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SUPPLEMENTARY INFORMATION

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Dengue virus NS2B protein targets cGAS for degradation and prevents mitochondrial DNA sensing during infection

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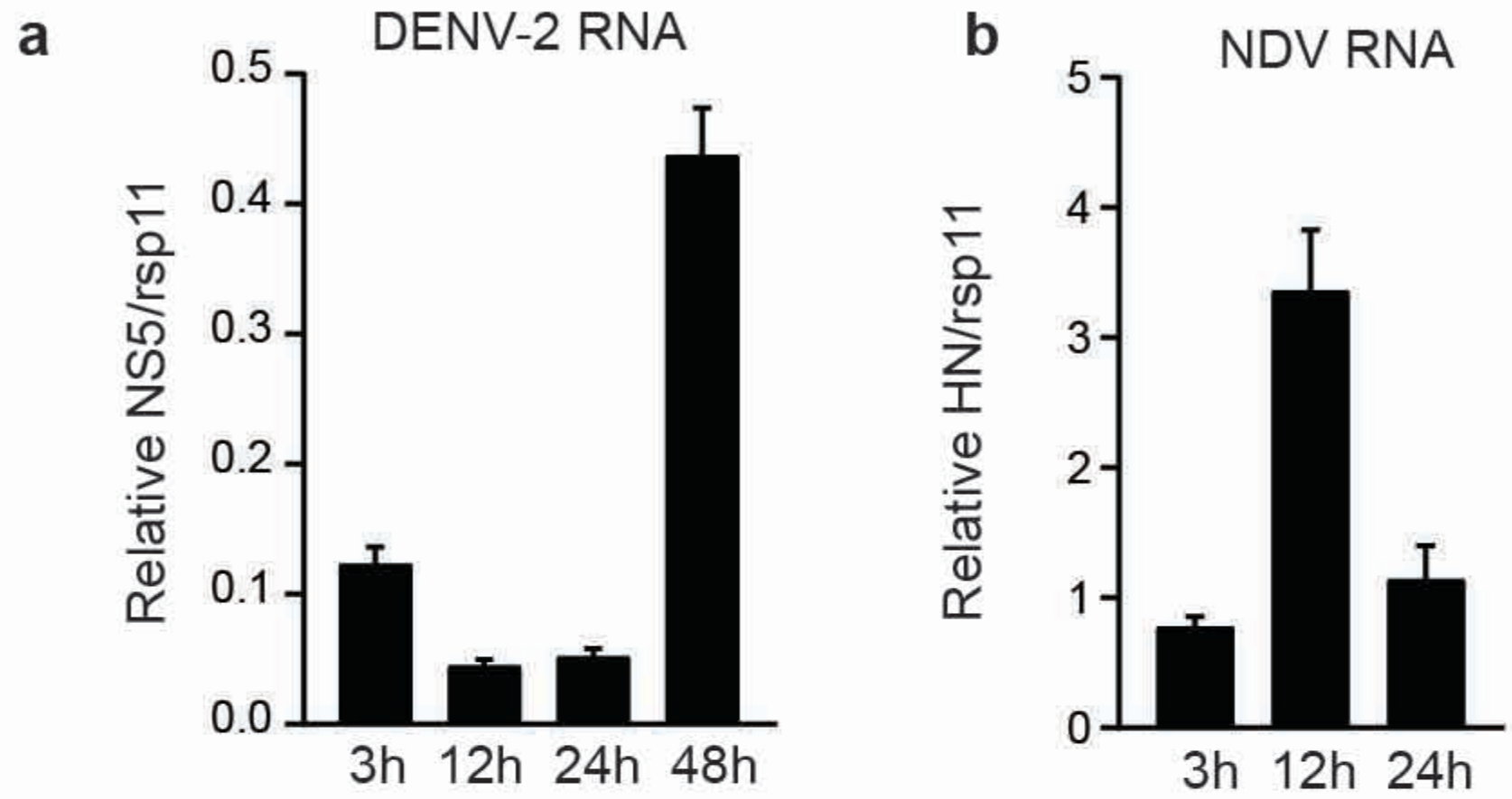
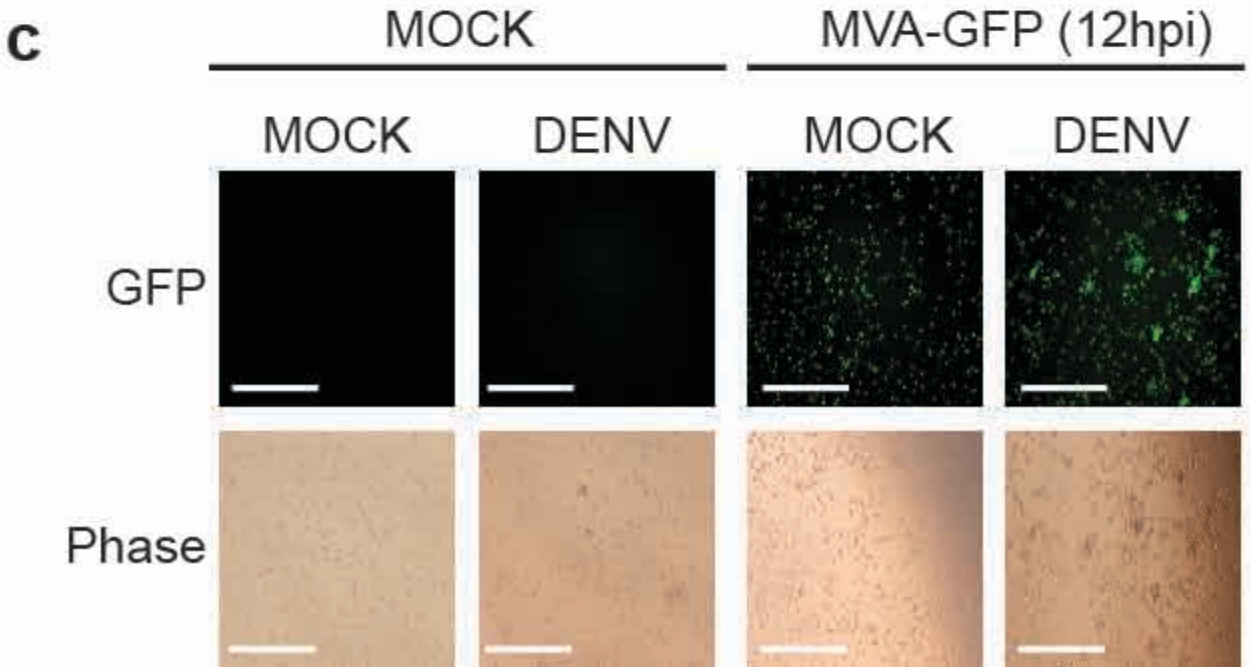
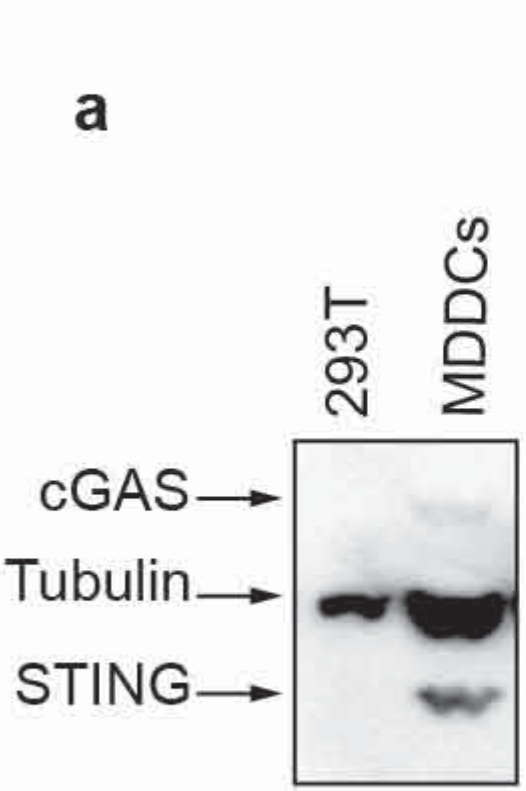
Supplementary figure 1

