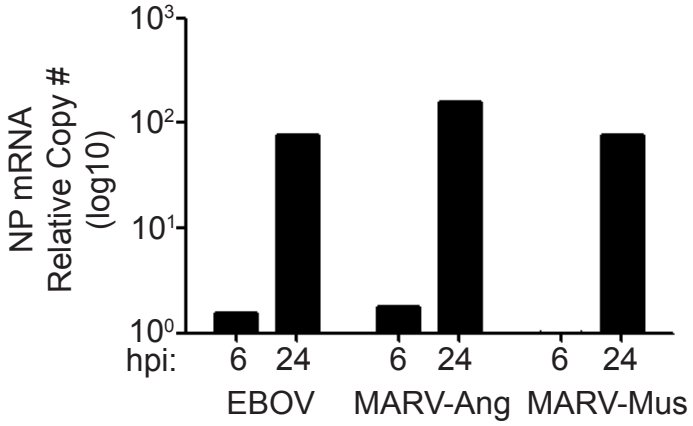
A


NP mRNA
Relative Copy #
(log10)
10^3
10^2
10^1
10^0
hpi: 6 24 6 24 6 24
EBOV
MARV-Ang
MARV-Mus

B

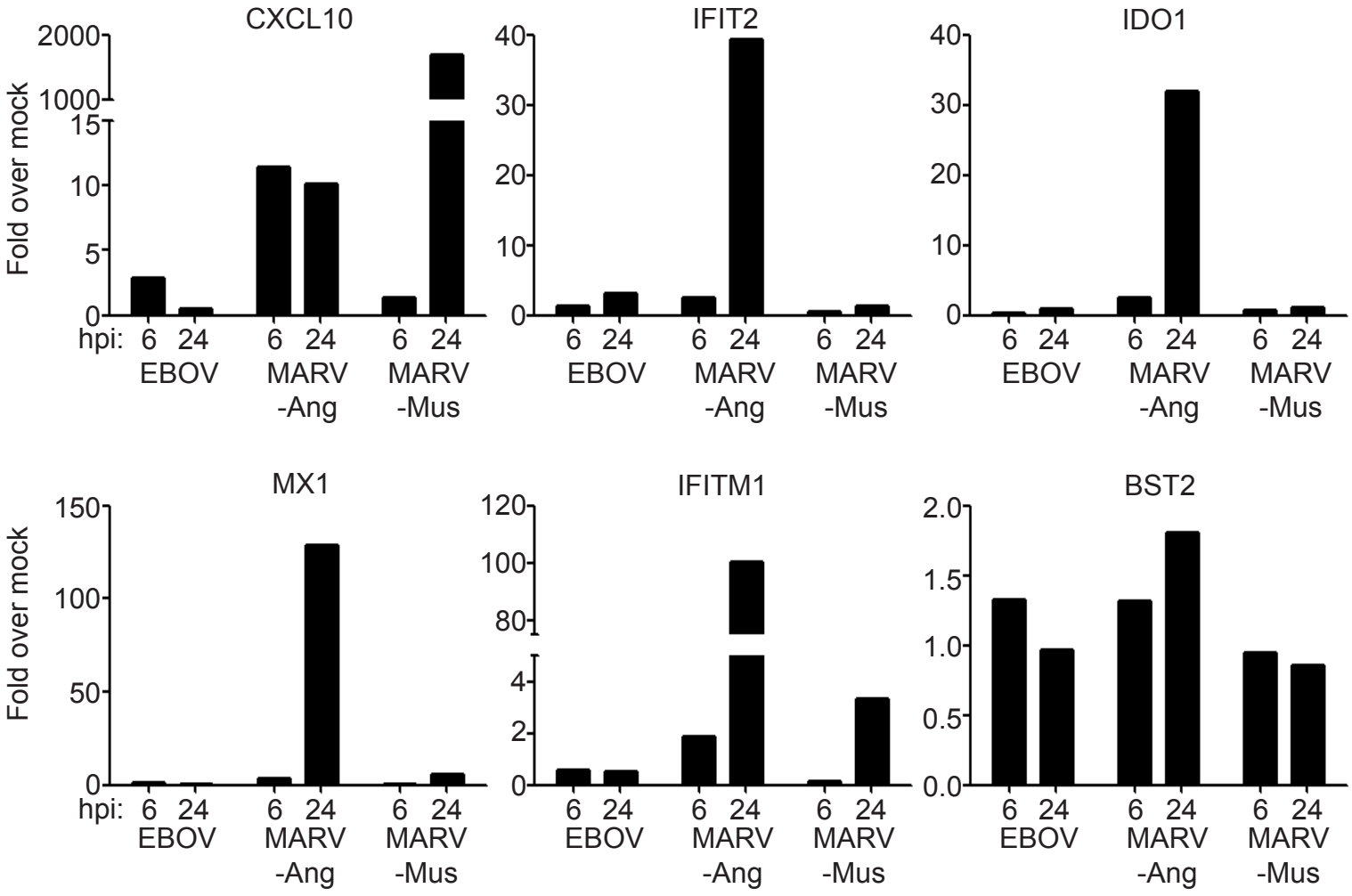

CXCL10
IFIT2
IDO1
MX1
IFITM1
BST2
Fold over mock
hpi: 6 24
EBOV
MARV -Ang
MARV -Mus

Figure S1 (related to Figure 1). EBOV and MARV show differential expression of IFN-regulated genes in THP-1 cells. (A) and (B) THP-1 cells were infected with EBOV, MARV-Ang and MARV-Mus (MOI=1). (A) Viral titers were monitored by filoviral NP mRNA present during infection using qRT-PCR at 6 and 24 hpi. (B) Expression levels of six ISGs at 6 and 24 hpi were analyzed by qRT-PCR. Values were normalized to *RPS11.*

