and proliferation of infected cells through the MAPK signaling pathway. IL-1A is a prototypical proinflammatory cytokine. Fig. 1A

Table 3. Gene anatology (GO) analysis of upregulated genes.

GO ID	Term	*p*-value
KDR, SHC1, IL-1A		
GO:0008283	Cell proliferation	0.004
GO:0065008	Regulation of biological quality	0.007
KDR, SHC1		
GO:0001525	Angiogenesis	0.030
GO:0048514	Blood vessel morphogenesis	0.038
GO:0008284	Positive regulation of cell proliferation	0.039
GO:0048646	Anatomical structure formation	0.041
GO:0001568	Blood vessel development	0.044
GO:0001944	Vasculature development	0.045
PCSK5, KDR		
GO:0007169	Transmembrane receptor protein tyrosine kinase signaling pathway	0.037

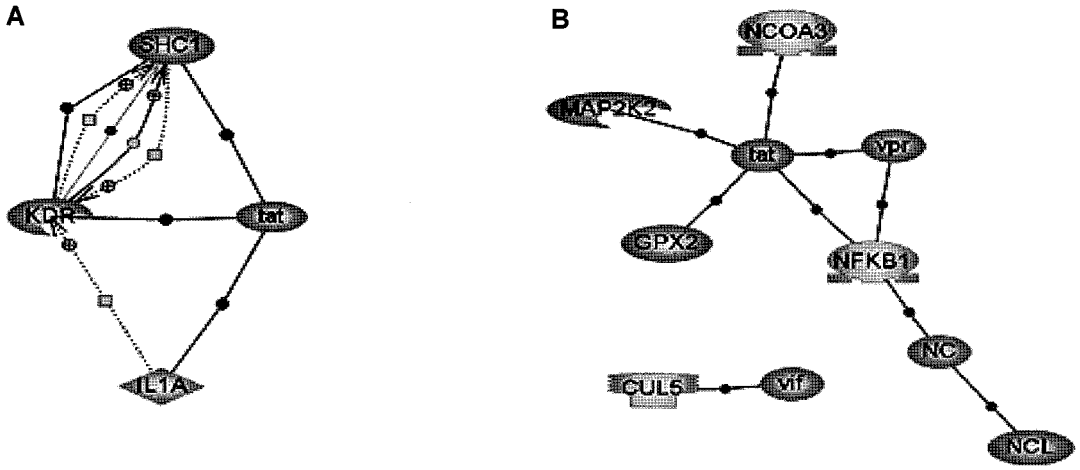

Fig. 1. Manually built cotransfection pathway of genes from flag NC and pTat cotransfection genes.
A. Manually built pathway of upregulated genes in flag NC and pTat cotransfected HEK 293T cells and HIV-1 protein. IL-1A is shown as a red rhombus (ligand), SHC1 and Tat are denoted by red ovals, KDR is denoted as a red sickle-vertex (kinase), binding is represented by violet links, ProtModification is represented by brown links, DirectRegulation is depicted by gray links with gray circles, and Regulation is depicted by gray links with gray rectangles. **B**. Downregulated genes and HIV-1 protein pathway. NFKB1 and NCOA3 are shown as a red O-vertex (transcription factor); CUL5 is depicted as a red stick-vertex (receptor); GPX2, NCL, Tat, NC, Vif, and Vpr are represented as red ovals; MAP2K2 is represented as a red sickle-vertex (kinase); binding is depicted by violet links.

also indicates IL-1A to be a positive regulator of KDR. However this regulatory mechanism is related to angiogenesis, not germane to HIV-1. We used the "find similar pathways for selected" tool to search for already curated signaling pathways that contained the selected (input) genes. We obtained 130 pathways similar to an upregulated pathway (Fig. 1A) and 217 pathways similar to the downregulated gene pathway (Fig. 1B) from this search function. We selected those pathways based on a significant association ($p<0.01$) and that had more than two overlapped molecules. In this way, we found seven pathways similar to upregulated gene pathways. Pathways involved in the downregulation of genes were not evident. Table 4 summarizes the seven signaling pathways that were similar to upregulated pathways in flag NC and pTat cotransfected HEK 293T cells. Six of the seven pathways were KDR signaling pathways that contained SHC1, and one contained IL-1A and SHC1 (IL-1 and IL-6 signaling) whose interaction was indirect. Although those signaling pathways displayed different upstream pathways, they had the same downstream pathway

Table 4. Signaling pathways similar to upregulated pathways in flag NC and pTat cotransfected HEK 293T cells.