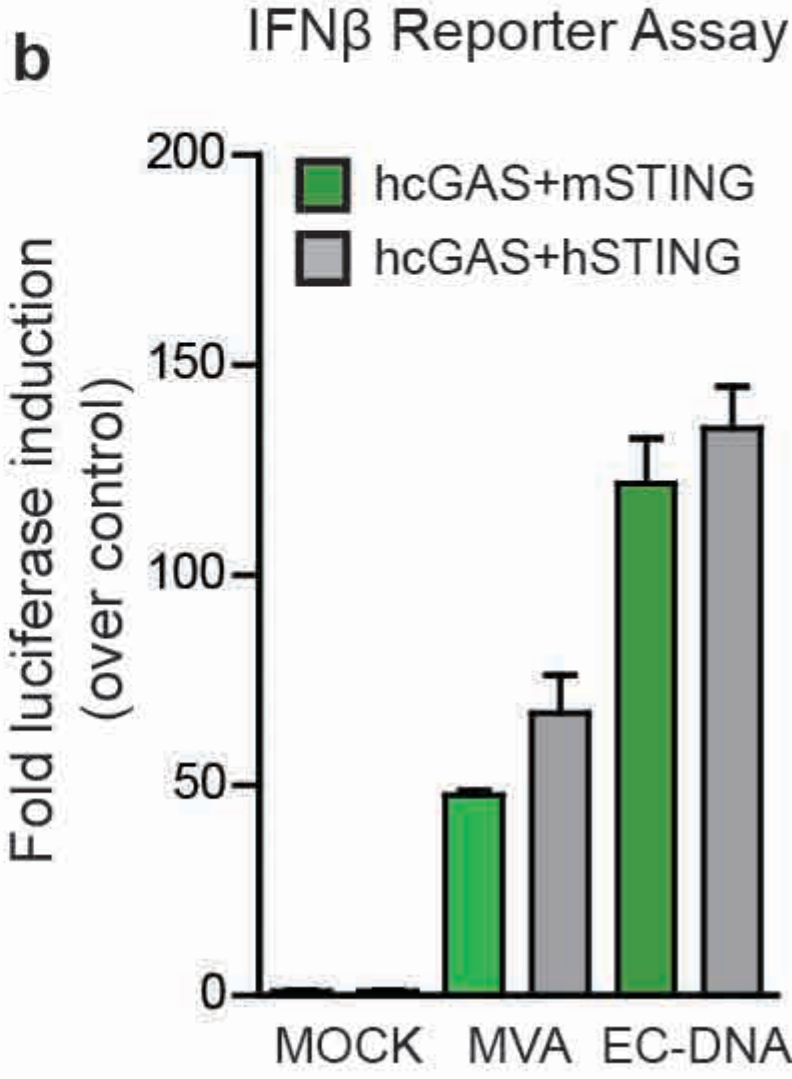




Supplementary figure 1. Replication kinetics of DENV, and NDV in MDDCs from experiment shown in Fig 1a and 1b. vRNA quantification using RT-qPCR for DENV-2 NS5 or NDV HN gene (b). Results are expressed as mean ± s.e.m. from three biological replicates. (c) Control of secondary infection with MVA-GFP for experiment described in Fig 1c. Detection of GFP in MDDCs previously infected MOCK or with DENV-2. Scale bar: 400µm.

