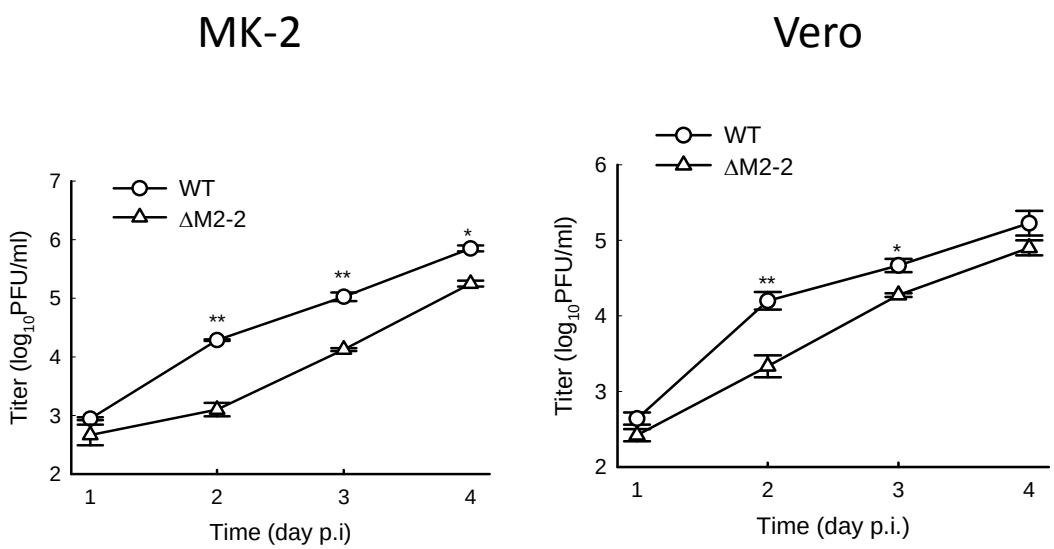



**Fig. S1**

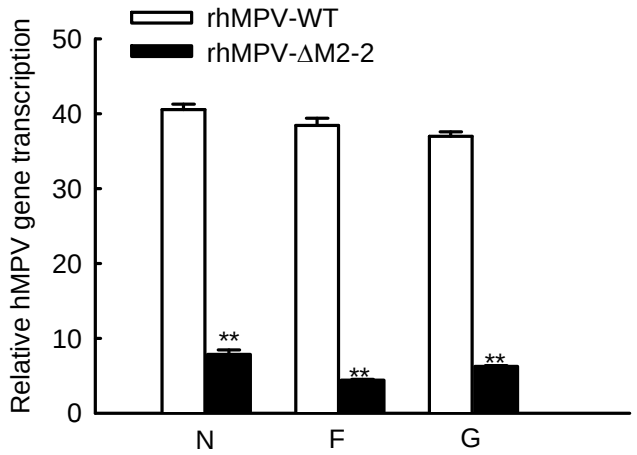

rhMPV-WT
rhMPV-ΔM2-2
Relative hMPV gene transcription
50
40
30
20
10
0
**
**
**
N
F
G