Name	Putative signaling pathway	*p*-value
IL-1 and IL-6	IL-1 and IL-6 signaling	9.6216e-005
KDR	NFATC1 signaling pathway	0.000213968
KDR	ATF2 signaling pathway	0.000500144
KDR	ELK4-SRF signaling pathway	0.00051212
KDR	ELK1-SRF signaling pathway	0.000524236
KDR	ATF1 signaling pathway	0.000561423
KDR	CREB1 signaling pathway	0.000574097

related with RAS that leads to proliferation or transcription. In this result, we suggest that upregulated genes were involved with proliferation, mitosis, and activation of transcription through the MAPK and RAS pathways in HIV-1-infected cells.

Fig. 1B summarizes the pathways implicated in the downregulated genes and HIV-1. Tat deregulates glutathione peroxidase 2 (gastrointestinal) (GPX2), a member of the glutathione peroxidase family that has glutathione-dependent hydrogen peroxide-reducing activity in the epithelium of the gastrointestinal tract [9, 22, 24]. Glutathione peroxidase GPX is used as a marker of oxidative damage and in antiretroviral therapy [27, 28]. Downregulated antioxidants such as GPX2 lead to oxidative stress, which may contribute to the pathogenesis of HIV infections in humans [30]. HIV pathogenesis involves depletion of oxidized glutathione (GSH) content, which leads to AIDS-associated Kaposi's sarcoma (the most common tumor in HIV-infected patients) [6, 9]. Therefore, we suggest that GPX2 is involved in oxidative-stress-related HIV-1 pathogenesis, and also related to AIDS-associated Kaposi's sarcoma. Vif is an HIV accessory protein in virus replication, suggesting that the gene encoding this protein (*vif*) counteracts the defect of a cellular factor that inhibits the formation of infectious virions [15, 26]. After HIV entry into cells, nucleolin (NCL) can be recovered also in fractions containing HIV DNA, viral matrix, and reverse transcriptase, consistent with the suggestion that NCL accompanies viral entry. Surface NCL is markedly downregulated within hours following HIV entry into permissive cells. This effect appears to be the consequence of its translocation into the cytoplasm [2, 21]. We suggest that NCL localization is

important to the HIV entry process into permissive cells in the early stage. Mitogen-activated protein kinase kinase 2 (MAP2K2), nuclear receptor coactivator 3 (NCOA3), cullin 5 (CUL5), and nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105) (NFKB1) also downregulated genes interacting with Tat, *vpr*, or NC. However, these regulatory relations were indicated as "activated by" or "enhanced by." The genes are more typically associated with the positive regulation of the initiation of viral replication and development, contrary to our findings.

In conclusion, we used a proteomic approach relating PathwayStudio to determine the interrelationships and cellular factors between the Tat and nucleocapsid proteins. Our results indicate that the up- and downregulated genes are important in the regulation of HIV-1 replication, proliferation, and viral entry.