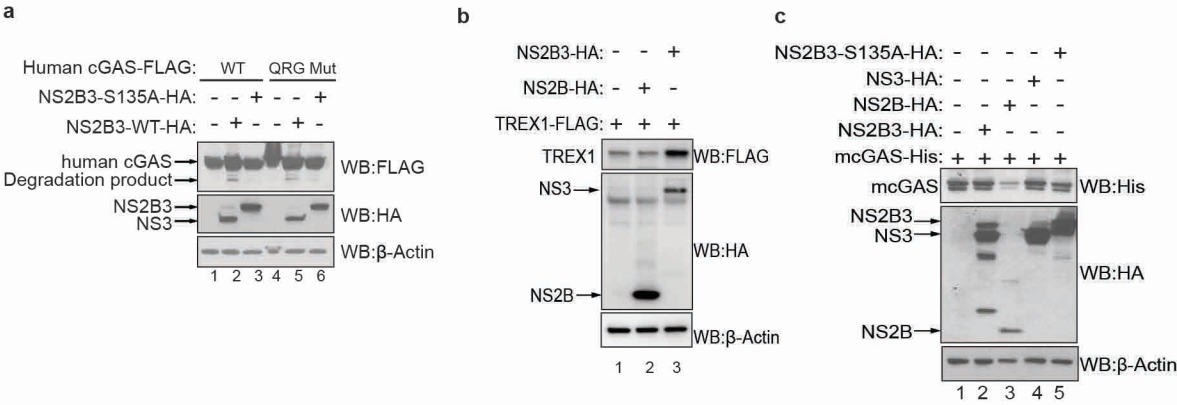
Supplementary figure 2





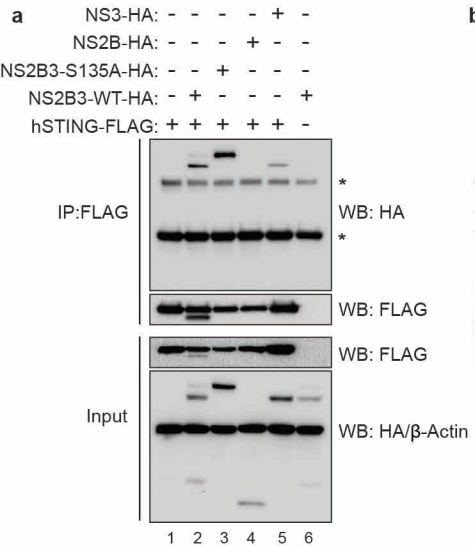
Supplementary figure 2: Endogenous cGAS and STING proteins abundance in 293T cells and primary MDDCs. Analysis of total cell lysates from 293T cells and MDDCs by SDS-PAGE/WB using anti-cGAS, anti-STING and anti-Tubulin antibodies. Evaluation of the murine STING to signal the activation of the IFNβ promoter in a human cell context. IFNβ promoter assay in 293T cells using (human cGAS + human STING) or (human cGAS + murine STING). The cGAS/STING pathway was stimulated by infection with MVA or transfection with purified E. coli DNA as described in materials and methods. Results are expressed as mean ± s.e.m. from three biological replicates.

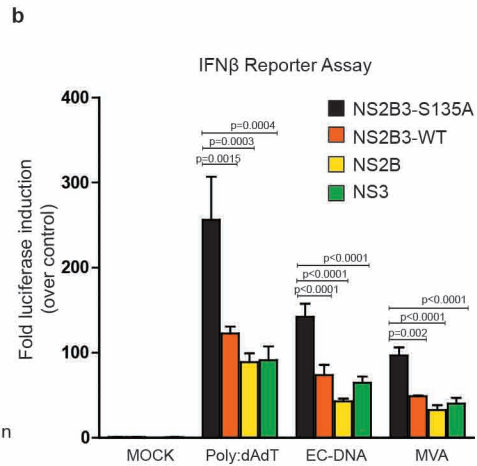
Supplementary figure 3



Supplementary figure 3: (a) Mapping of cGAS cleavage by DENV NS2B3. WB analysis of the degradation rendered by co-expression of DENV NS2B3-WT or NS2B3-S135A with human cGAS WT or mutant QRG/AAA proteins. (b) DENV NS2B degradation specificity analysis with unrelated protein control: human TREX1 was co-transfected with empty vector, DENV NS2B or NS2B3-WT. Protein status was evaluated by SDS-PAGE/WB. (c) Degradation of mcGAS by DENV NS2B. Expression analysis of murine cGAS with empty vector, DENV NS2B3, NS2B, NS3 or NS2B3-S135A by SDS-PAGE/WB.

