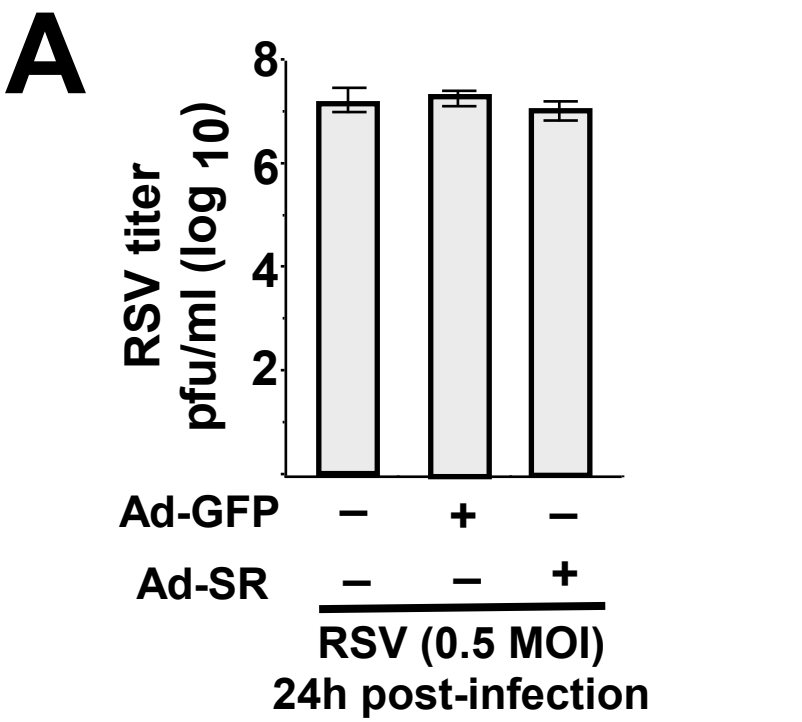
Fig. S1


A
RSV titer
pfu/ml (log 10)
8
6
4
2
Ad-GFP – + –
Ad-SR – – +
RSV (0.5 MOI)
24h post-infection

