NEF FROM PRIMARY HIV INFECTION COUNTERACTS IFITM3 AND RESTORES VIRION INFECTIVITY

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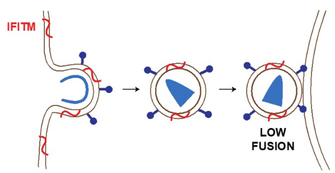
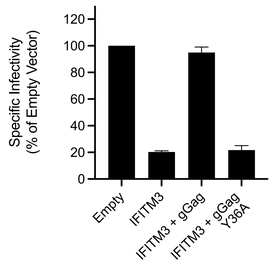
BACKGROUND

Interferon-induced transmembrane protein 3 (IFITM3) inhibits diverse range of viruses by inhibiting viral membrane fusion with host cellular membranes.



IFITM3 restricts HIV-1 spread between cells



MLV Glycogag enables viral evasion of IFITM3

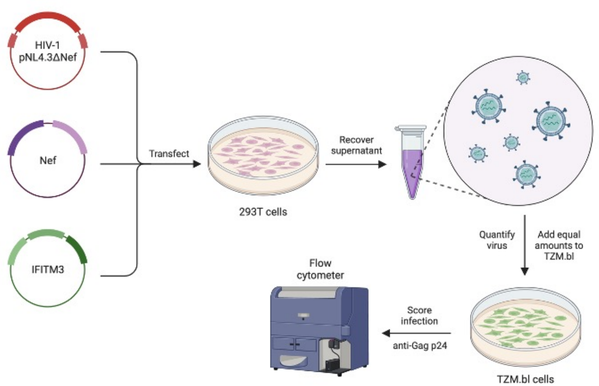
Compton et al. *Cell Host & Microbe*, 2014
Ahi, Yimer et al. *mBio*, 2020

Whether other retroviruses encode the means for IFITM3 counteraction is unclear.

We investigated whether HIV-1 Nef reduces the impact of IFITM3 on virion infectivity.

METHODS

Nef-deficient HIV-1 (NL4.3) was produced by transient transfection of HEK293T cells -/+ pCMV-hIFITM3 and -/+ pBJ-HA-Nef. Infectivity of produced virions was measured by infecting TZM.bl cells (input virus normalized by p24 CA ELISA). Interactions between Nef and IFITM3 were determined by co-immunoprecipitation and western blot analysis.



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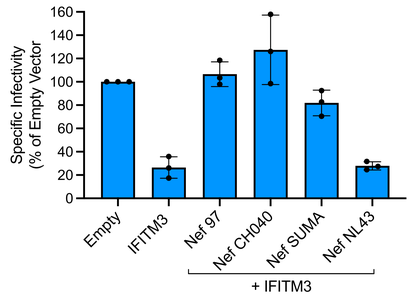
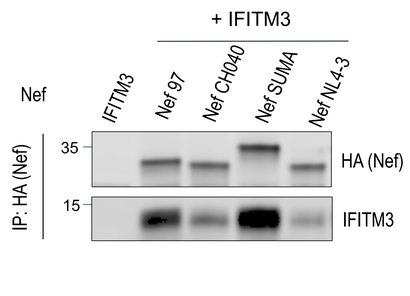
RESULTS

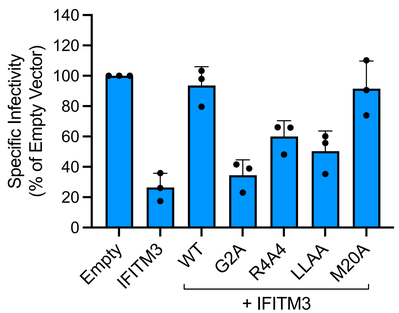
Antagonism of IFITM3 by Nef from different HIV-1 isolates

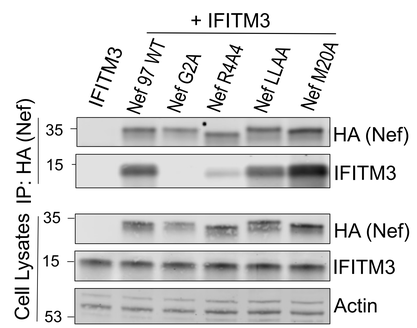


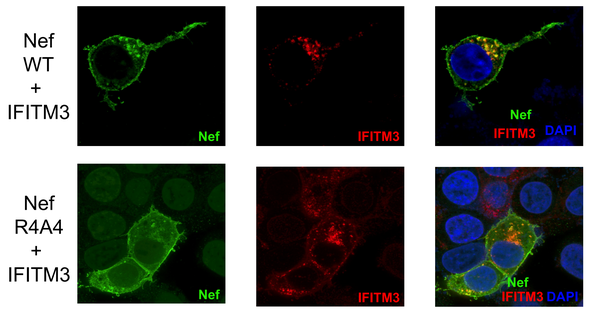


Mechanism of IFITM3 antagonism

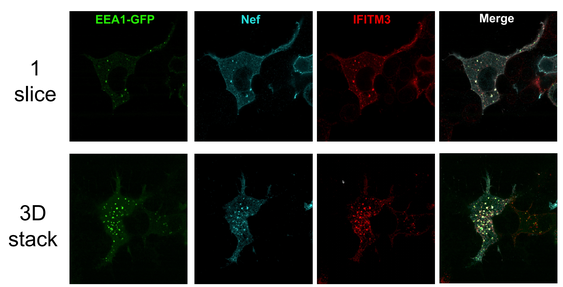
2	17 19-22	28-35	155-156	168-169	175-176
G	R RMRR	EPAADGVG	EE	LL	ED
Myr	Nef-associated kinase complex (NAKC)		COP1	AP-2	V-ATPase/Raf-1



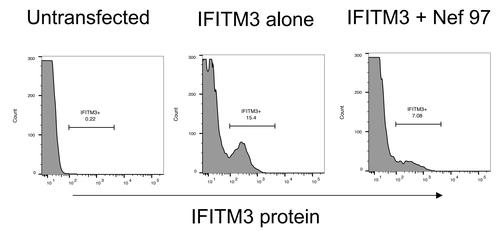




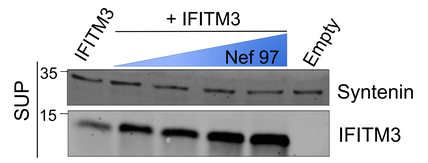
Nef colocalizes with IFITM3 in early endosomes



Nef downmodulates IFITM3 from the cell surface

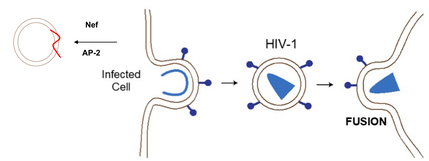


Nef promotes the exocytosis of IFITM3



CONCLUSIONS

- Certain Nef from primary HIV-1 have evolved to counteract human IFITM3 in virus-producing cells, while lab-adapted HIV-1 lost this activity.



FUTURE DIRECTIONS

- Determine whether IFITM3 and Nef interact directly in a cell-free system
- Study how Nef affects the cell surface abundance of endogenous IFITM3 in primary T cells and macrophages
- Assess whether Nef restores membrane fluidity in cells expressing IFITM3
- Determine whether IFITM3 is dependent upon SERINC3/5 for its antiviral function