Supplementary figure 4





Supplementary figure 4: Interaction of human STING with the DENV protease complex or its components (DENV NS2B3-WT-HA, NS2B3-S135A-HA, NS2B-HA or NS3-HA). (a) Co-immunoprecipitation of human STING-FLAG using anti-FLAG antibody. Analysis by SDS-PAGE/WB. (b) Evaluation of the DENV protease components as antagonists of the cGAS/STING pathway by IFNβ reporter assay. 293T reporter cells were co-transfected with plasmids encoding human STING, cGAS, proteolytically inactive NS2B3 mutant (NS2B3-S135A) as control, DENV wild type protease (NS2B3-WT), NS2B or NS3 expressed individually. The pathway was triggered by Poly-dA:dT [1ug/well], purified E. coli DNA [1ug/well], or infection by MVA [MOI=1]. Results are expressed as mean ± s.e.m. from three biological replicates (one-way ANOVA).

Supplementary figure 5

a

293T cells expressing:

GFP-FLAG
cGAS-FLAG
RIG-I-FLAG

DENV infection

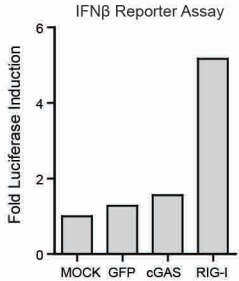
24 hpi

IP: anti-FLAG

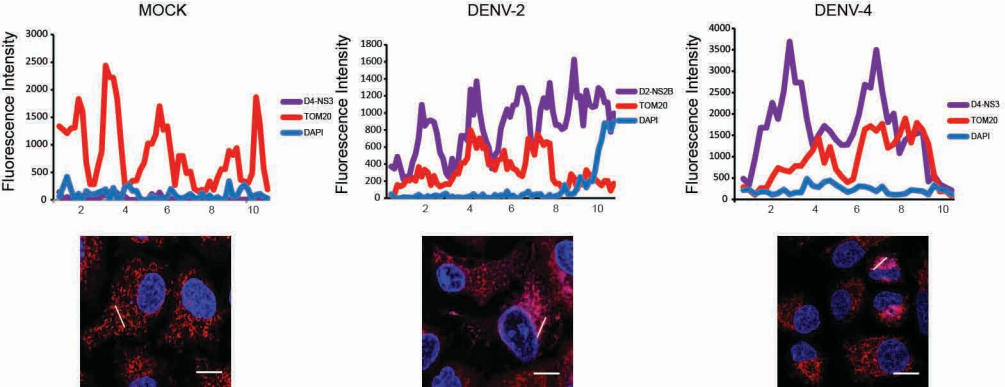
RNA extraction/analysis

RNA recovery from immunoprecipitation:

	GFP	cGAS	RIG-I
RNA (ng/ul)	5	7	110



b



Supplementary figure 5: RNA recovery from cGAS pulldowns. 293T cells expressing GFP-FLAG, cGAS-FLAG, and RIG-I-FLAG were infected with DENV-2 for 24h. Proteins were immunoprecipitated using anti-FLAG antibody and total RNA was extracted as described in materials and methods. (a) RNA concentration recovered from three described conditions. RNA immunogenicity was evaluated by IFNβ reporter assay. Mitochondria distribution and DENV proteins and mitochondrial marker co-localization. (b) Fluorescence intensity profile of A549 cells treated MOCK, DENV-2 and DENV-4 (scale bar: 10μm). Graphs were analyzed using ImageJ software.