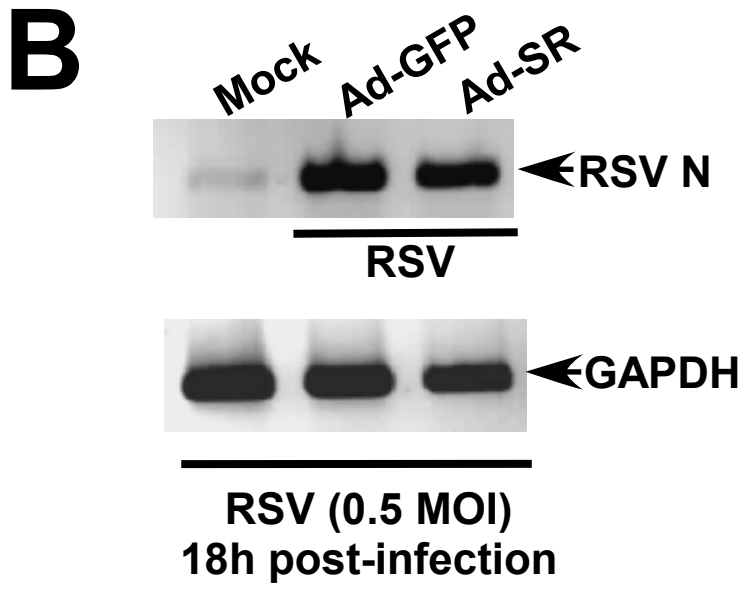

B
Mock
Ad-GFP
Ad-SR
RSV N
RSV
GAPDH
RSV (0.5 MOI)
18h post-infection

Fig. S2

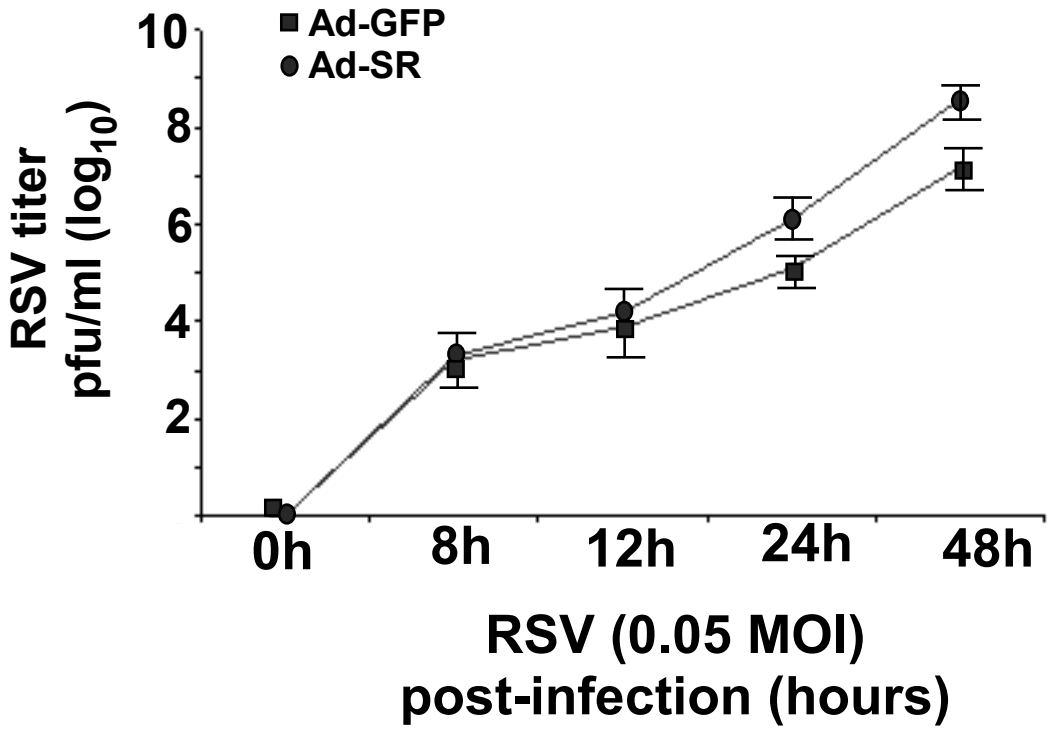

Ad-GFP
Ad-SR
RSV titer
pfu/ml (log10)
10
8
6
4
2
0h
8h
12h
24h
48h
RSV (0.05 MOI)
post-infection (hours)