**Fig. S2**

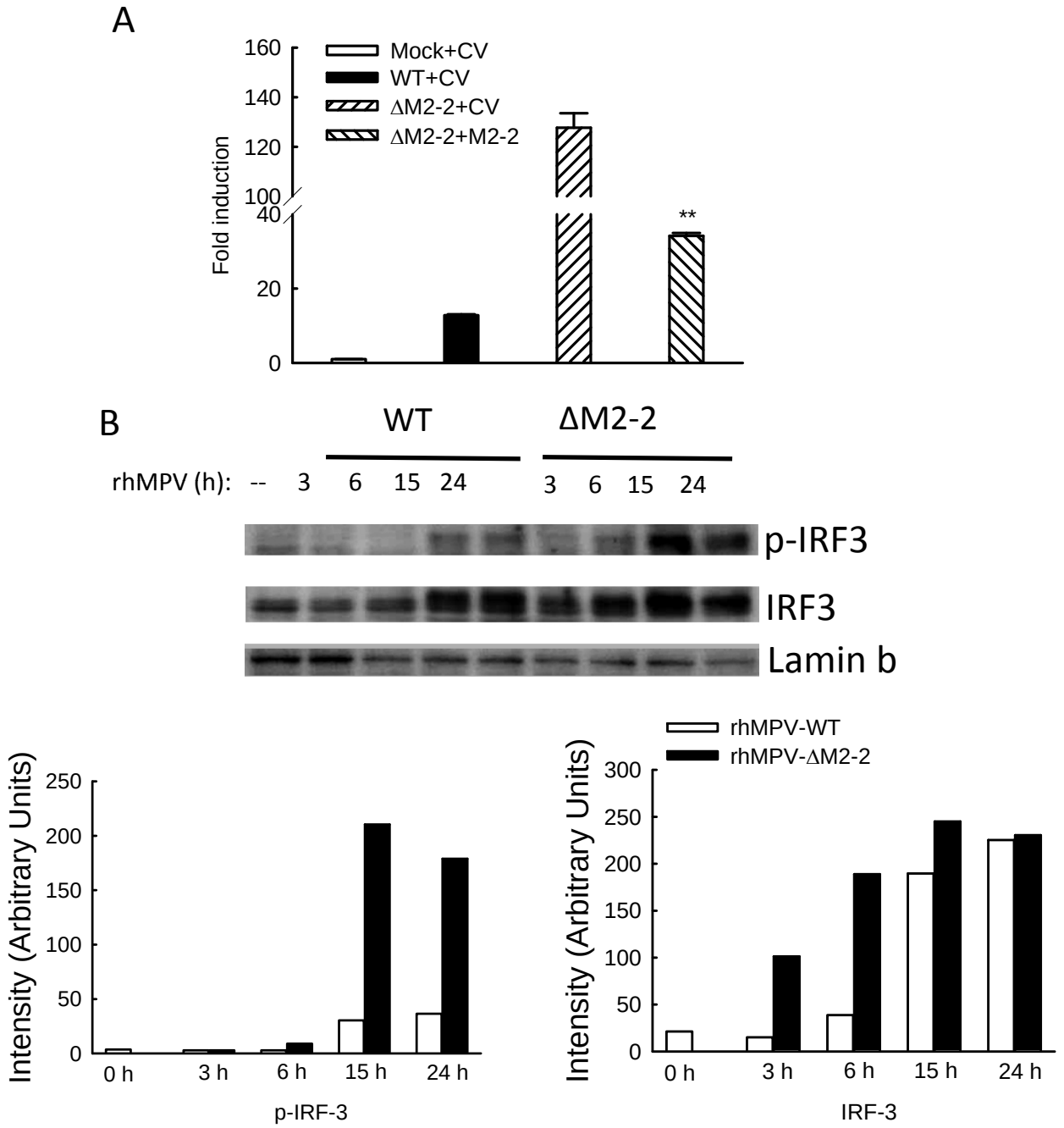

A
Mock+CV
WT+CV
ΔM2-2+CV
ΔM2-2+M2-2
Fold induction
160
140
120
100
40
20
0
**
B
WT
ΔM2-2
rhMPV (h): -- 3 6 15 24 3 6 15 24
p-IRF3
IRF3
Lamin b
rhMPV-WT
rhMPV-ΔM2-2
Intensity (Arbitrary Units)
250
200
150
100
50
0
0 h
3 h
6 h
15 h
24 h
p-IRF-3
Intensity (Arbitrary Units)
300
250
200
150
100
50
0
0 h
3 h
6 h
15 h
24 h
IRF-3

