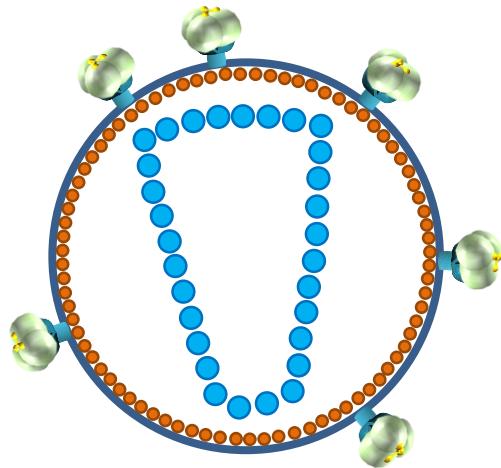


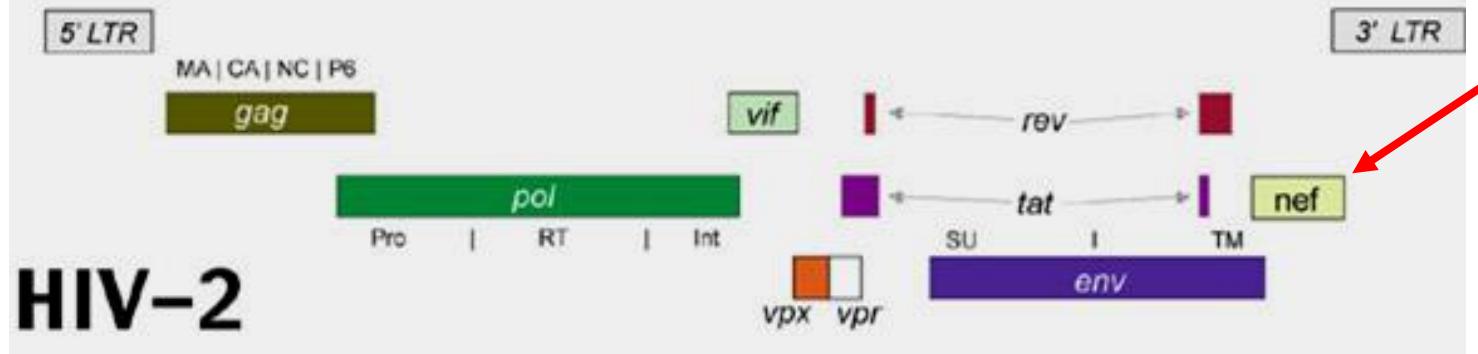
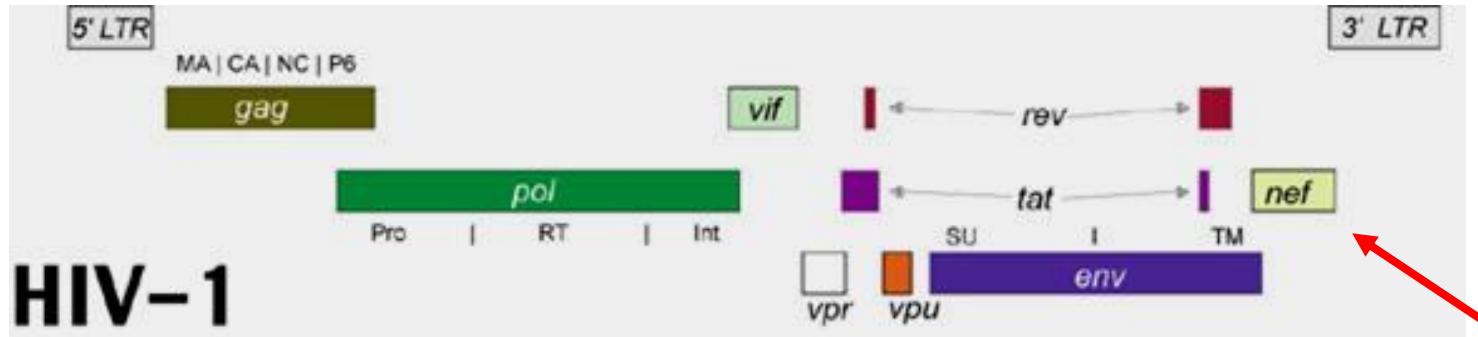
SERINCs: novel restriction factors counteracted by HIV Nef



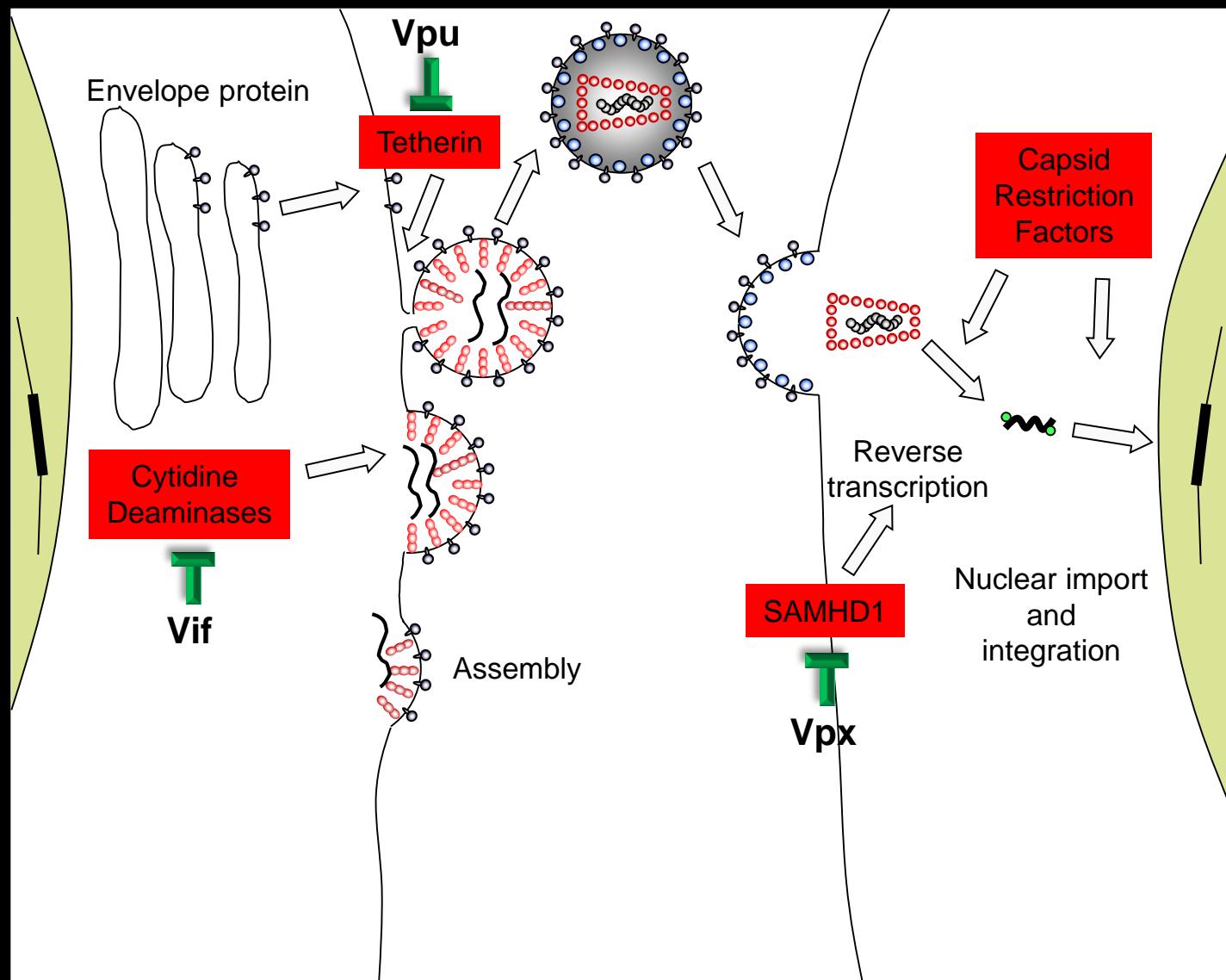
Heinrich Göttlinger, MD
Molecular, Cell and Cancer Biology
UMass Medical School



HIV-1 Genes and Proteins



Intrinsic cellular-defense against retroviruses



Role of HIV-1 Nef

- Crucial for high viral loads and disease progression
- In cell culture:
 - Robustly down-regulates CD4
 - Down-regulates MHC-I
 - **Enhances infectivity**
 - CD4-independent
 - No obvious effect on virion
 - Producer cell-dependent
 - Effect depends on **dynamin, clathrin, AP2**
 - Effect mimicked by **MLV glycoGag**
 - Effect determined by **Env**