Fig. S3

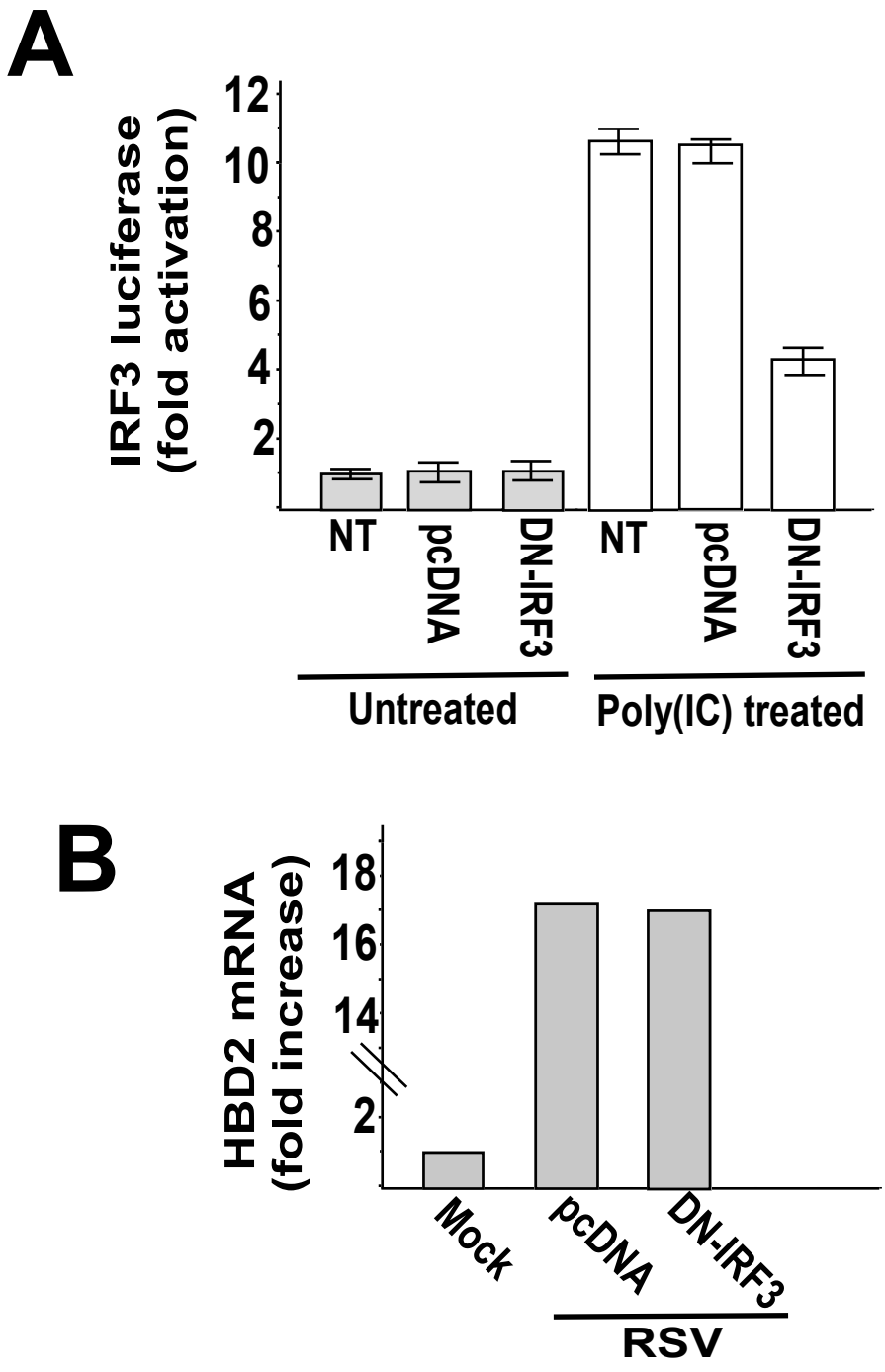

A
IRF3 luciferase
(fold activation)
12
10
8
6
4
2
NT
pcDNA
DN-IRF3
NT
pcDNA
DN-IRF3
Untreated
Poly(IC) treated
B
HBD2 mRNA
(fold increase)
18
16
14
2
Mock
pcDNA
DN-IRF3
RSV