**Fig. S3**

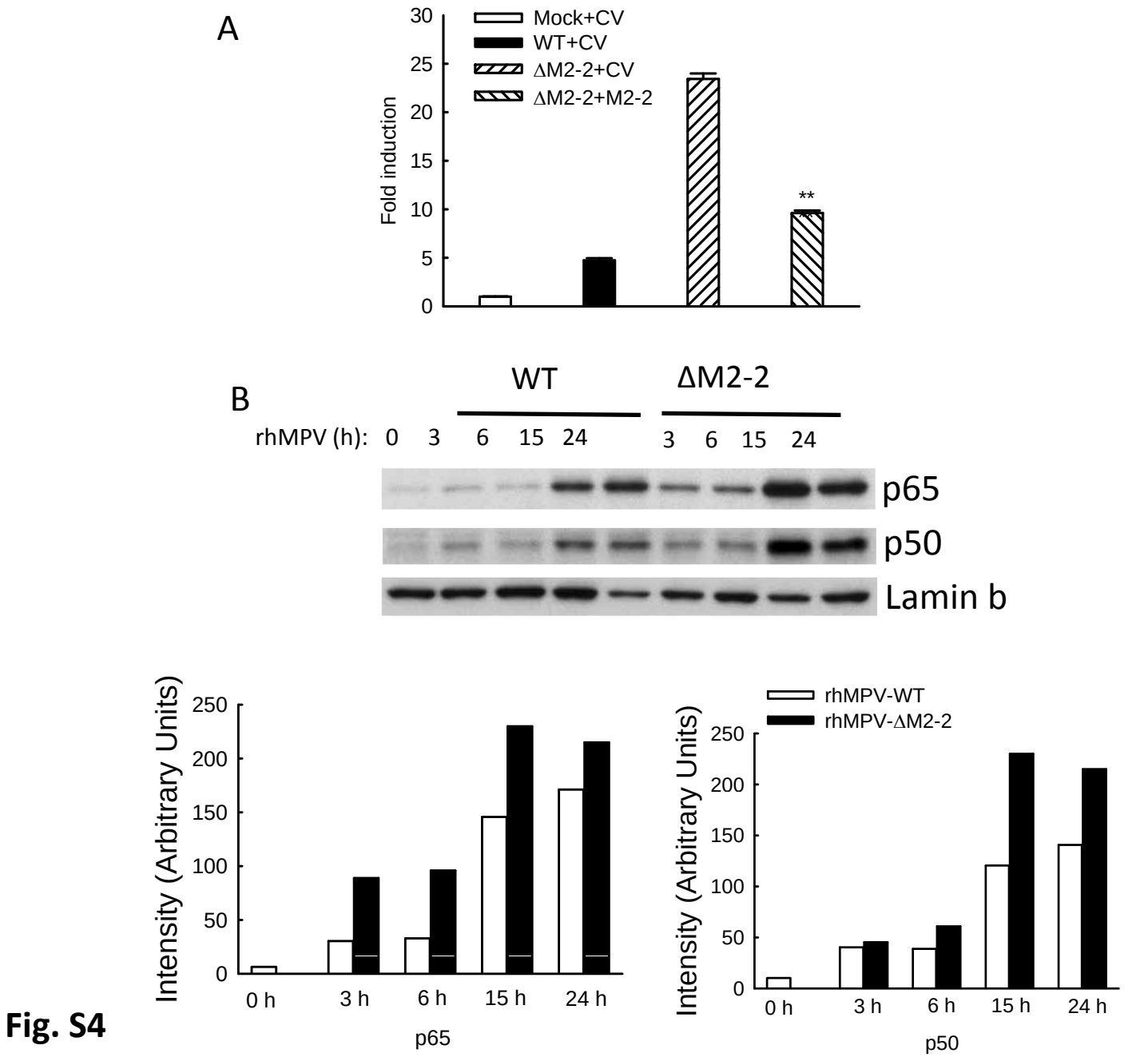

A
Mock+CV
WT+CV
ΔM2-2+CV
ΔM2-2+M2-2
Fold induction
30
25
20
15
10
5
0
**
B
WT
ΔM2-2
rhMPV (h): 0 3 6 15 24 3 6 15 24
p65
p50
Lamin b
Intensity (Arbitrary Units)
250
200
150
100
50
0
0 h
3 h
6 h
15 h
24 h
p65
rhMPV-WT
rhMPV-ΔM2-2
Intensity (Arbitrary Units)
p50

