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HIV-1 Nef promotes infection by excluding SERINC5 from virion incorporation

Annachiara Rosa, Ajit Chande, Serena Ziglio, Veronica De Sanctis, Roberto Bertorelli, Shih Lin Goh, Sean M. McCauley, Anetta Nowosielska, Stylianos E. Antonarakis, Jeremy Luban, Federico Andrea Santoni & Massimo Pizzato

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Abstract

HIV-1 Nef, a protein important for the development of AIDS, has well-characterized effects on host membrane trafficking and receptor downregulation. By an unidentified mechanism, Nef increases the intrinsic infectivity of HIV-1 virions in a host-cell-dependent manner. Here we identify the host transmembrane protein SERINC5, and to a lesser extent SERINC3, as a potent inhibitor of HIV-1 particle infectivity that is counteracted by Nef. SERINC5 localizes to the plasma membrane, where it is efficiently incorporated into budding HIV-1 virions and impairs subsequent virion penetration of susceptible target cells. Nef redirects SERINC5 to a Rab7-positive endosomal compartment and thereby excludes it from HIV-1 particles. The ability to counteract SERINC5 was conserved in Nef encoded by diverse primate immunodeficiency viruses, as well as in the structurally unrelated glycosylated Gag from murine leukaemia virus. These examples of functional conservation and convergent evolution emphasize the fundamental importance of SERINC5 as a potent anti-retroviral factor.

Subject terms: Restriction factors Virus-host interactions

Accession codes

Primary accessions

Sequence Read Archive

SRP062444

Referenced accessions

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Author information

These authors contributed equally to this work.
Annachiara Rosa, Ajit Chande & Serena Ziglio

Affiliations

University of Trento, Centre for Integrative Biology, 38123 Trento, Italy
Annachiara Rosa, Ajit Chande, Serena Ziglio & Massimo Pizzato

University of Trento, Laboratory of Biomolecular Sequence and Structure Analysis for Health, NGS facility, 38123 Trento, Italy
Veronica De Sanctis & Roberto Bertorelli

University of Massachusetts Medical School, Program in Molecular Medicine, Worcester, Massachusetts 01605, USA
Shih Lin Goh, Sean M. McCauley, Anetta Nowosielska & Jeremy Luban

University of Geneva, Department of Genetic Medicine and Development, Geneva 1211, Switzerland
Stylianos E. Antonarakis & Federico Andrea Santoni

iGE3 Institute of Genetics and Genomics of Geneva, Geneva 1211, Switzerland
Stylianos E. Antonarakis

Contributions

A.R., A.C., S.Z., V.D.S., R.B., S.E.A., J.L., F.A.S. and M.P. designed the experiments. A.R., S.Z., A.C., V.D.S., R.B., S.L.G.,

S.M.M., A.N., F.A.S. and M.P. performed the experiments. All authors contributed to the assembly and writing of the manuscript. A.R., A.C. and S.Z. contributed equally to the study.

Competing financial interests

The authors declare no competing financial interests.

Corresponding author

Correspondence to: Massimo Pizzato

RNA-seq fatsq data have been deposited in NCBI Sequence Read Archive (SRA) under accession code SRP062444.

Extended data figures and tables

Extended Data Figures

1. Extended Data Figure 1: SERINC5 is an inhibitor of HIV-1 infectivity. (333 KB)
   a, Mapping of the INDELS in the genomic locus spanning SERINC5 exon 2 in JTAg cell clonal populations from Fig. 2a. **b**, Infectivity of HIV-1 from JTAg cells stably transduced with lentiCRISPR targeting GFP or SERINC5 in three different exons (n = 4, experiment replicated twice). **c**, Relative expression of *SERINC5* in primary cells and in cell lines measured by qPCR normalized by expression of *ACTB* (n = 3). **d**, Infectivity of HIV-1 from the indicated cell lines expressing SERINC5 (n = 4, experiments were replicated twice). Mean ± s.d., unpaired two-tailed t-test, ***P < 0.001 **e**, Expression levels of the five *SERINC* genes in JTAg cells obtained from RNA-seq.

2. Extended Data Figure 2: Nef and glycoGag expression result in relocalization of SERINC5 to an endosomal compartment and prevent its incorporation into virions. (289 KB)
   a, Single round Nef-defective NL4-3 produced by cotransfection of HEK293T cells with plasmids expressing Nef proteins or the empty vector control, and PBJ6-SERINC5–HA: immunoblotting of virions and cell lysates from producer cells. **b**, Immunofluorescence staining of JTAg cells transfected to express SERINC5–GFP, Nef–HA from HIV-1 isolate 97ZA012 (clade C), from SIV^{mac239}, HA–glycoGag or an empty vector control. Scale bar, 10 μm.

3. Extended Data Figure 3: SERINC5 inhibits cytoplasmic delivery of virion content. (257 KB)
   a, Immunodetection of Cre-recombinase (38 kDa) and p24 in HIV-1 particles. **b**, Effect of 1 μM AZT or 100 nM T20 on Cre-delivery and virus infectivity (TU, transducing units). **c**, Immunoblotting of HIV-1 virus particles produced from HEK293T expressing increasing levels of SERINC5–HA. **d**, Effect of SERINC5 on virus fusion measured with BLAM assay T20 served as a negative control. (n = 4, experiment replicated twice). **e**, Cre delivery by EBOV-GP pseudotyped HIV-1 particles. **f**, Inhibition of Cre delivery and counteraction by Nef on HIV-1 from HEK293T expressing SERINC5. Mean ± s.d., n = 4, unpaired two-tailed t-test, *P < 0.05, **P < 0.01, ***P < 0.001. Scale bar, 100 μm.

4. Extended Data Figure 4: SERINC3 and SERINC5 expression is not induced by interferon nor LPS treatments. (206 KB)
   a–**d**, Relative gene expression levels of *SERINC3*, *SERINC5* and *CXCL10* in response to treatment with IFN-β and LPS in Jurkat (**a**), monocyte-derived dendritic cells from two donors (MDDC, **b**), $CD4^+$ primary T cells unstimulated (**c**) or stimulated with PHA (**d**) from two donors. Expression of the housekeeping gene *OAZ1* was used as a normalization control. Mean ± s.d., n = 3.

Extended Data Tables

1. Extended Data Table 1: Description of the cells lines used in Fig. 1a (973 KB)

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