Supplementary Figure 6

Fig 1g

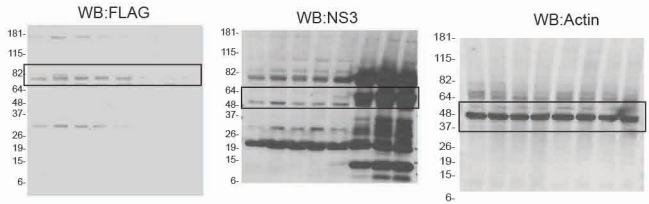


Fig 1h

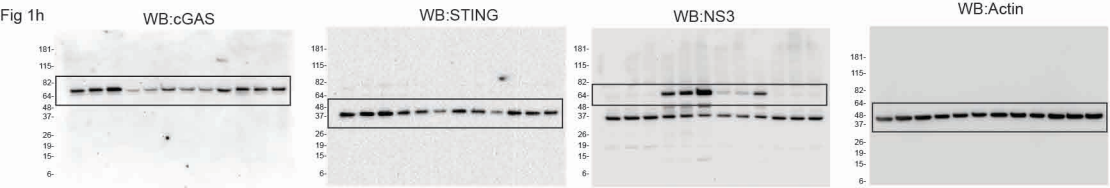


Fig 2b

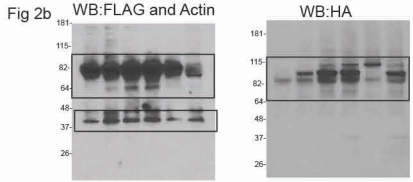


Fig 2c

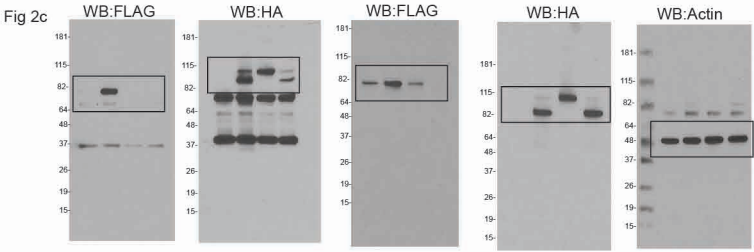


Fig 2d

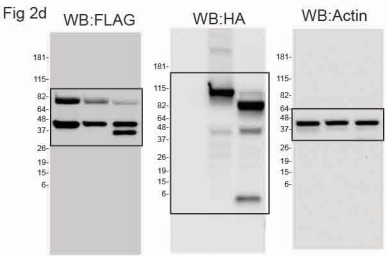


Fig 2e

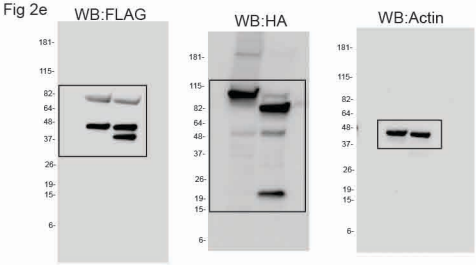
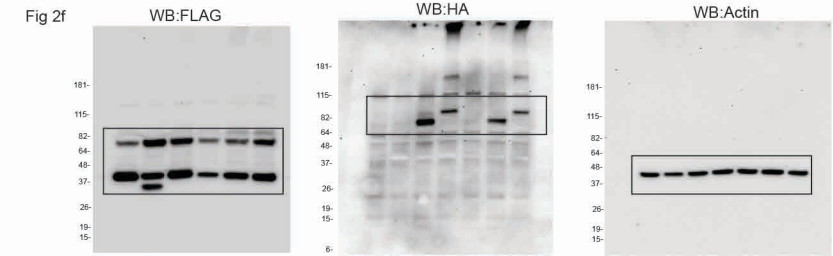