ARTICLE

doi:10.1038/nature15399

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

Annachiara Rosa^{1*}, Ajit Chande^{1*}, Serena Ziglio^{1*}, Veronica De Sanctis², Roberto Bertorelli², Shih Lin Goh³, Sean M. McCauley³, Anetta Nowosielska³, Stylianos E. Antonarakis^{4,5}, Jeremy Luban³, Federico Andrea Santoni⁴ & Massimo Pizzato¹

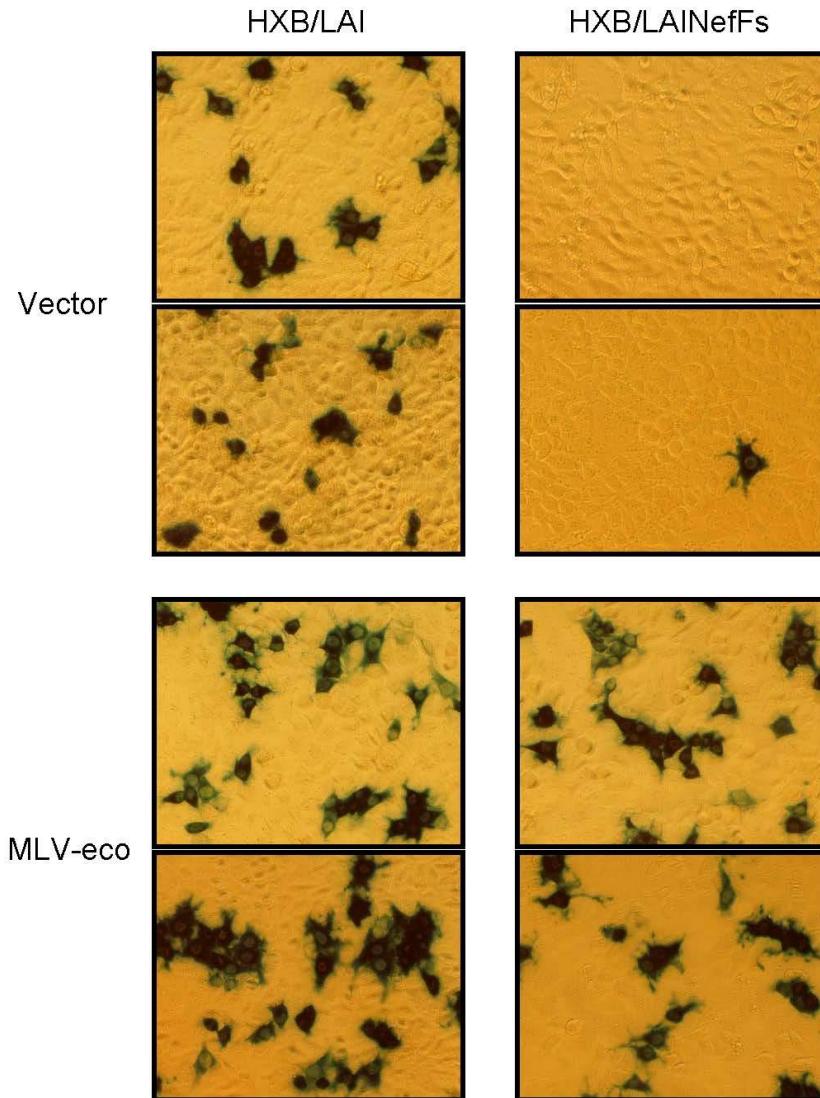
ARTICLE

doi:10.1038/nature15400

SERINC3 and SERINC5 restrict HIV-1 infectivity and are counteracted by Nef

Yoshiko Usami^{1*}, Yuanfei Wu^{1*} & Heinrich G. Göttlinger¹

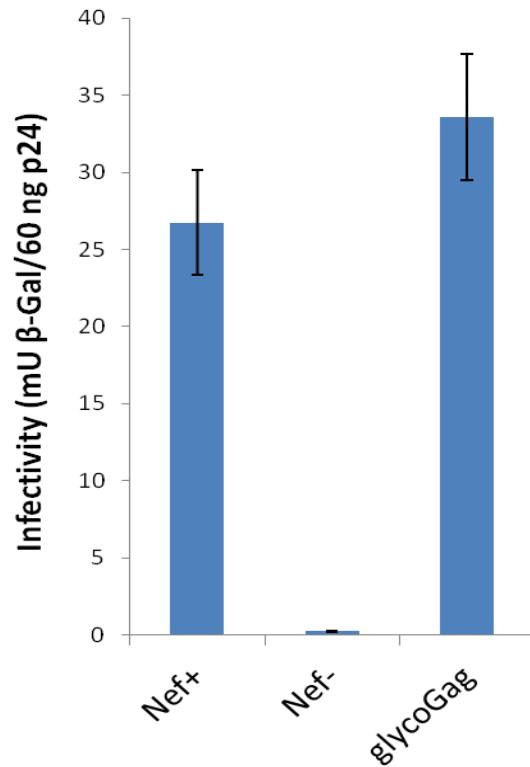
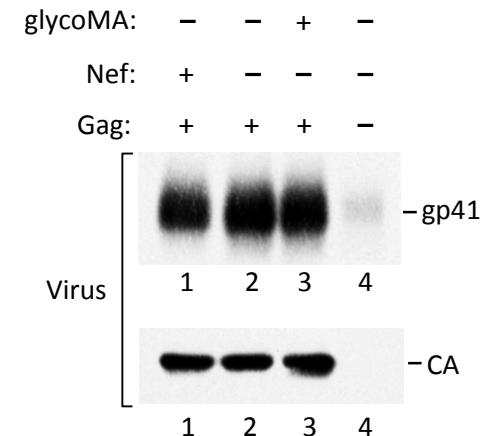
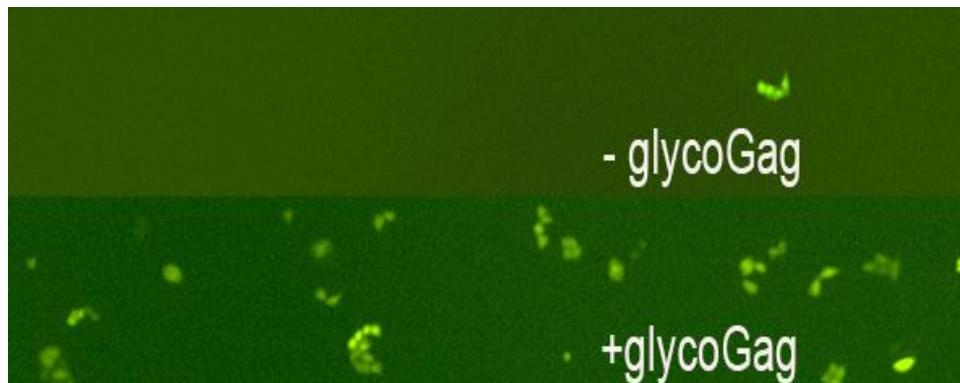
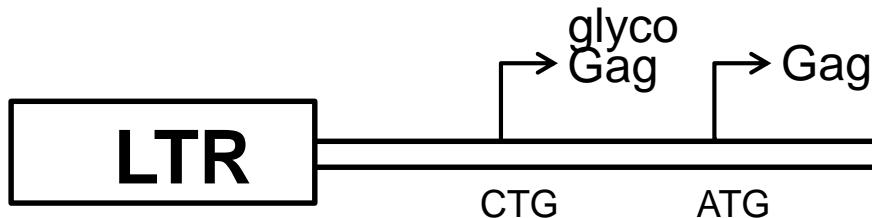




provided by Massimo Pizzato

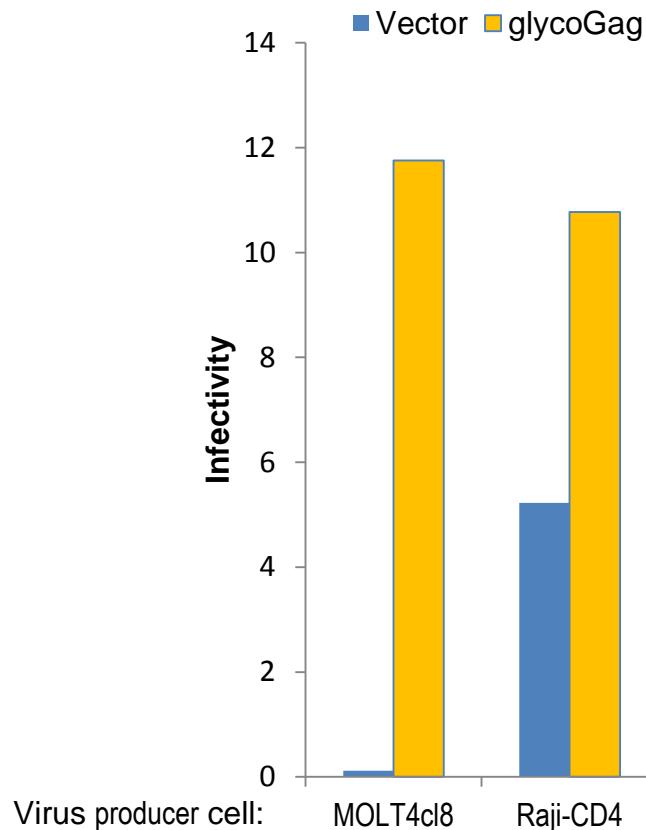
MLV glycoGag has Nef-like effect on HIV infectivity