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REFERENCES

1. Albini, A., R. Soldi, D. Giunciuglio, E. Giraudo, R. Benelli, L. Primo, *et al.* 1996. The angiogenesis induced by HIV-1 tat protein is mediated by the Flk-1/KDR receptor on vascular endothelial cells. *Nat. Med.* **2:** 1371–1375.
2. Bacharach, E., J. Gonsky, K. Alin, M. Orlova, and S. P. Goff. 2000. The carboxy-terminal fragment of nucleolin interacts with the nucleocapsid domain of retroviral Gag proteins and inhibits virion assembly. *J. Virol.* **74:** 11027–11039.
3. Belka, C., C. Gruber, V. Jendrossek, S. Wesselborg, and W. Budach. 2003. The tyrosine kinase Lck is involved in regulation of mitochondrial apoptosis pathways. *Oncogene* **22:** 176–185.
4. Bettaccini, A. A., A. Baj, R. S. Accolla, F. Basolo, and A. Q. Toniolo. 2005. Proliferative activity of extracellular HIV-1 Tat protein in human epithelial cells: Expression profile of pathogenetically relevant genes. *BMC Microbiol.* **5:** 20.
5. Biswas, D. K., T. R. Salas, F. Wang, C. M. Ahlers, B. J. Dezube, and A. B. Pardee. 1995. A Tat-induced auto-up-regulatory loop for superactivation of the human immunodeficiency virus type 1 promoter. *J. Virol.* **69:** 7437–7444.
6. Boshoff, C. and R. Weiss. 2002. AIDS-related malignancies. *Nat. Rev. Cancer* **2:** 373–382.
7. Brigati, C., M. Giacca, D. M. Noonan, and A. Albini. 2003. HIV Tat, its TARgets and the control of viral gene expression. *FEMS Microbiol. Lett.* **220:** 57–65.
8. Cakmak, A. and G. Ozsoyoglu. 2007. Mining biological networks for unknown pathways. *Bioinformatics* **23:** 2775–2783.
9. Choi, J., R. M. Liu, R. K. Kundu, F. Sangiorgi, W. Wu, R. Maxson, and H. J. Forman. 2000. Molecular mechanism of decreased glutathione content in human immunodeficiency virus type 1 Tat-transgenic mice. *J. Biol. Chem.* **275:** 3693–3698.
10. Cohen, K. B. and L. Hunter. 2008. Getting started in text mining. *PLoS Comput. Biol.* **4:** e20.
11. Dayton, A. I., J. G. Sodroski, C. A. Rosen, W. C. Goh, and W. A. Haseltine. 1986. The trans-activator gene of the human T cell lymphotropic virus type III is required for replication. *Cell* **44:** 941–947.
12. Deregibus, M. C., V. Cantaluppi, S. Doublier, M. F. Brizzi, I. Deambrosis, A. Albini, and G. Camussi. 2002. HIV-1-Tat protein activates phosphatidylinositol 3-kinase/AKT-dependent survival pathways in Kaposi's sarcoma cells. *J. Biol. Chem.* **277:** 25195–25202.
13. Ensoli, B., L. Buonaguro, G. Barillari, V. Fiorelli, R. Gendelman, R. A. Morgan, P. Wingfield, and R. C. Gallo. 1993. Release, uptake, and effects of extracellular human immunodeficiency virus type 1 Tat protein on cell growth and viral transactivation. *J. Virol.* **67:** 277–287.
14. Fisher, A. G., M. B. Feinberg, S. F. Josephs, M. E. Harper, L. M. Marselle, G. Reyes, *et al.* 1986. The trans-activator gene of HTLV-III is essential for virus replication. *Nature* **320:** 367–371.
15. Freed, E. O. 2001. HIV-1 replication. *Somat. Cell Mol. Genet.* **26:** 13–33.
16. Freed, E. O. and M. A. Martin. 2007. HIVs and their replication. pp. 2107, *In* D. M. Knipe and P. M. Howley (eds.). *Fields Virology.* Lippincott Williams & Wilkins, Philadelphia.
17. Ganju, R. K., N. Munshi, B. C. Nair, Z. Y. Liu, P. Gill, and J. E. Groopman. 1998. Human immunodeficiency virus Tat modulates the Flk-1/KDR receptor, mitogen-activated protein kinases, and components of focal adhesion in Kaposi's sarcoma cells. *J. Virol.* **72:** 6131–6137.
18. Gupta, S. and D. Mitra. 2007. Human immunodeficiency virus-1 Tat protein: Immunological facets of a transcriptional activator. *Indian J. Biochem. Biophys.* **44:** 269–275.
19. Ideker, T. and R. Sharan. 2008. Protein networks in disease. *Genome Res.* **18:** 644–652.
20. Menegon, A., C. Leoni, F. Benfenati, and F. Valtorta. 1997. Tat protein from HIV-1 activates MAP kinase in granular neurons and glial cells from rat cerebellum. *Biochem. Biophys. Res. Commun.* **238:** 800–805.
21. Nisole, S., B. Krust, and A. G. Hovanessian. 2002. Anchorage of HIV on permissive cells leads to coaggregation of viral particles with surface nucleolin at membrane raft microdomains. *Exp. Cell Res.* **276:** 155–173.
22. Opalenik, S. R., Q. Ding, S. R. Mallery, and J. A. Thompson. 1998. Glutathione depletion associated with the HIV-1 TAT protein mediates the extracellular appearance of acidic fibroblast growth factor. *Arch. Biochem. Biophys.* **351:** 17–26.
23. Peruzzi, F. 2006. The multiple functions of HIV-1 Tat: Proliferation versus apoptosis. *Front. Biosci.* **11:** 708–717.
24. Richard, M. J., P. Guiraud, C. Didier, M. Seve, S. C. Flores, and A. Favier. 2001. Human immunodeficiency virus type 1 Tat protein impairs selenoglutathione peroxidase expression and activity by a mechanism independent of cellular selenium uptake: Consequences on cellular resistance to UV-A radiation. *Arch. Biochem. Biophys.* **386:** 213–220.
25. Rusnati, M. and M. Presta. 2002. HIV-1 Tat protein and endothelium: From protein/cell interaction to AIDS-associated pathologies. *Angiogenesis* **5:** 141–151.
26. Simon, J. H., N. C. Gaddis, R. A. Fouchier, and M. H. Malim. 1998. Evidence for a newly discovered cellular anti-HIV-1 phenotype. *Nat. Med.* **4:** 1397–1400.