**Fig. S4**

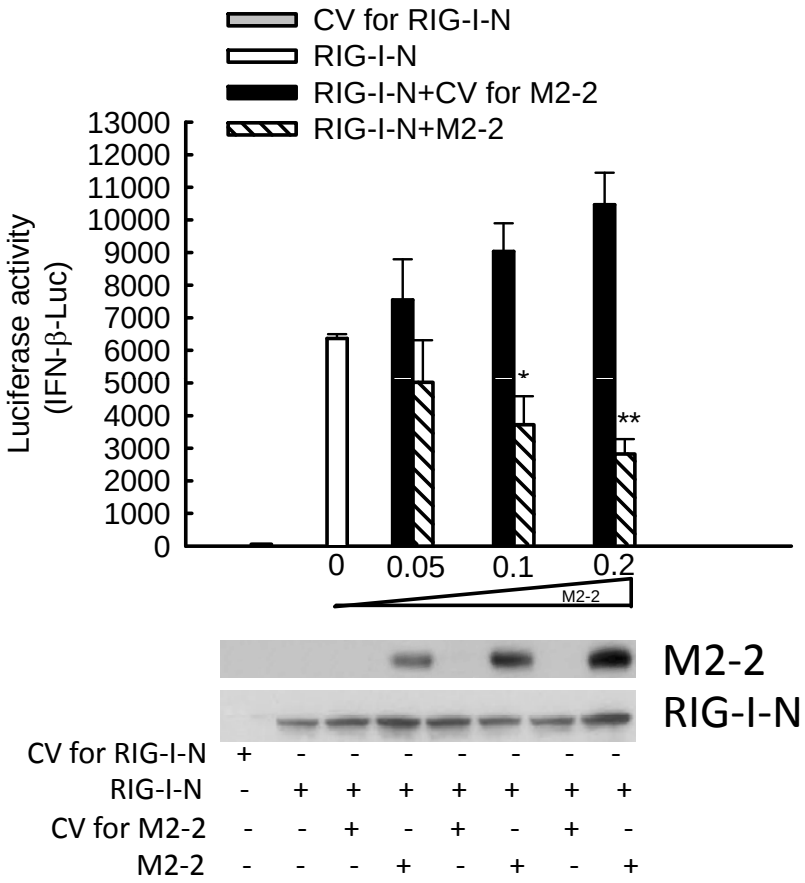
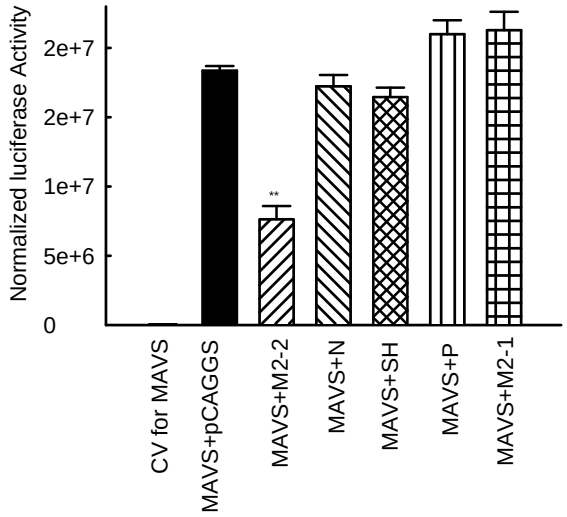

CV for RIG-I-N
RIG-I-N
RIG-I-N+CV for M2-2
RIG-I-N+M2-2
Luciferase activity (IFN-β-Luc)
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
*
**
0
0.05
0.1
0.2
M2-2
M2-2
RIG-I-N
CV for RIG-I-N + - - - - - - -
RIG-I-N - + + + + + + +
CV for M2-2 - - + - + - + -
M2-2 - - - + - + - +

**Fig. S5**


Normalized luciferase Activity
2e+7
2e+7
1e+7
5e+6
0
**
CV for MAVS
MAVS+pCAGGS
MAVS+M2-2
MAVS+N
MAVS+SH
MAVS+P
MAVS+M2-1