5' end of murine leukemia virus:

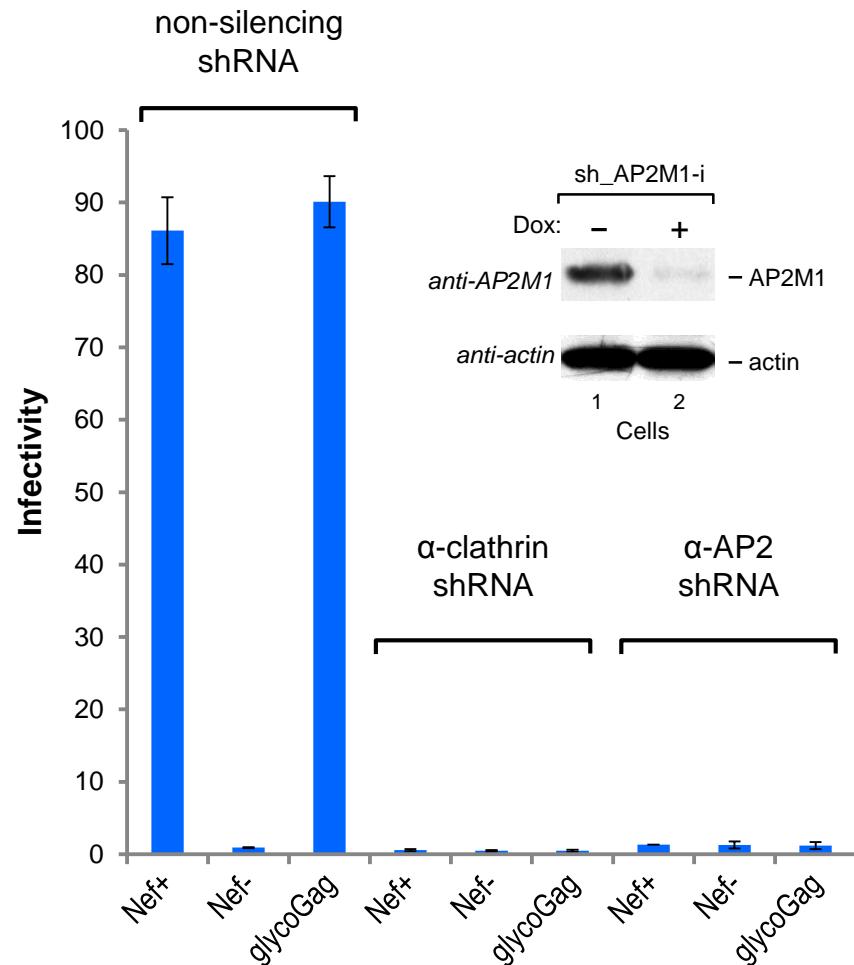


HIV-1 infectivity enhancement by Nef and glycoGag

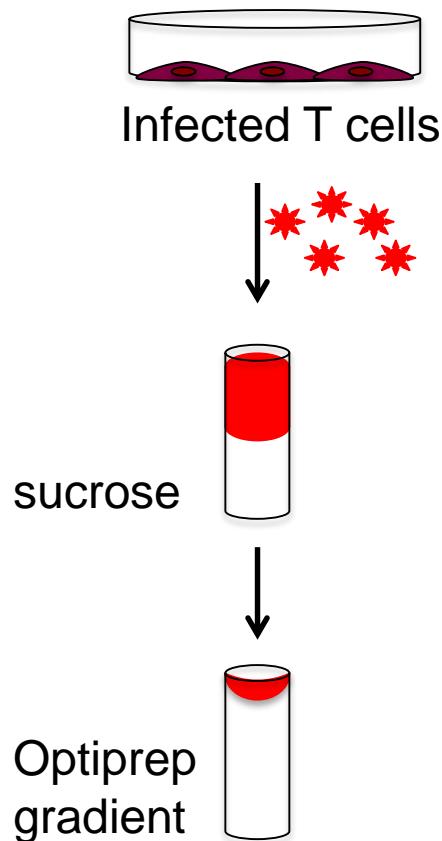
Dependence on producer cell type:



Dependence on clathrin-mediated endocytosis:



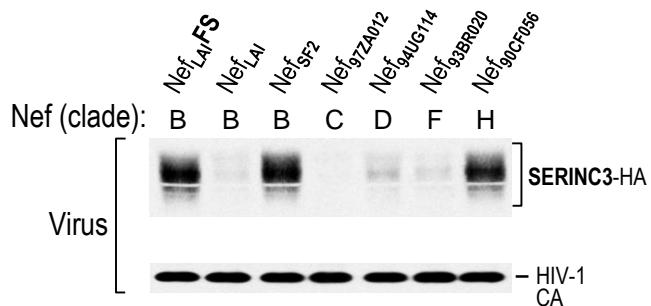
- HIV Nef and MLV glycoGag similarly enhance HIV-1 infectivity
- Effects are similarly dependent on producer cell type
- Effects exhibit a similar reliance on clathrin-mediated endocytosis



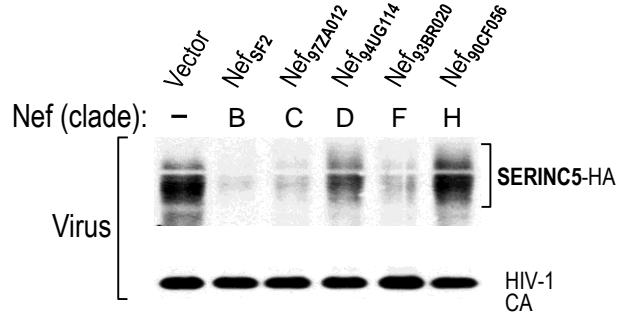
Proteins identified only in Nef- virions				
	Protein Symbol	Gradient Fraction	Total Spectrum Count	% Coverage
Experiment 1	SERINC3	8	7	15
		9	11	16
	STOM	8	2	7
		9	6	16
Experiment 2	PFKP	8	5	5
		9	10	8
	SERINC3	8	5	9
		9	9	9

Inhibition of SERINC incorporation by Nef correlates with infectivity enhancement