Fig 2f



Supplementary Figure 6

Fig 3a

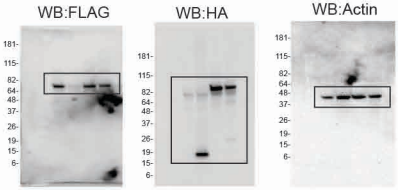


Fig 3b

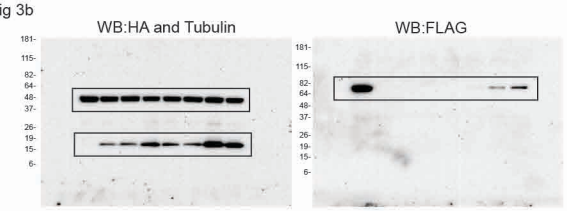


Fig 3c and d

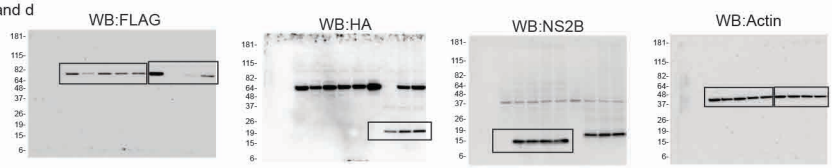
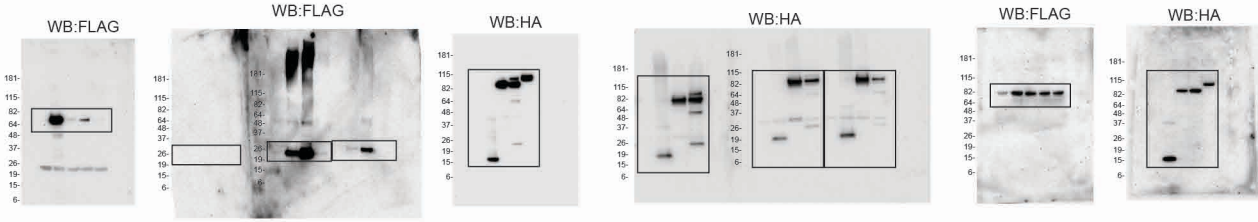
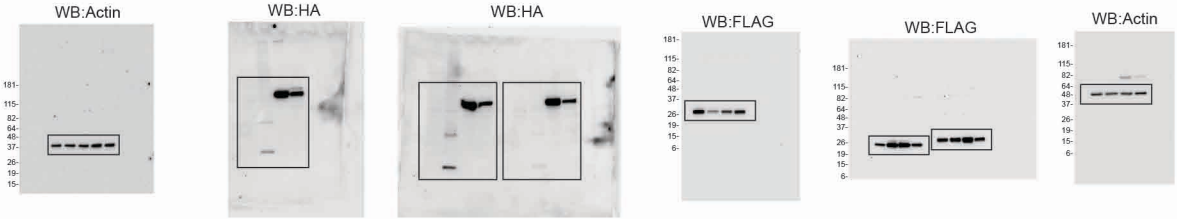
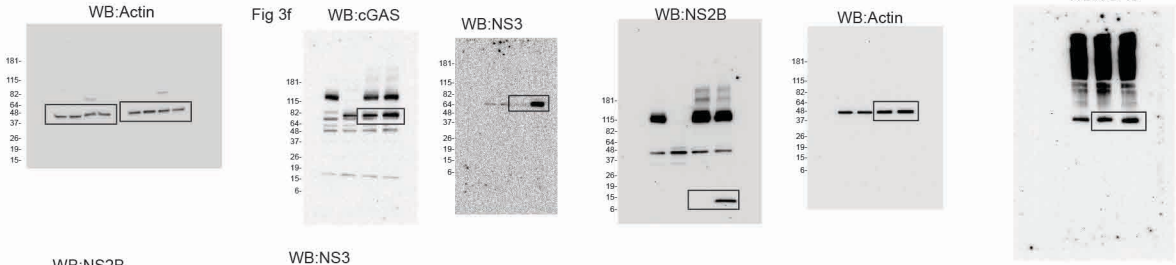