Fig. S4

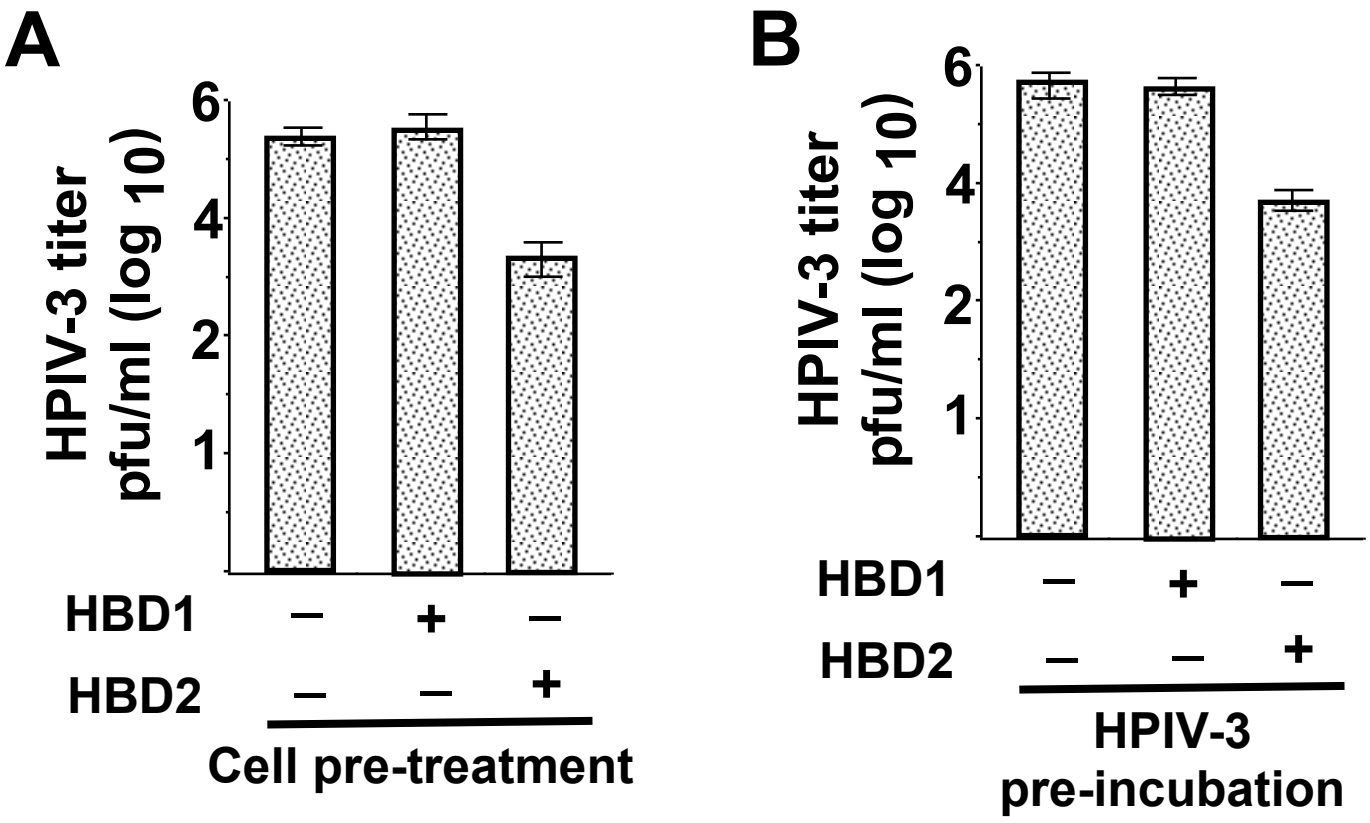

A
HPIV-3 titer
pfu/ml (log 10)
6
4
2
1
HBD1
HBD2
–
+
–
–
–
+
Cell pre-treatment
B
HPIV-3 titer
pfu/ml (log 10)
6
4
2
1
HBD1
HBD2
–
+
–
–
–
+
HPIV-3
pre-incubation