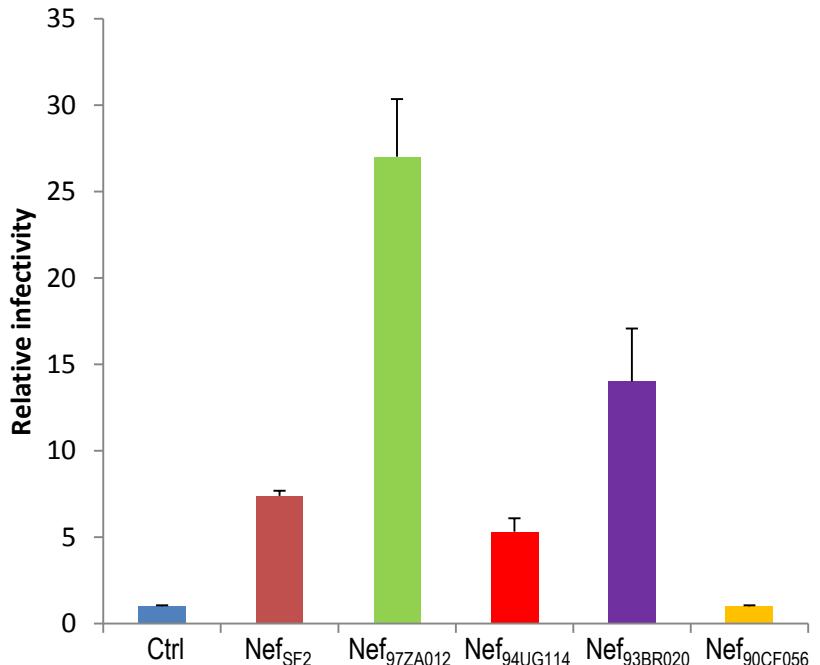
Inhibition of SERINC3 incorporation



Inhibition of SERINC5 incorporation



Enhancement of HIV-1 infectivity



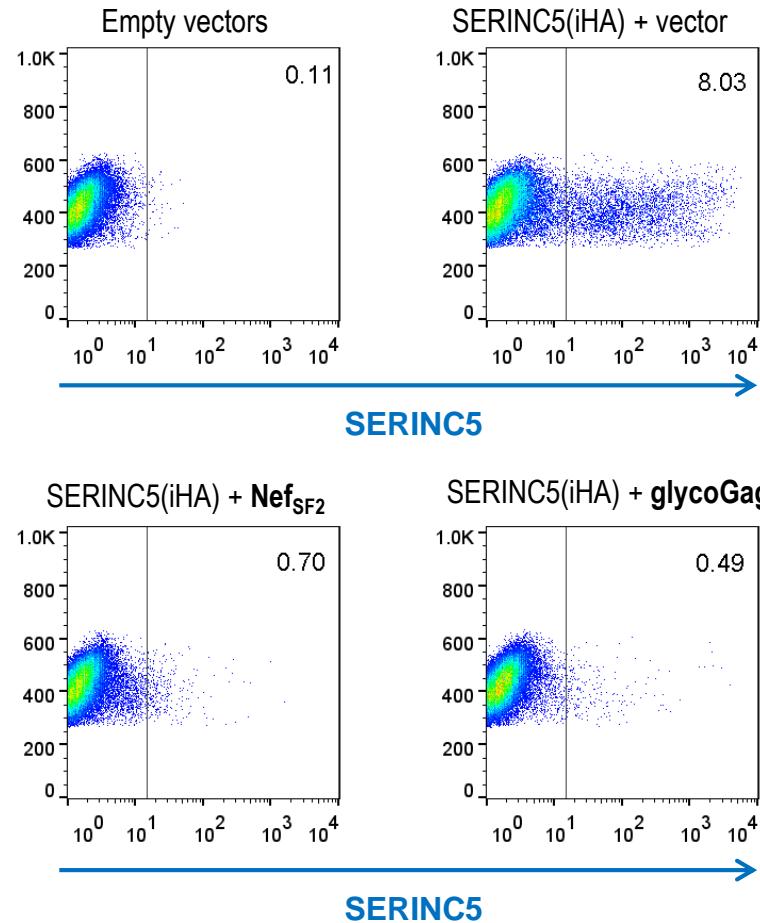
Nef and glycoGag remove SERINC5 from the cell surface

Effect on subcellular localization



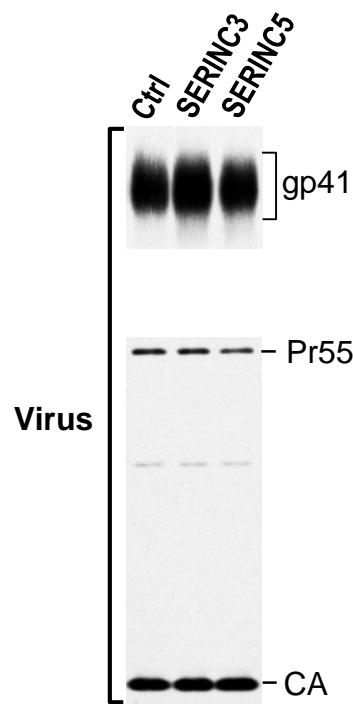
A fluorescence microscopy image showing numerous bright, circular spots of varying sizes distributed across a dark background. The spots are concentrated along a diagonal axis, suggesting a specific cellular localization or distribution pattern.