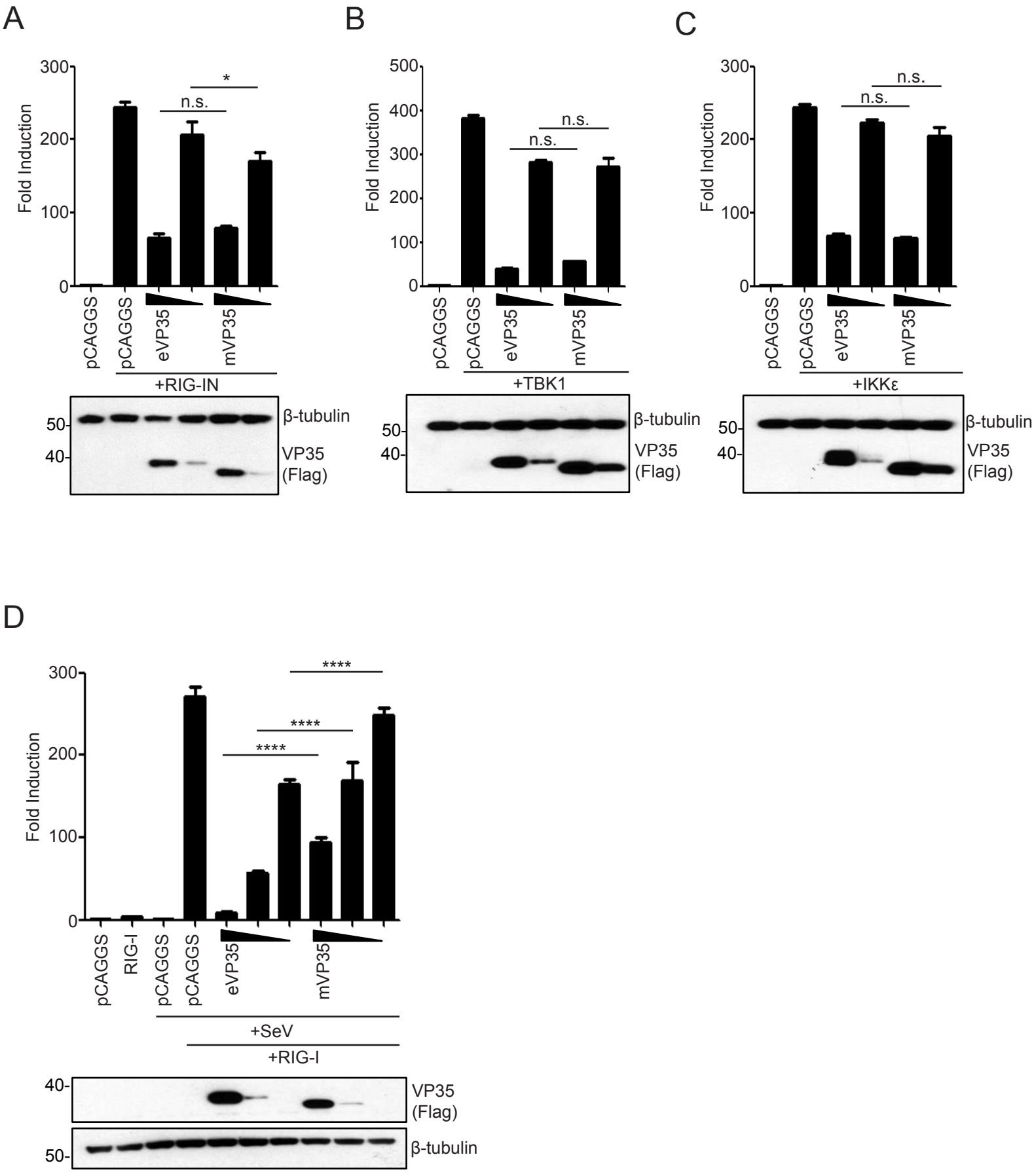

A
B
C
D
Fold Induction
300
200
100
0
500
400
300
n.s.
*
****
pCAGGS
RIG-I
eVP35
mVP35
+RIG-IN
+TBK1
+IKKε
+SeV
+RIG-I
50
40
β-tubulin
VP35
(Flag)

Figure S2 (related to Figure 2). eVP35 and mVP35 inhibit dsRNA independent activation of the IFN-β pathway similarly. (A-C) HEK293T cells were transfected with IFN-β firefly luciferase reporter plasmid, a constitutively expressed *Renilla* luciferase reporter plasmid and empty vector (pCAGGS) or decreasing concentrations of eVP35 or mVP35 (250ng and 25ng). IFN-β reporter activity was stimulated by overexpression of (A) RIG-IN (25ng), (B) TBK1 (10ng) or (C) IKKε (10ng). (D) Rig-I KO HEK293T cells were transfected with IFN-β firefly luciferase reporter plasmid, a constitutively expressed *Renilla* luciferase reporter plasmid and empty vector (pCAGGS) or decreasing concentrations of eVP35 or mVP35 (500ng, 50ng and 5ng) in the presence of transfected RIG-I (25ng). 24 h post transfection cells were infected with SeV. Values represent the mean and SEM of triplicate samples, and statistical significance was assessed by a one-way ANOVA comparing columns as indicated; n.s. not significant, *$p<0.05$ and ****$p<0.0001$.