Fig. S5

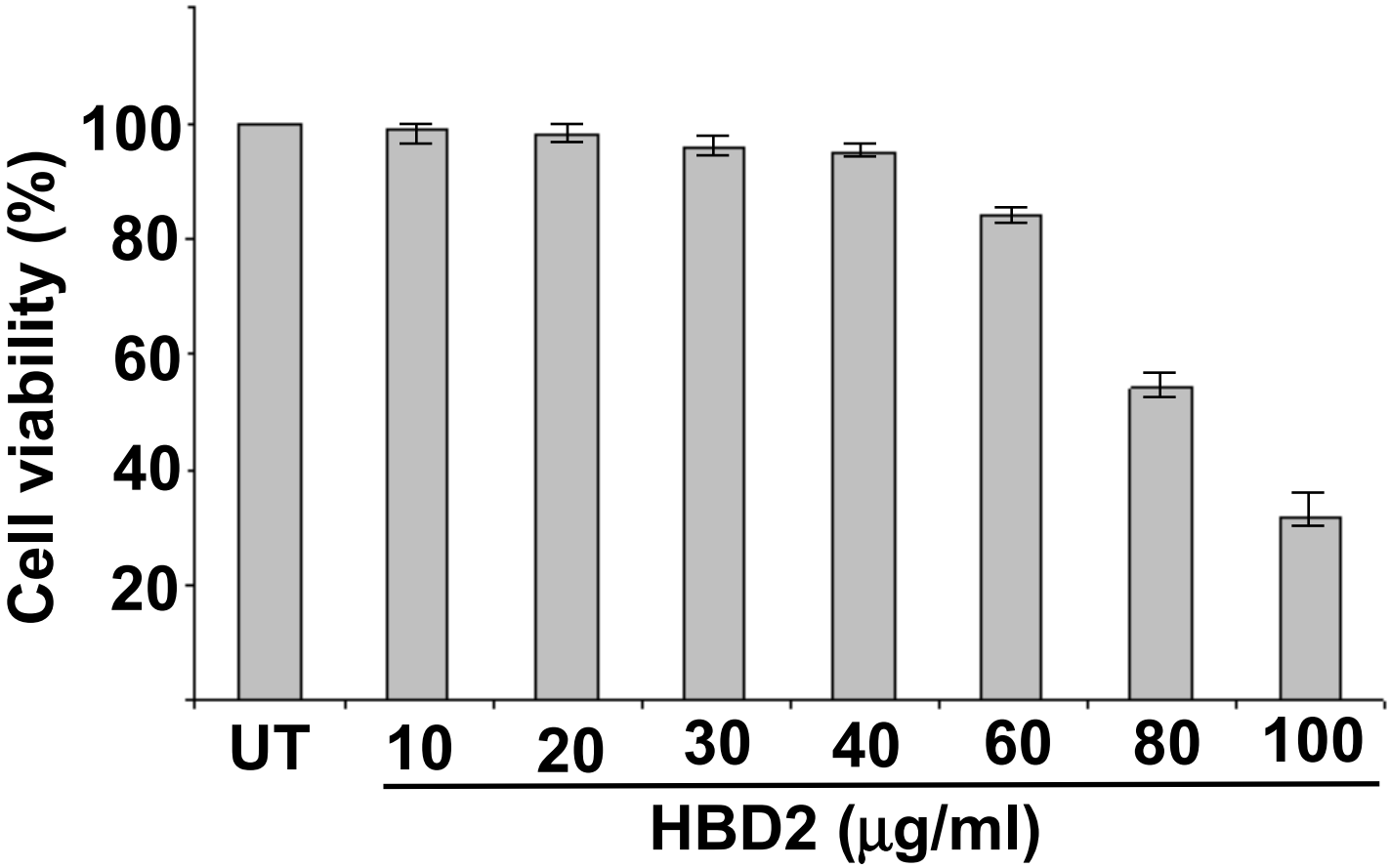


Fig. S6

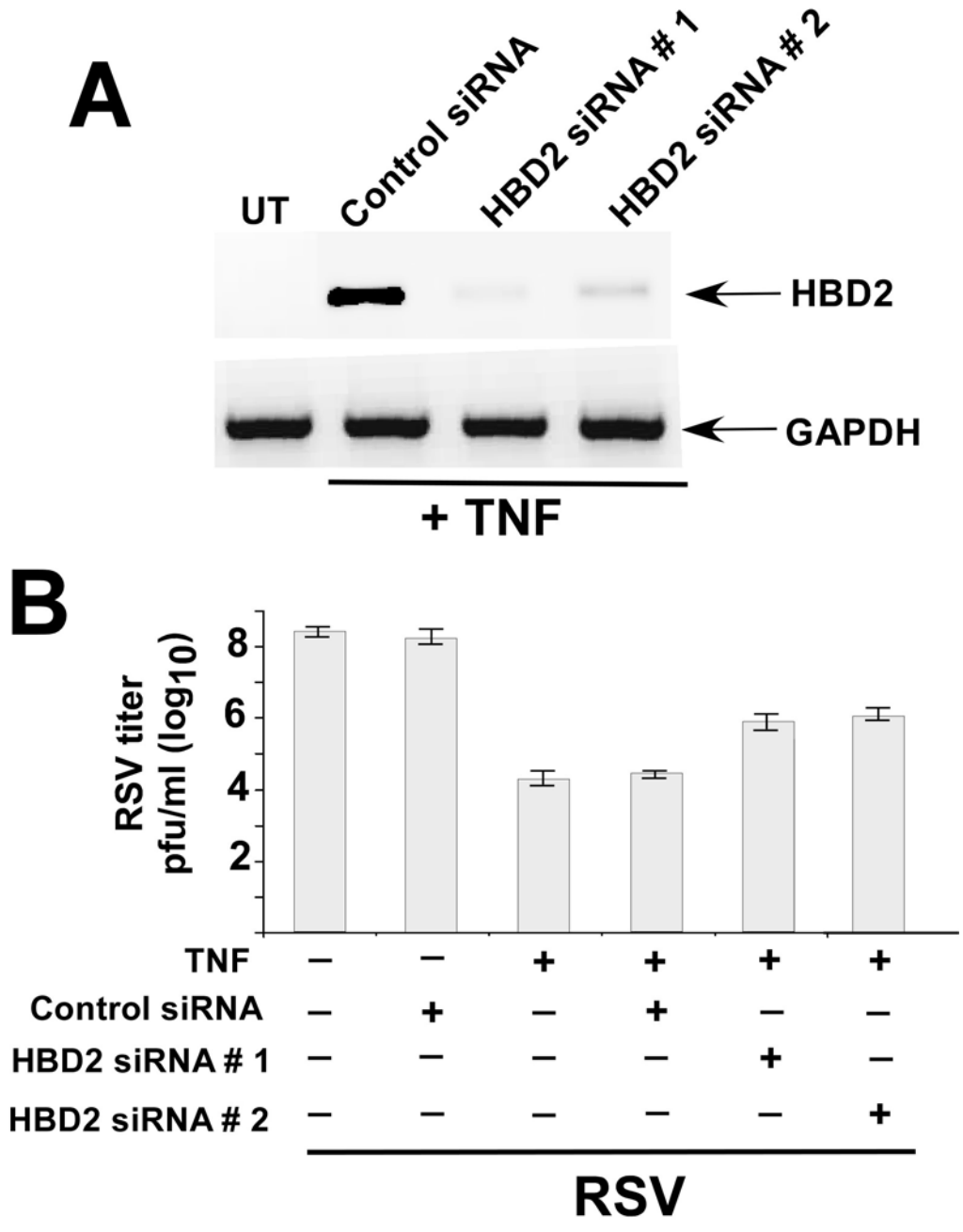

A
UT
Control siRNA
HBD2 siRNA # 1
HBD2 siRNA # 2
HBD2
GAPDH
+ TNF
B
RSV titer
pfu/ml (log10)
8
6
4
2
TNF
Control siRNA
HBD2 siRNA # 1
HBD2 siRNA # 2
RSV

Fig. S1. RSV infection in A549 cells expressing GFP and IκB-super repressor (IκB-SR). A. Medium supernatant collected from RSV infected (infected with 0.5 MOI RSV for 24h) A549 cells that were previously infected with recombinant adenovirus expressing either GFP (control) (Ad-GFP) or IκB-super repressor (Ad-SR) was subjected to plaque assay analysis. The plaque assay values for RSV are expressed as pfu/ml and each value represents the mean ± standard deviation for three determinations. B. Total RNA collected from mock infected and RSV (0.5 MOI) infected A549 cells (at 18h post-infection) infected with either Ad-GFP or Ad-SR were subjected to RT-PCR to detect RSV nucleocapsid (N protein) protein and GAPDH (loading control).