Effect on cell surface expression

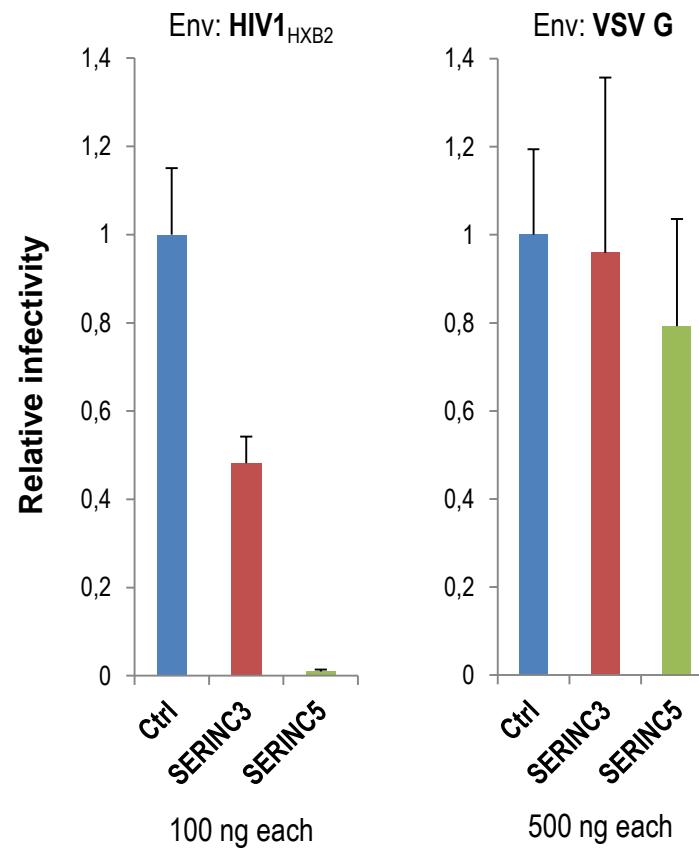


Exogenous SERINC5 specifically blocks HIV-1 Env-mediated infectivity

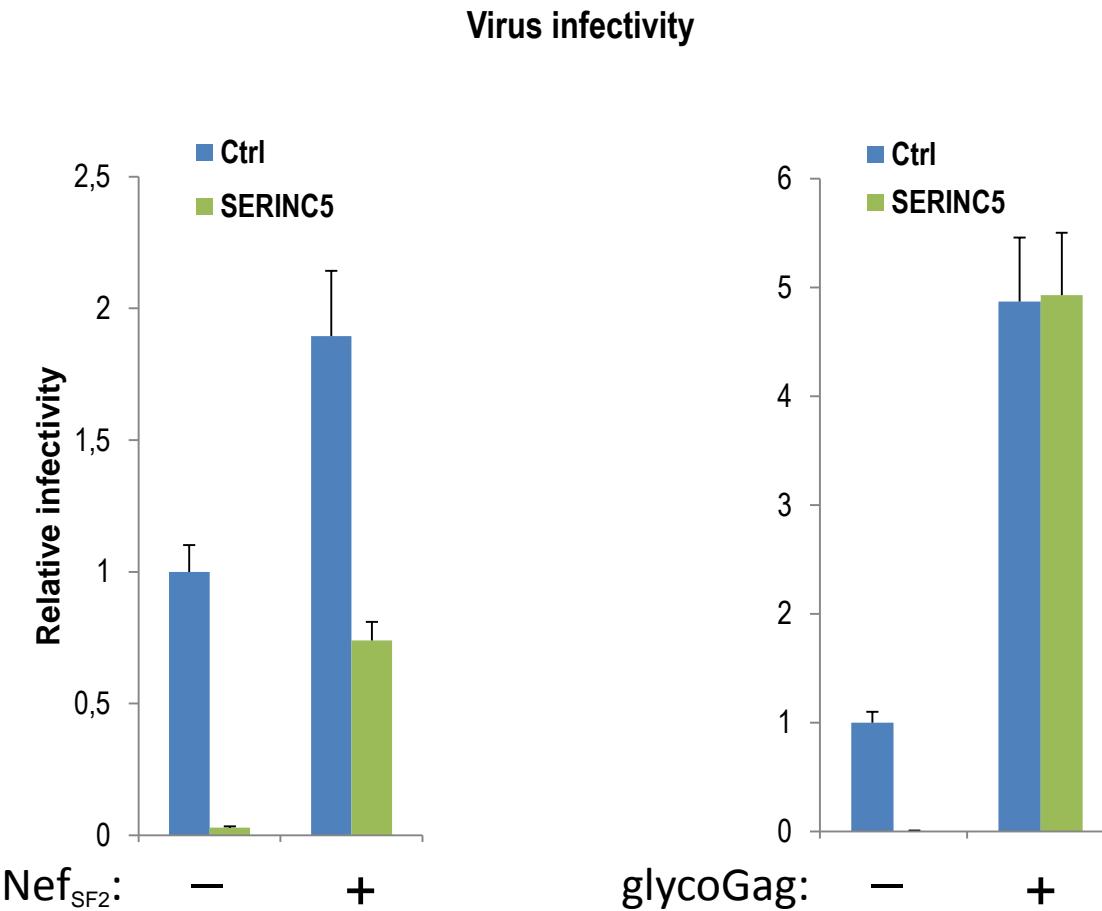
Virus morphogenesis



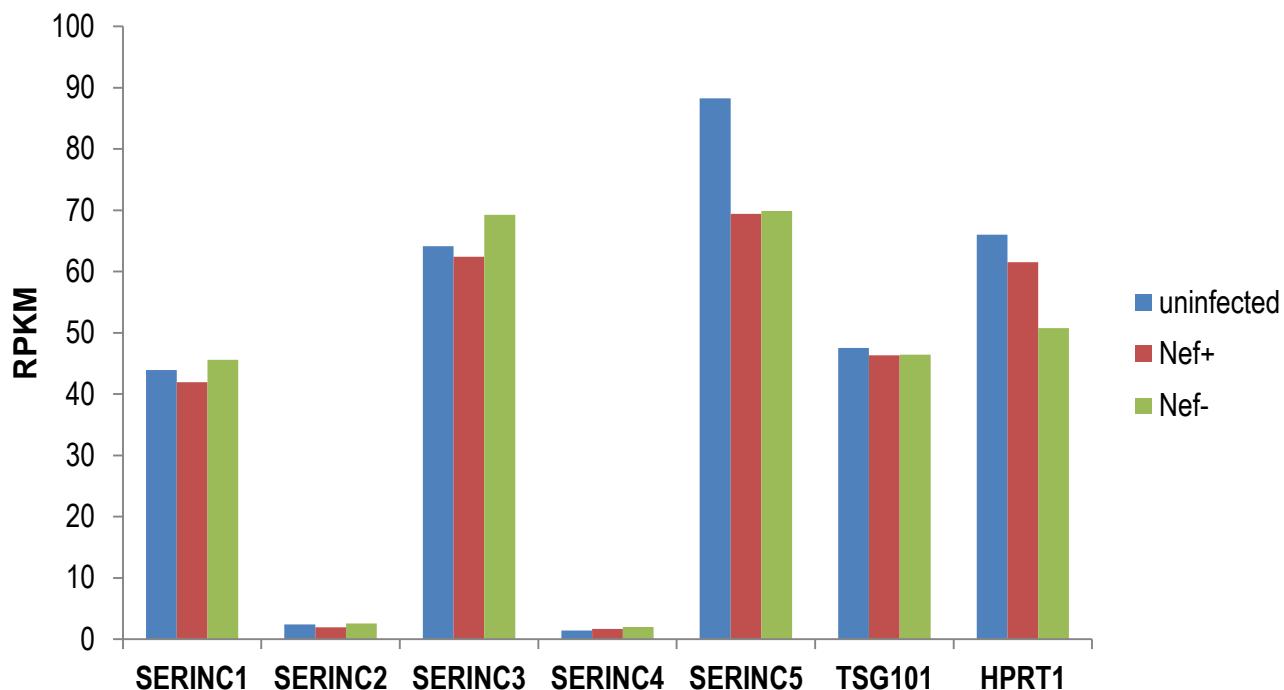
Virus infectivity



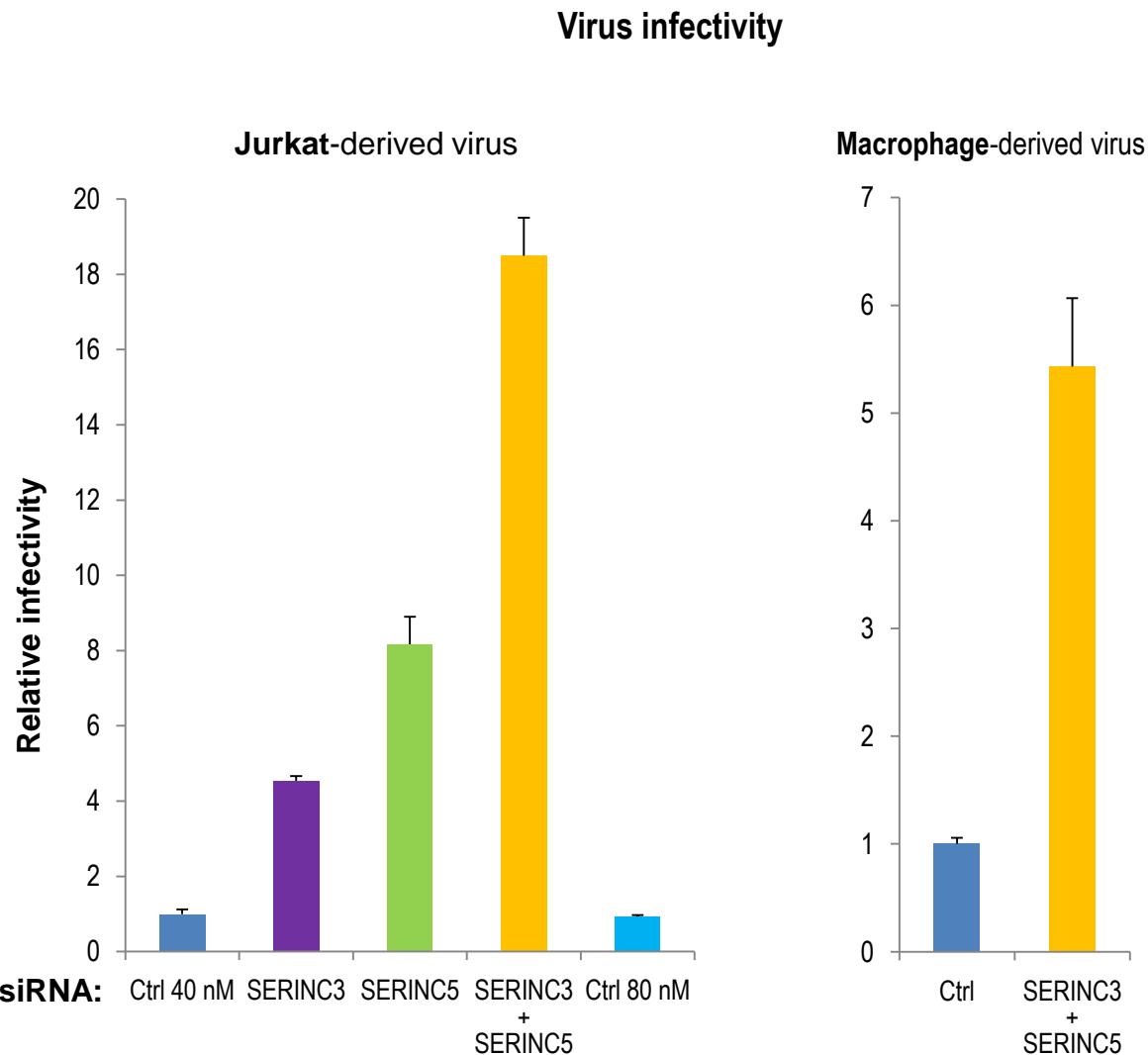
Exogenous SERINC5 is counteracted by Nef and glycoGag



