Nipah virus impairs autocrine IFN signaling by sequestering STAT1 and STAT2 into inclusion bodies

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Supplementary Material

In order of appearance in the manuscript

Supplementary Table 1

Table S1. List of primary and secondary antibodies.

Primary antibodies	Source	Dilution	Provider	Catalogue number	Permeabilization after 4% PFA fixation
anti-NiV N/P (GP3)	guinea pig	1:500	Heinz Feldmann (Hamilton, MT, USA)		0.1% Triton X-100
anti-STAT1	mouse	1:50	Santa Cruz	sc-464	0.1% Triton X-100
anti-STAT2	mouse	1:50	Santa Cruz	sc-1668	0.1% Triton X-100
anti-pSTAT1	rabbit	1:100	Invitrogen	MA5-15071	100% methanol
anti-pSTAT2	rabbit	1:50	Cell Signaling Technology	88410	100% methanol
anti-IRF3	rabbit	1:400	Cell Signaling Technology	11904	0.1% Triton X-100
anti-Nf-κB	rabbit	1:400	Cell Signaling Technology	8242	0.1% Triton X-100

Secondary antibodies	Source	Dilution	Provider	Catalogue number
anti-mouse IgG Alexa Fluor 568	goat	1:300	ThermoFisher	A-11031
anti-rabbit IgG Alexa Fluor 568	goat	1:300	ThermoFisher	A-11036
anti-guinea pig IgG Alexa Fluor 488	goat	1:300	ThermoFisher	A-11073

Supplementary Table 2

Table S2. Primer pairs used for qPCR.

Genes	Primer pairs	Primer sequences	Reference
Human α-Tubulin	hu TubA1b forward hu TubA1b reverse	5′-ACCACCCTGGAGCACTCTGA-3′ 5′-GACAGATGTCATAGATGGCCTCAT-3′	[51]
Human IFN-β	hu IFNβ forward hu IFNβ reverse	5′-TGCCTCAAGGACAGGATGAAC-3′ 5′-GGAACTGCTGCAGCTGCTTA-3′	[51]
Human IFN-λ2,3	hu IFNλ2/3 forward hu IFNλ2/3 reverse	5'-GCCAAAGATGCCTTAGAAGAG-3' 5'-CAGAACCTTCAGCGTCAGG-3'	[51]
Human ISG56	hu ISG56 forward hu ISG56 reverse	5′-CAGCAACCATGAGTACAAAT-3′ 5′-AAGTGACATCTCAATTGCTC-3′	[51]
Human OAS	hu OAS forward hu OAS reverse	5′-GCCCTGGGTCAGTTGACTGG-3′ 5′-TGAAGCAGGTGGAGAACTCGC-3′	[51]
Human PKR	hu PKR forward hu PKR reverse	5′-ACACTCGTCTCTGAATCATC-3′ 5′-GAGACCATTCATAAGCAACG-3′	This study
Human MxA	hu MxA forward hu MxA reverse	5′-ATGAGCTAATCACCCTGGAG-3′ 5′-ATACCCAATGTCAGCAGGC-3′	This study

Supplementary Figure 1

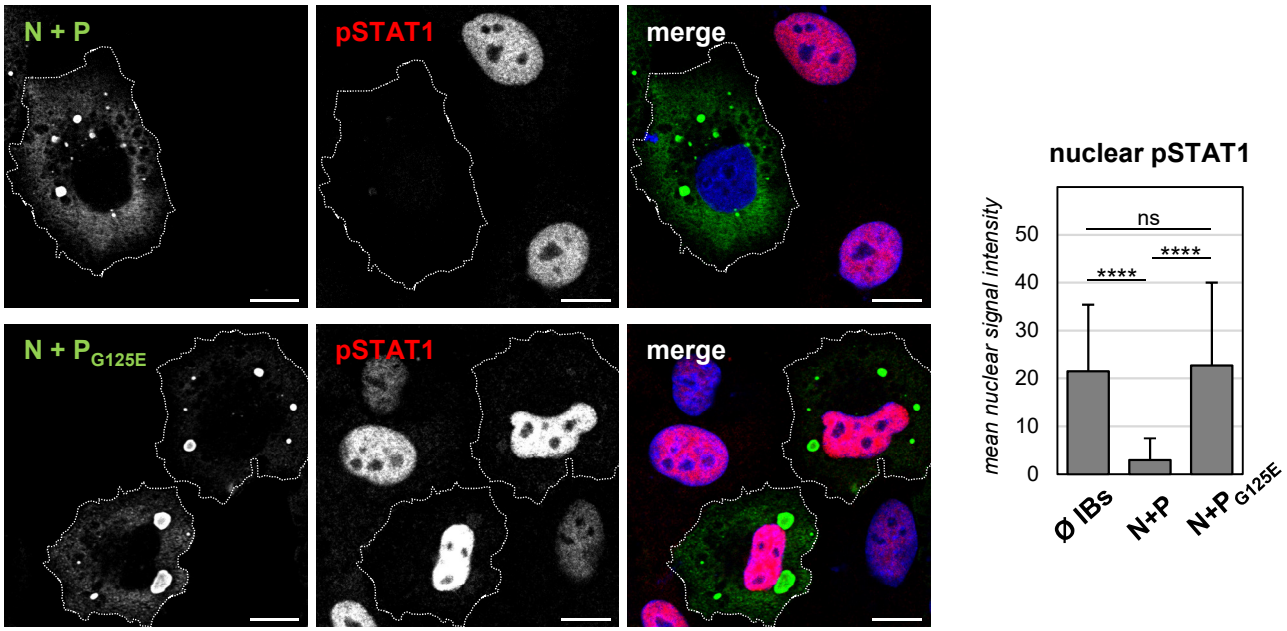

Figure S1. Wildtype NiV P but not STAT1-binding defective NiV P_{G125E} blocks nuclear pSTAT1 accumulation in IFN-treated cells.

A549 cells were transfected with plasmids encoding NiV N and NiV P (N+P) or NiV N and NiV P_{G125E} ($N+P_{G125E}$), a mutant P protein which is known to be STAT1-binding defective [41].

After 22 h, IBs had formed and cells were treated with 1000 U/ml of human IFN-β for 40 min and fixed with 4% PFA. After permeabilization with methanol at -20°C , endogenous pSTAT1 was stained with specific antibodies (red). IBs were detected with a NiV N/P specific antiserum (green). Nuclei were counterstained with DAPI (blue). Greyscale single-channel images and colorized merged images are shown. The dashed lines indicate IB-containing cells. Scale bars, 10 μm.

Quantification (right panel): To quantify the nuclear pSTAT1 fluorescence in IFN-treated cells, the signal intensities of pSTAT1 in the nucleus of cells without IBs (Ø IBs), cells with IBs containing wildtype P (N+P) and cells with IBs containing STAT1-binding defective P_{G125E} ($N+P_{G125E}$) were analyzed (> 800 cells in total). The mean nuclear signal intensity of pSTAT1 is shown. Error bars indicate standard deviation (SD); ****, $p < 0.0001$; ns, not significant.