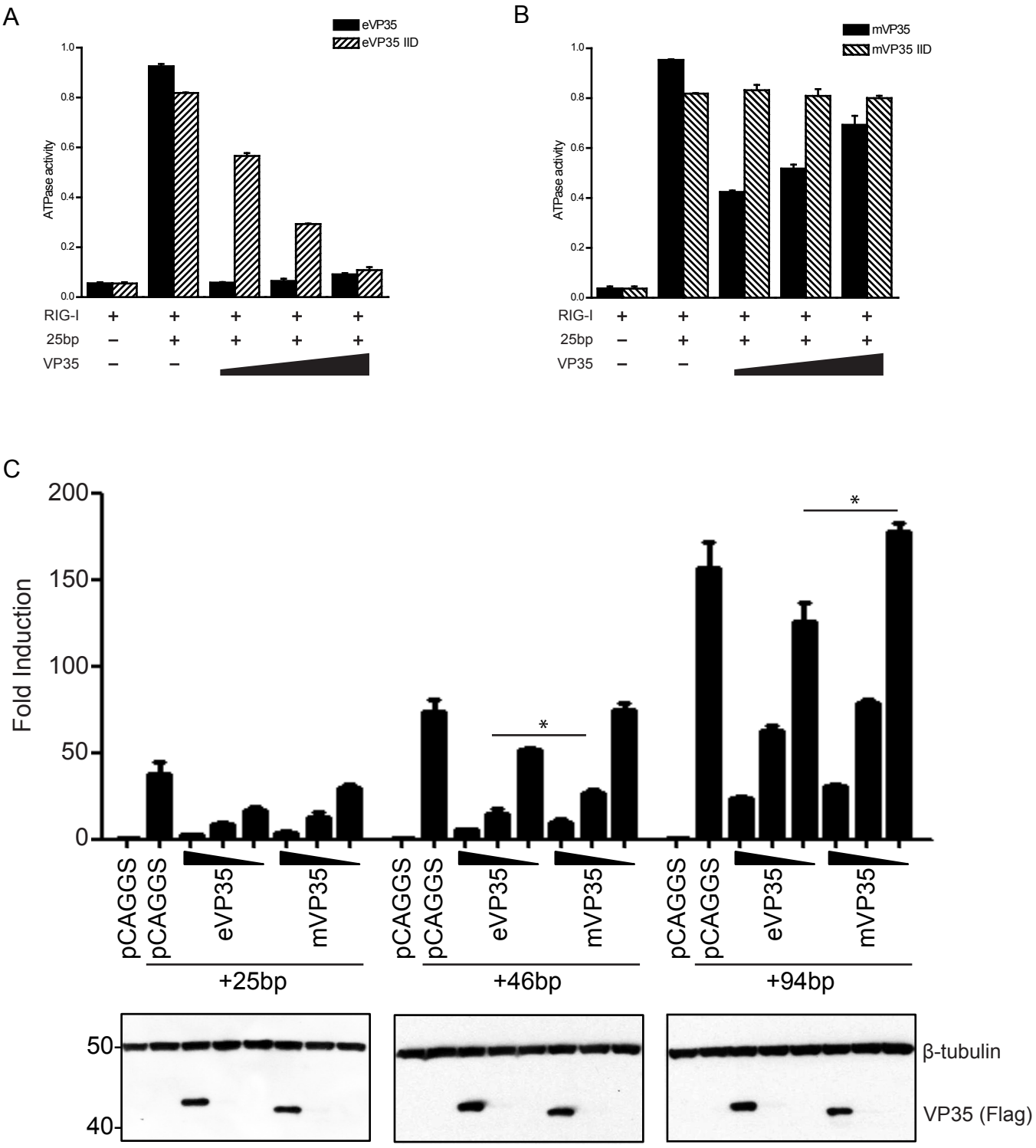

A
eVP35
eVP35 IID
ATPase activity
1.0
0.8
0.6
0.4
0.2
0.0
RIG-I + + + + +
25bp – + + + +
VP35 – –
B
mVP35
mVP35 IID
ATPase activity
1.0
0.8
0.6
0.4
0.2
0.0
RIG-I + + + + +
25bp – + + + +
VP35 – –
C
Fold Induction
200
150
100
50
0
*
*
pCAGGS
pCAGGS
eVP35
mVP35
pCAGGS
pCAGGS
eVP35
mVP35
pCAGGS
pCAGGS
eVP35
mVP35
+25bp
+46bp
+94bp
50
40
β-tubulin
VP35 (Flag)