Expression of SERINCs in Jurkat cells

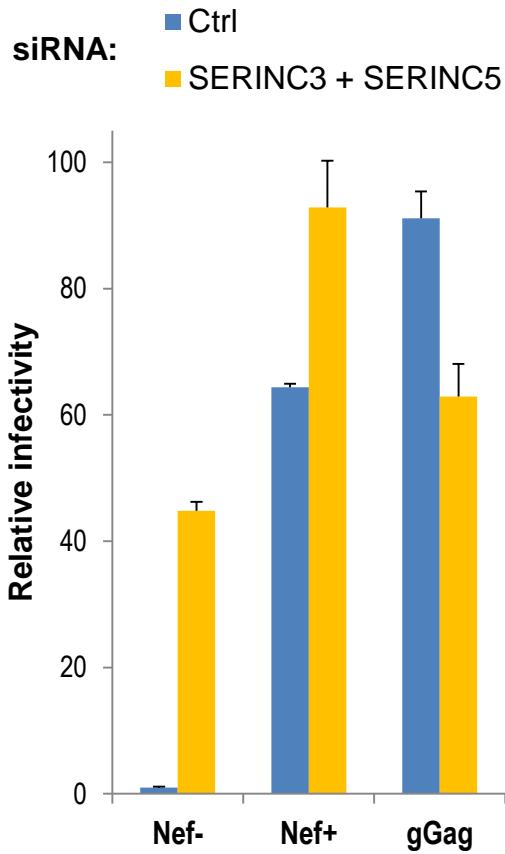


Endogenous SERINC3 and SERINC5 synergistically restrict Nef⁻ HIV-1 infectivity

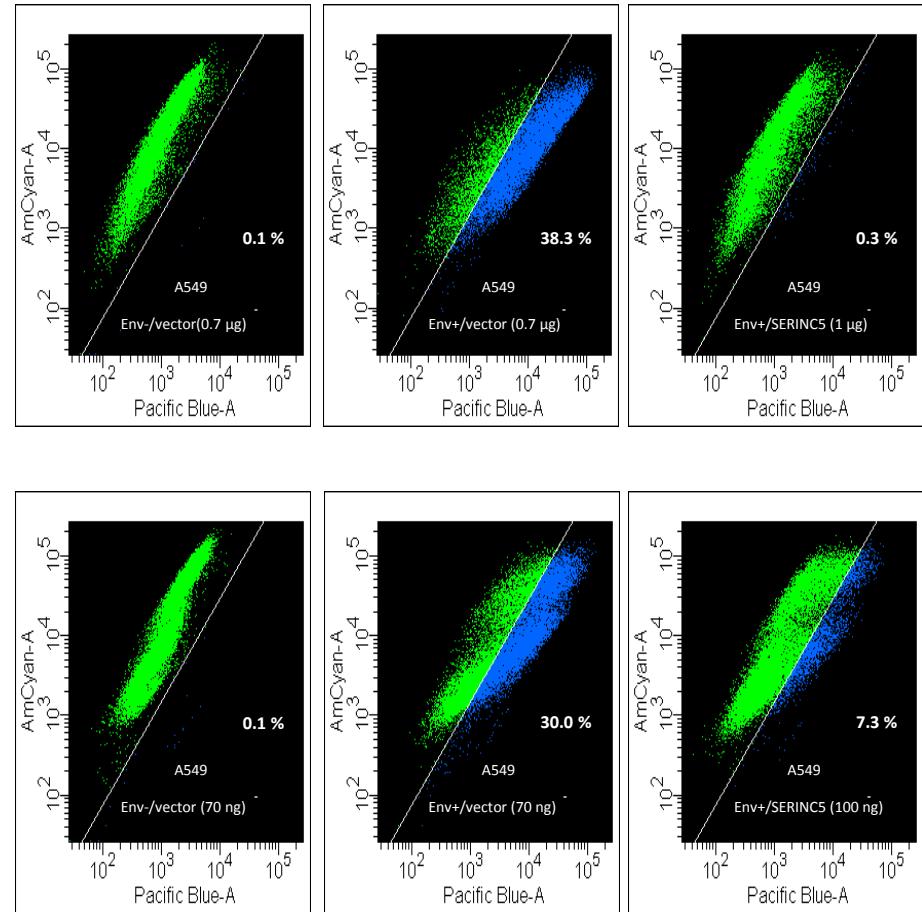
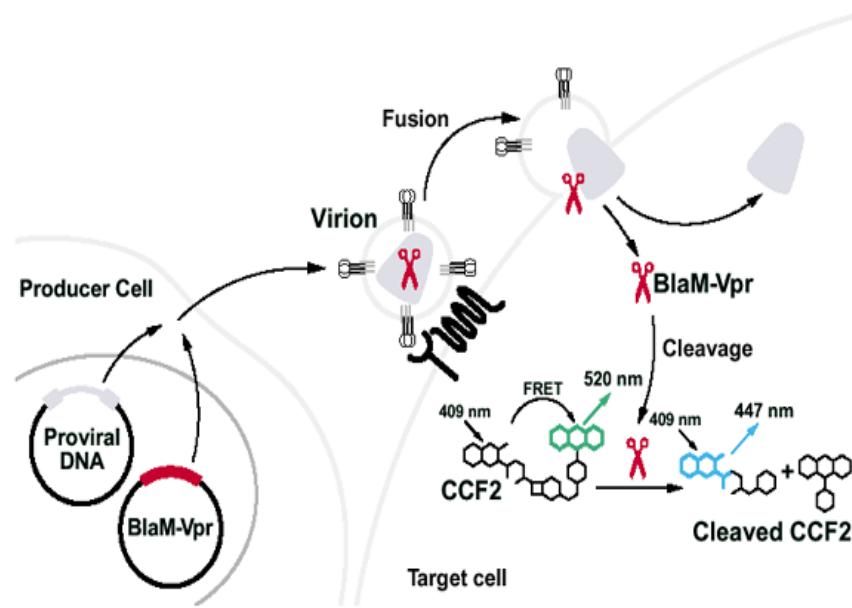


SERINC depletion has negligible effects on HIV infectivity
in the presence of Nef or glycoGag

Infectivity of JurkatTAg-derived virus



Exogenous SERINC5 blocks HIV-1 entry



CRISPR/Cas9-mediated knockout of SERINC3 and SERINC5 in Jurkat-TAg cells

SERINC3

Gene sequence

Target site A
TGT **GTATCGGATCAGCTTGCCATGGCCATCT**

JurkatTAg S3 -/- (1)

TGT **GTATCGGATCAGCTTG**GC**CA**TGGCCATCT (1 bp insertion)
TGT **GTATCG**T**CT** (20 bp deletion)