Fig 3e







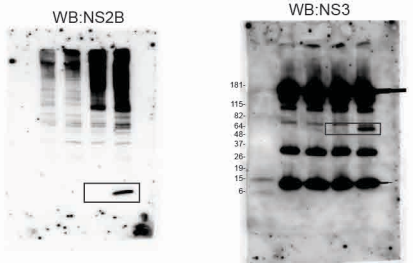


Fig 4a

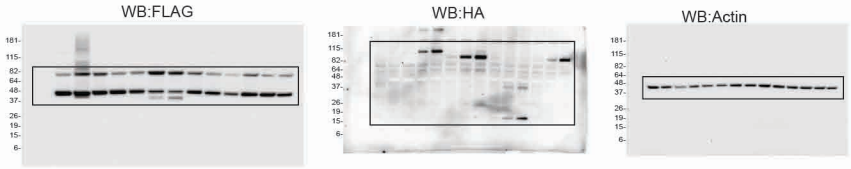


Fig 4b

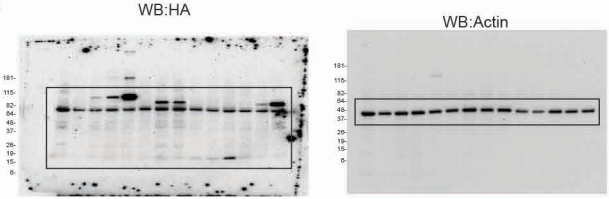


Fig S2a

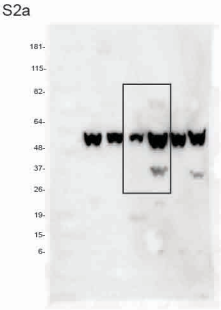


Fig 6a

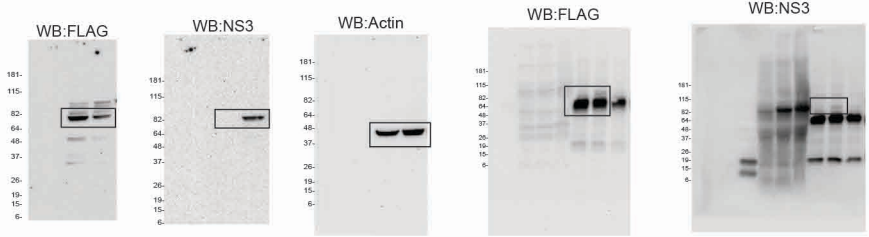


Fig S3a

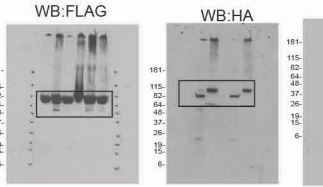


Fig S3b

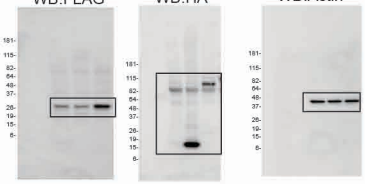


Fig S3c

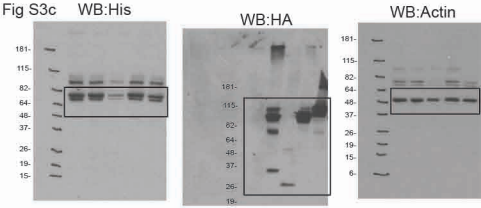
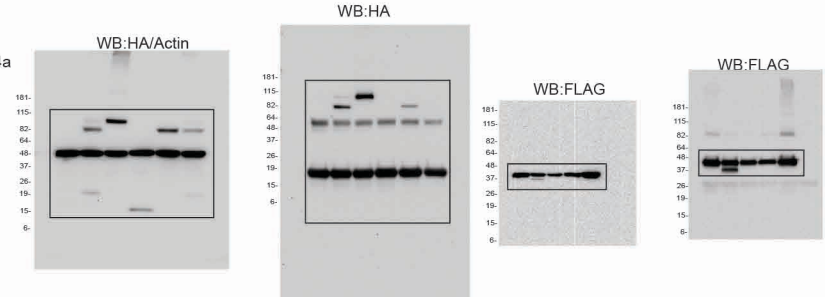


Fig S4a



Supplementary figure 6: Complete images from blots used throughout the manuscript with the corresponding molecular weight markers are shown. Cropped areas in every blot are marked and the antibody used in each case is named.