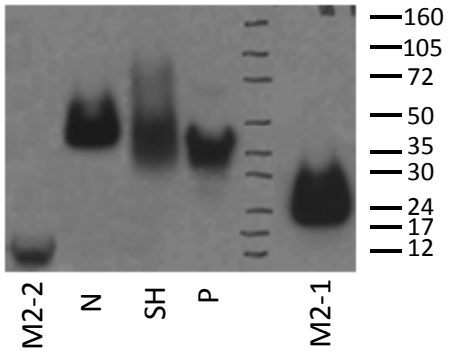

160
105
72
50
35
30
24
17
12
M2-2
N
SH
P
M2-1

**Fig. S6**

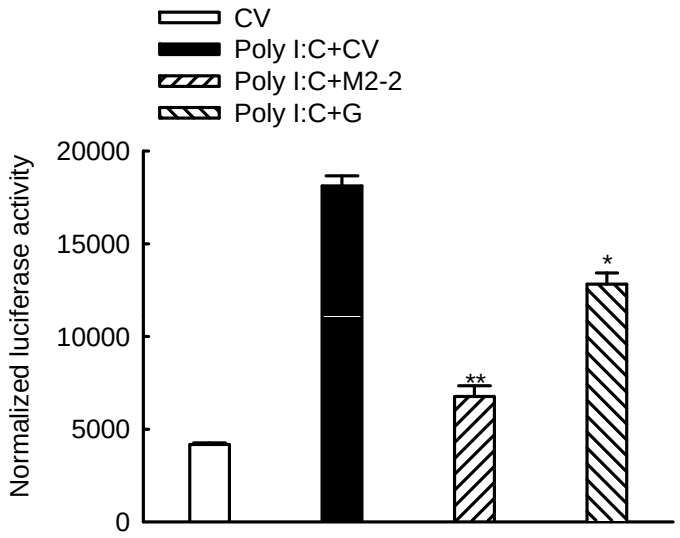

CV
Poly I:C+CV
Poly I:C+M2-2
Poly I:C+G
Normalized luciferase activity
20000
15000
10000
5000
0
**
*

**Fig. S7**

| Net (pg/ml) | **IFN-β** | **RANTES** | **KC** |
|---|---|---|---|
| **WT** | 170.08±9.07 | 231.95±42.66 | 217.52±32.68 |
| **MAVS-/-** | N.D** | N.D** | 35.26±19.48* |

**Table I. The effect of MAVS on chemokines/cytokine induction following hMPV infection.** Confluent MEFs derived from wild type (WT) or MAVS-/- mice were infected with hMPV and harvested supernatant at 24 h p.i. IFN-β was quantified by IFN-β ELISA kit from PBL, while RANTES and KC were measured by ELISA kits from R&D. Data are from two independent experiments and are expressed as mean± SE of net induction.*, $P<0.05$; **, $P<0.01$, relative to WT.

**Fig. S1. Multiple-cycle growth kinetics of WT and ΔM2-2**. MK-2 or Vero cells were infected at MOI of 0.1 PFU/cell with rhMPV-WT or rhMPV-M2-2. At 24-h intervals, cells in suspension were sonicated, followed by centrifugation. Viral particles in supernatant were analyzed by immunostaining with an anti-hMPV antibody. Each time point was represented by two wells, and each virus titration was done in triplicate. Data are expressed as mean±SE. **, $P<0.01$, relative to rhMPV-WT-infected cells.

**Fig. S2. Viral gene transcription of WT and ΔM2-2.** Vero cells were mock infected or infected with rhMPV at MOI of 2 for 15 h, followed by total RNA extraction using Trizol. The extracted RNAs in triplicate were then subjected to real-time PCR to assay the relative gene transcription of N, F, and G. The transcribed genes were first normalized by 18S, followed by the normalization by respective gene transcription in WT-infected cells at 3 h p.i. The results are the representative of two independent experiments and are expressed as mean±SE of transcribed genes. **, $P<0.01$, relative to rhMPV-WT-infected Vero cells.