SERINC 5

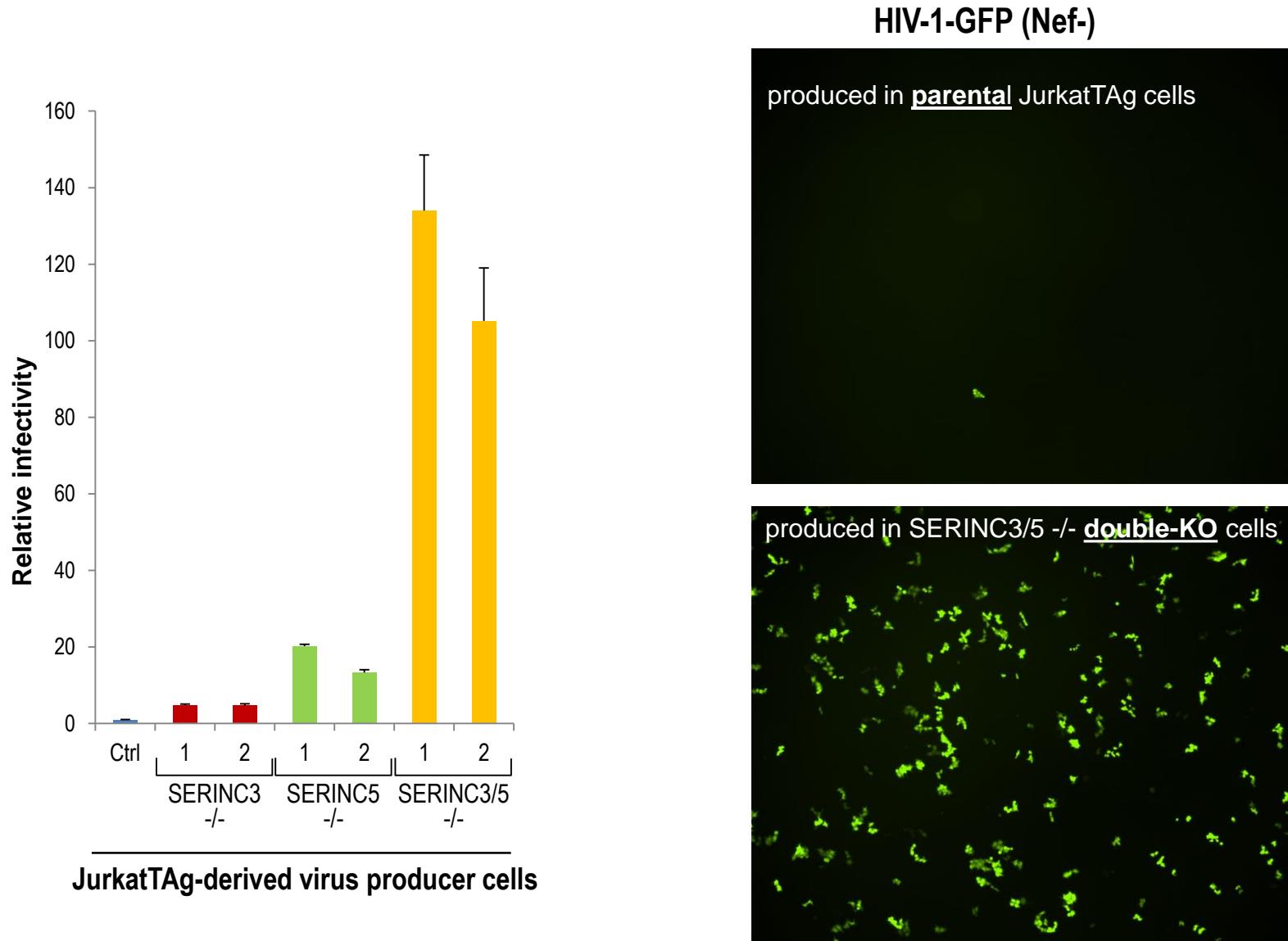
Gene sequence

Target site A
GGT **GACACCTGTGAGAAGCTGGTGGATATT** /11.5 kb / **GACAGCCA**CACTCGGGCTTTACAAT**CAGGGT**

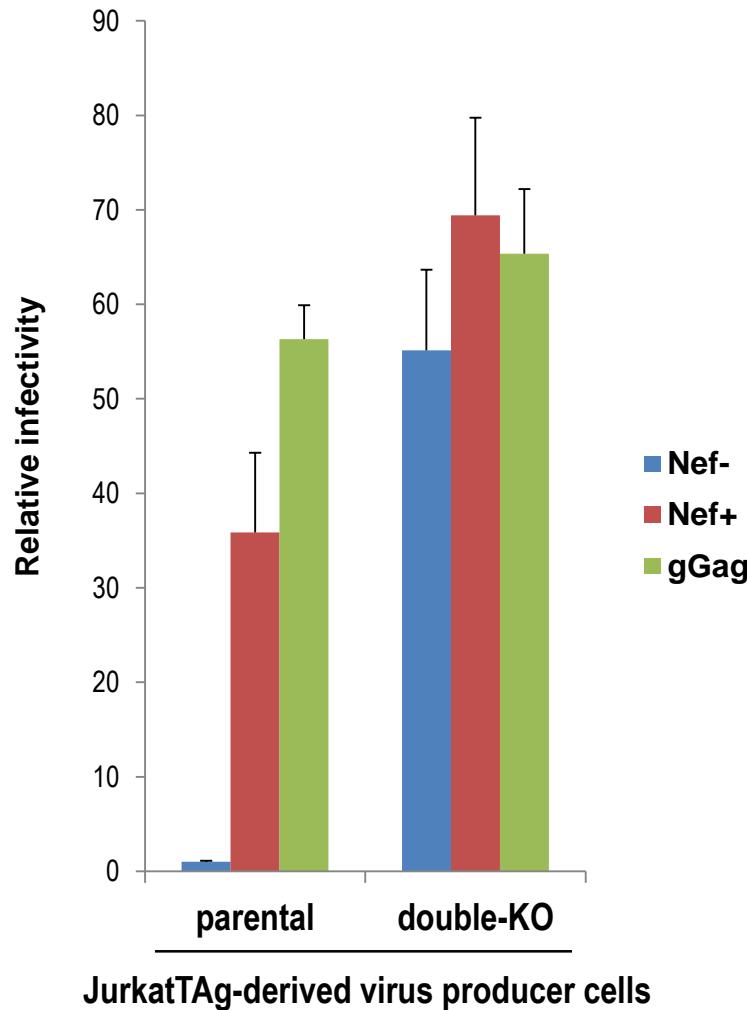
JurkatTAg S3+5 -/- (1)

GGT **GACACCTGTGAGAAGCT**A**TG**T**GGCT**TGTC**** /11.5 kb / **AATATCCC**ACC**GGGGCTTTACAAT**CAGGGT****
GGT **GACACCTGTGAGAAGCT**T**GG**T**GG**TGGATATT**** /11.5 kb / **GACAGCCA****CAC**A**T**CGGGCTTTACAAT**CAGGGT**

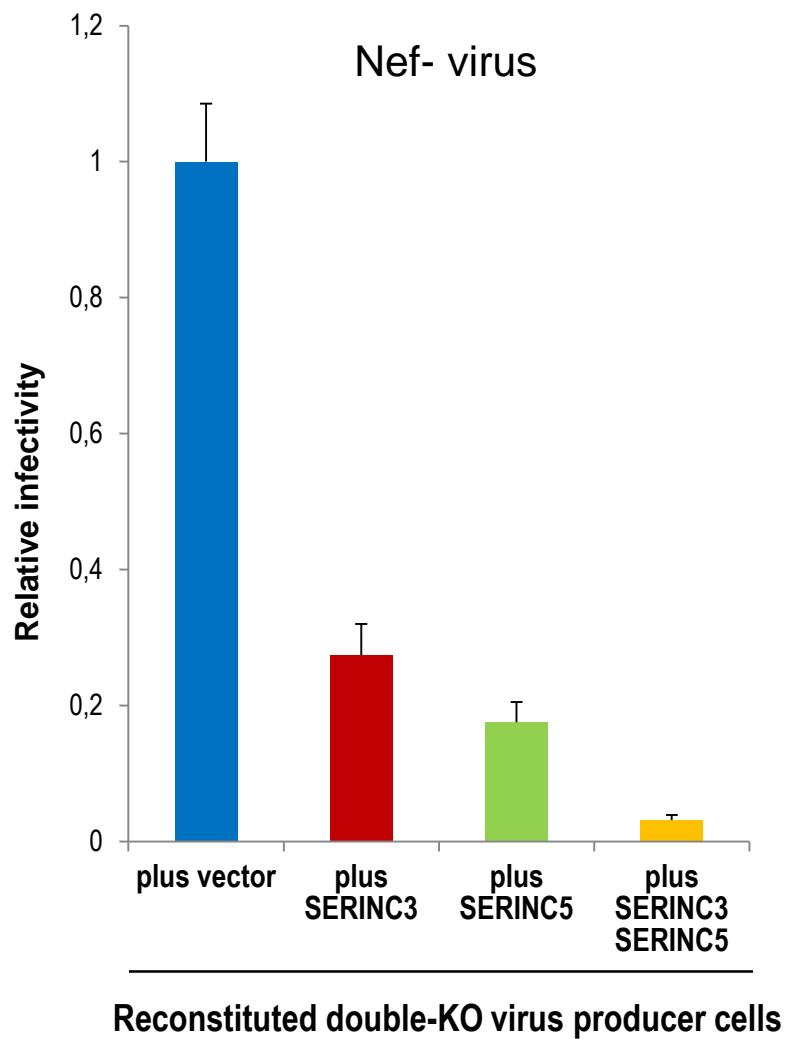
Infectivity of Nef⁻ HIV-1 produced in SERINC knockout JurkatTAg cells