Figure S3 (related to Figure 3). VP35 inhibition of RIG-I activation is dependent on VP35 oligomerization state and dsRNA length. (A-B) Full length VP35 is a more effective inhibitor of the RIG-I activation than IID alone. 5, 10, and 25 μM of full length (A) eVP35 (solid gray) and eVP35 IID (gray stripes) or (B) mVP35 (solid black) or mVP35 IID (black stripes) were used to inhibit the activation of RIG-I (1 μM) by 25 nM of 25 bp dsRNA. The ATPase activity was normalized to that of RIG-I in the absence of VP35. (C) HEK293T cells were transfected with IFN-β firefly luciferase reporter plasmid, a constitutively expressed *Renilla* luciferase reporter plasmid and empty vector (pCAGGS) or decreasing concentrations of eVP35 or mVP35 (250ng, 25ng and 2.5ng). 24 h post transfection IFN-β reporter activity was stimulated by the transfection of 35 fmol of SeV DI RNA of different lengths (25, 46, or 94 bp). Values represent the mean and SEM of triplicate samples, and statistical significance was assessed by a one-way ANOVA comparing columns as indicated; *$p<0.05$.

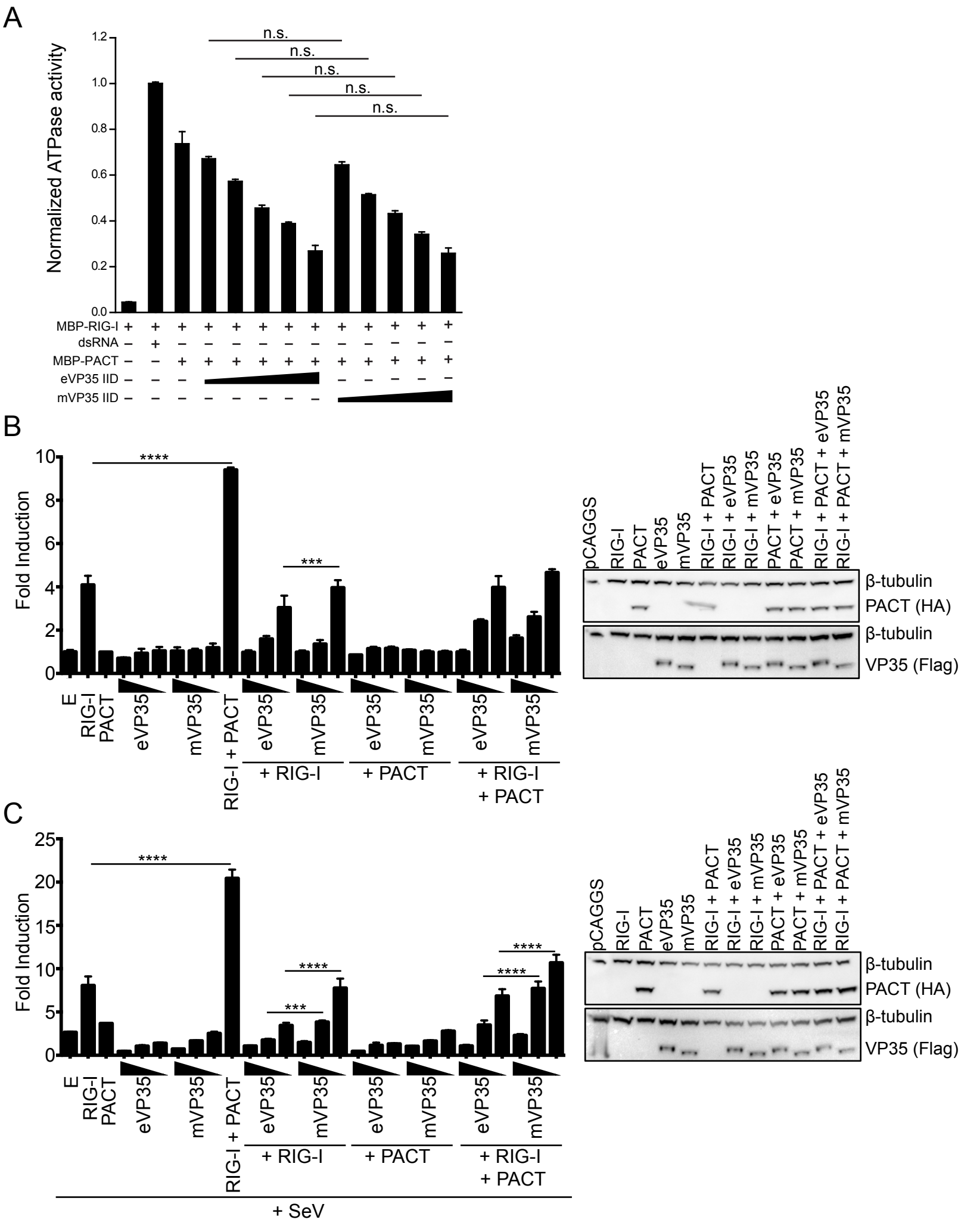

A
Normalized ATPase activity
n.s.
n.s.
n.s.
n.s.
n.s.
MBP-RIG-I
dsRNA
MBP-PACT
eVP35 IID
mVP35 IID
B
Fold Induction
****
***
E
RIG-I
PACT
eVP35
mVP35
RIG-I + PACT
+ RIG-I
+ PACT
+ RIG-I + PACT
pCAGGS
RIG-I
PACT
eVP35
mVP35
RIG-I + PACT
RIG-I + eVP35
RIG-I + mVP35
PACT + eVP35
PACT + mVP35
RIG-I + PACT + eVP35
RIG-I + PACT + mVP35
β-tubulin
PACT (HA)
β-tubulin
VP35 (Flag)
C
Fold Induction