**Fig. S3. hMPV M2-2 protein modulates viral-induced IRF-3 activation.** (**A**) HEK 293 cells in triplicate were transfected with a luciferase report plasmid IRF-3-Luc, a plasmid encoding M2-2 protein or its control vector, followed by the infection with rhMPV-WT or -ΔM2-2, at MOI of 2, for 15 h. Mock infection served as controls. Cells were lysed to measure luciferase activity. For each plate, luciferase was normalized to the β-galactosidase reporter activity. Data are representative of two independent experiments and are expressed as mean± SE of normalized luciferase activity. **, $P<0.01$ relative to rhMPV-WT. (**B**) A549 cells were infected with rhMPV-WT or rhMPV-ΔM2-2, at MOI of 2, for various lengths of time and harvested to prepare nuclear extracts. Equal amounts of protein from uninfected and infected cells were analyzed by Western blot using either an anti-phosphorylated IRF-3 or anti-IRF-3 antibody. Membranes were stripped and reprobed for lamin b, as control for equal loading of the samples. The band intensity was analyzed using Vision Works, a software associated with a Western image machine from UVP (Upland, CA). Data are representative of two independent experiments.

**Fig. S4. hMPV M2-2 protein modulates viral-induced NF-κB activation.** (**A**) HEK 293 cells in triplicate were transfected with a luciferase plasmid Kb-5-Luc, and a plasmid expressing M2-2 or its control vector, followed by the infection with rhMPV-WT or -ΔM2-2, at MOI of 2, for 15 h. Mock infection served as controls. Cells were lysed to measure luciferase activity. For each plate, luciferase was normalized to the β-galactosidase reporter activity. Data are representative of two independent experiments and are expressed as mean± SE of normalized luciferase activity. **, $P<0.01$ relative to rhMPV-WT. (**B**) A549 cells were infected with viruses as described in **Fig. S3B**. Nuclear extracts from uninfected and infected cells were analyzed by Western blot using either an anti-p50 or anti-p65 antibody. Membranes were stripped and reprobed for lamin b, as

control for equal loading of the samples. The band intensity was analyzed using Vision Works. Data are representative of two independent experiments.

**Fig. S5. M2-2 inhibits RIG-I-mediated pathway in a dose-dependent manner**. 293 cells in triplicate were transfected with a luciferase plasmid IFN-β-Luc, plasmids encoding CARD domain of RIG-I (RIG-I-N) or its control vectors, and a plasmid expressing hMPV M2-2 or N or the empty vector in an increasing gradient. Cells were harvested 30 h post-transfection to measure luciferase activity. Luciferase was normalized to the β-galactosidase reporter activity. Data are representative of two independent experiments and are expressed as mean±SE of normalized luciferase activity. * $P<0.05$ and ** $P<0.01$: relative to RIG-I-N +CV. CV: control vector for M2-2.

**Fig. S6. The inhibitory effect of M2-2 on MAVS-induced signaling is viral protein specific**. 293 cells in triplicates were transfected with a luciferase plasmid IFN-β-Luc, a plasmid encoding MAVS or its control vector, and a plasmid encoding hMPV proteins as indicated. After 36 h, cells were harvested to measure the luciferase activity. Luciferase was normalized to the β-galactosidase reporter activity. Data are representative of two independent experiments and are expressed as mean±SE of normalized luciferase activity. * $P<0.05$ and ** $P<0.01$: relative to MAVS +pCAGGS.

**Fig. S7. M2-2 and G exhibited different sufficiency in inhibiting poly I:C-induced IFN-β transcription**. 293 cells in triplicates were transfected with a luciferase plasmid IFN-β-Luc, and a plasmid encoding M2-2 or G or its empty control vector. After 24 h, cells were untreated or treated with 0.1 μg/well poly I:C (LMW, Invivogen, San Diego, California) for additional 15 h, and then harvested to measure the luciferase activity. Luciferase was normalized to the β-galactosidase reporter activity. Data are representative of two independent experiments and are

expressed as mean±SE of normalized luciferase activity. * $P<0.05$ and ** $P<0.01$: relative to Poly I:C +CV. CV: control vector for M2-2.