27. Stephensen, C. B., G. S. Marquis, S. D. Douglas, L. A. Kruzich, and C. M. Wilson. 2007. Glutathione, glutathione peroxidase, and selenium status in HIV-positive and HIV-negative adolescents and young adults. *Am. J. Clin. Nutr.* **85:** 173–181.
28. Treitinger, A., C. Spada, J. C. Verdi, A. F. Miranda, O. V. Oliveira, M. V. Silveira, P. Moriel, and D. S. Abdalla. 2000. Decreased antioxidant defence in individuals infected by the human immunodeficiency virus. *Eur. J. Clin. Invest.* **30:** 454–459.
29. Wain-Hobson, S., P. Sonigo, O. Danos, S. Cole, and M. Alizon. 1985. Nucleotide sequence of the AIDS virus, LAV. *Cell* **40:** 9–17.
30. Webb, C., T. Lehman, K. McCord, P. Avery, and S. Dow. 2008. Oxidative stress during acute FIV infection in cats. *Vet. Immunol. Immunopathol.* **122:** 16–24.
31. White, M. K., T. S. Gorrill, and K. Khalili. 2006. Reciprocal transactivation between HIV-1 and other human viruses. *Virology* **352:** 1–13.
32. Yuryev, A., Z. Mulyukov, E. Kotelnikova, S. Maslov, S. Egorov, A. Nikitin, N. Daraselia, and I. Mazo. 2006. Automatic pathway building in biological association networks. *BMC Bioinformatics* **7:** 171.
33. Zanetti, A., M. G. Lampugnani, G. Balconi, F. Breviario, M. Corada, L. Lanfrancone, and E. Dejana. 2002. Vascular endothelial growth factor induces SHC association with vascular endothelial cadherin: A potential feedback mechanism to control vascular endothelial growth factor receptor-2 signaling. *Arterioscler. Thromb. Vasc. Biol.* **22:** 617–622.