****
***
****
****
****
+ SeV

Figure S4 (related to Figure 4). PACT does not show any significant impact on the inhibition of RIG-I ATPase activity by eVP35 IID and mVP35 IID. (A) MBP-RIGI full length at 1 μM is activated by 700nM MBP-PACT in the absence and presence of IID between 5 and 25 μM. dsRNA (8 bp, 50 nM) was used as a positive control. Statistical significance is as indicated: n.s. not significant. (B) and (C) HEK293T cells were transfected with IFN-β firefly luciferase reporter plasmid, a constitutively expressed *Renilla* luciferase reporter plasmid and the indicated plasmids. 24 h post transfection cells were (B) mock infected or (C) infected with Sendai virus (SeV). Luciferase activity was assayed at 18 hpi. VP35 and PACT expression for the controls and highest concentration of VP35 was assessed by western blot for Flag and HA as indicated. Values represent the mean and SEM of triplicate samples, and statistical significance was assessed for one-way ANOVA comparing columns as indicated; ***$p<0.001$ and ****$p<0.0001$.

Table S1 (related to Figure 3). Full length VP35 form oligomers

construct	Mw of monomer (kDa)	Apparent Mw (kDa) (± percentage error (%))	Polydispersity Mw/Mn (percentage error (%))	Oligomeric state
eIID	13.86	15.07 (± 9.97%)	1.044 (± 12.551%)	Monomer
mIID	13.81	13.76 (± 2.78%)	1.009 (± 3.981%)	Monomer
Full length MBP-eVP35	81.39	324.9 (± 4.9%)	1.017 (± 6.489%)	Tetramer
Full length MBP-mVP35	80.17	346.4 (± 2.8%)	1.003 (± 3.927%)	Tetramer