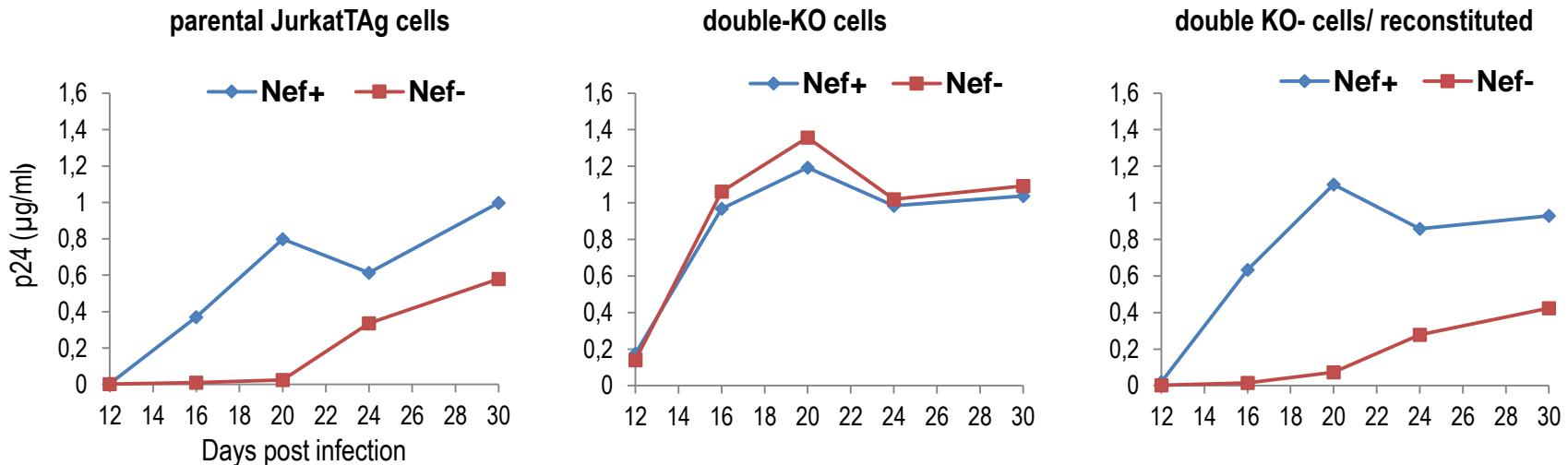
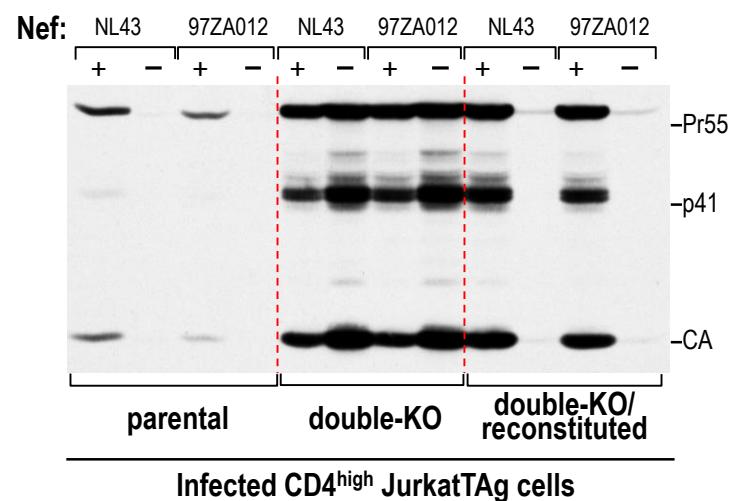
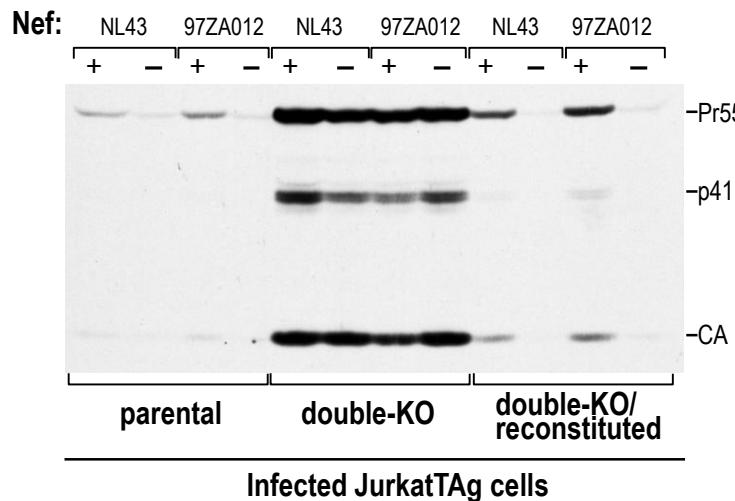
Nef and glycoGag have negligible effects on HIV infectivity
in double-KO cells lacking SERINC3 and SERINC5



Re-expressed SERINCs restrict HIV-1 infectivity



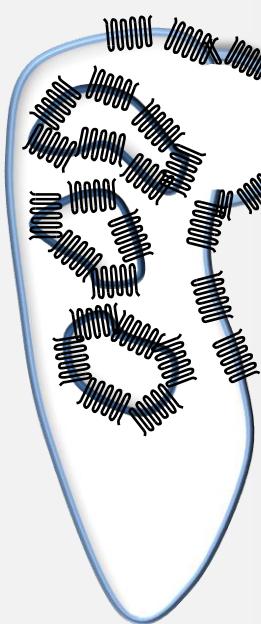
Nef counteracts inhibition of HIV-1 replication by SERINC3 and SERINC5



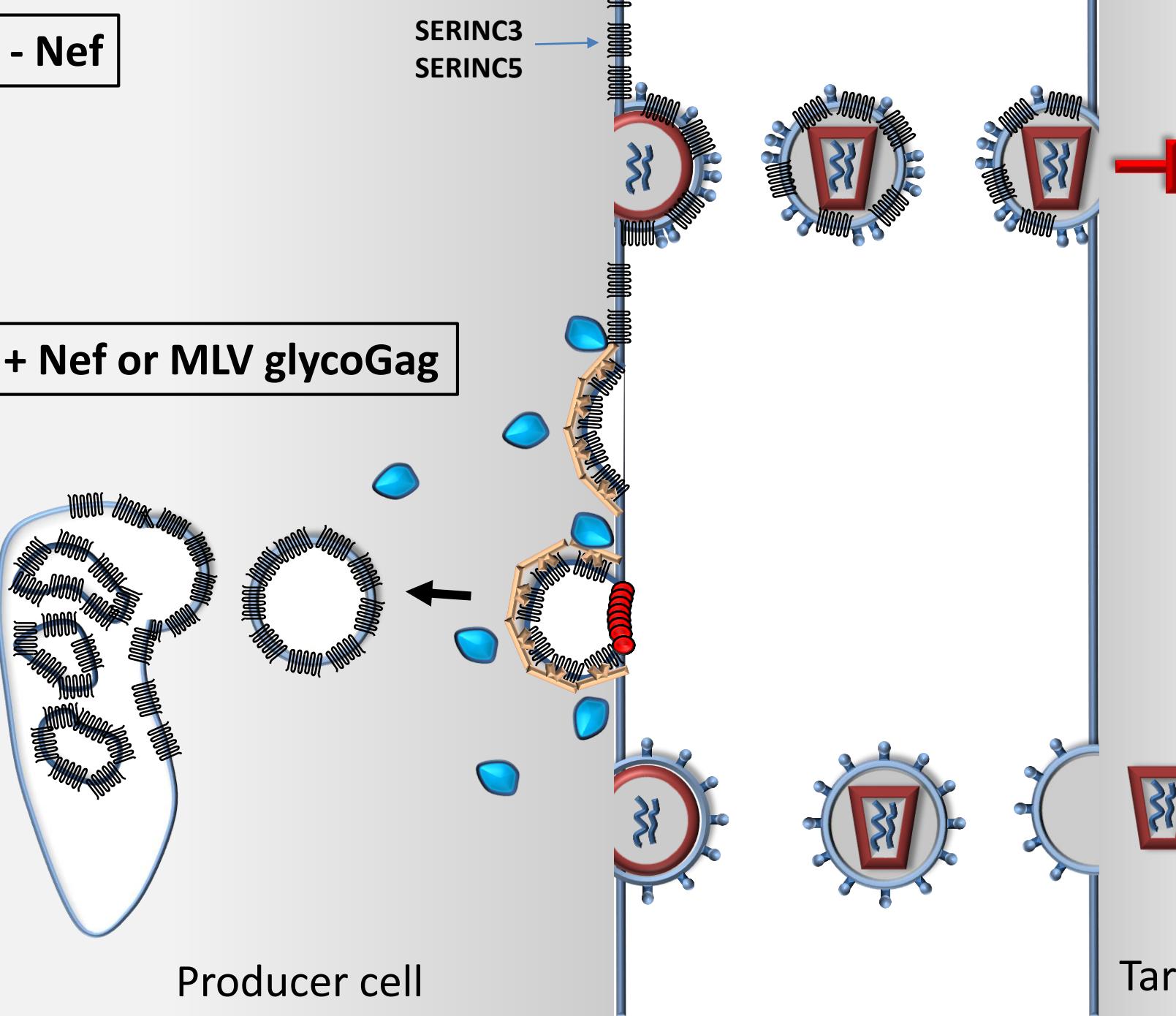
- Nef

SERINC3
SERINC5

+ Nef or MLV glycoGag



Producer cell



Target cell



NIDA
NATIONAL INSTITUTE
ON DRUG ABUSE