Fig. S2. RSV infection in A549 cells expressing GFP and IκB-SR. Medium supernatant collected from RSV infected (infected with 0.05 MOI RSV for 0h - 48h) A549 cells that were previously infected with recombinant adenovirus expressing either GFP (control) (Ad-GFP, black square) or IκB-super repressor (Ad-SR, black circle) was subjected to plaque assay analysis. The plaque assay values for RSV are expressed as pfu/ml and each value represents the mean ± standard deviation for three determinations.

Fig. S3. Role of interferon regulatory factor – 3 (IRF3) in HBD2 induction following RSV infection. A. Induction of luciferase activity (fold induction) in poly(IC) treated [treated with 120 μg/ml poly(IC) for 5h] A549 cells that were transfected (for 36h) with either pcDNA or dominant-negative IRF3 (DN-IRF3) and IRF3 luciferase reporter gene (firefly luciferase). The luciferase activity was normalized to the *Renilla* luciferase activity (dual luciferase assay from Promega) and the fold induction values represent the mean ± standard deviation for three determinations. B. A549 cells transfected with either pcDNA or DN-IRF3 for 36h were infected with RSV (0.5 MOI) for 16h. Total RNA collected from mock and RSV infected cells were subjected to real-time quantitative RT-PCR (qPCR) to detect HBD2 expression. The HBD2 transcript levels were normalized to human keratin 5 and the results are representative of three independent experiments with similar values.

Fig. S4. Anti-viral activity of HBD2 against human parainfluenza virus type 3 (HPIV-3) infection. A. A549 cells pre-treated with either HBD1 or HBD2 (3 μg/ml) were infected with HPIV-3 (in the absence of HBDs) and at 36h post-infection, viral titer was measured by plaque assay analysis. B. HPIV-3 virion particles pre-incubated with either HBD1 or HBD2 (3 μg/ml) were used to infect A549 cells (in the absence of HBDs) and at 36h post-infection, viral titer was measured by plaque assay analysis. The plaque assay values are expressed as pfu/ml and each value represents the mean ± standard deviation for three determinations.

Fig. S5. Cellular toxicity of HBD2. A549 cells treated with different concentrations of HBD2 (10 μg/ml – 100 μg/ml) for 60h were subjected to cell viability assay using MTT as described below. The results are expressed as percent (%) viable cells, where 100% viability represents the value obtained from untreated (UT) cells. The result represents the mean ± standard deviation for three determinations.

MTT cell viability assay - MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide] cell viability assay is based on the conversion of yellow colored MTT to purple colored formazan in living cells by mitochondrial reductase enzymes. The change in color (representing cell viability) is examined by measuring optical density (OD) at 540 nm. For the MTT assay, 5mg/ml of MTT was freshly prepared in 1X PBS. Following incubation of A549 cells with different concentrations of HBD2, MTT solution was added to each well. Following 4h incubation (at 37^{o}C), the media was discarded and the cells were washed with 1X PBS. DMSO was then added to each well and the cells were incubated (in a shaker) with DMSO at room temperature for 45

min. The DMSO solution collected from each well was centrifuged at 3000 RPM for 5 min to remove cell debris. The OD of supernatant (DMSO solution) was measured at 540 nm (DMSO was used as a blank).

Fig. S6. Silencing efficiency of HBD2 specific siRNAs and diminished anti-viral activity of TNF in HBD2 silenced cells. A. Total RNA collected from untreated (UT), and TNF treated (10 ng/ml TNF treatment for 16h) A549 cells transfected with either control siRNA or two different HBD2 specific siRNAs (HBD2 siRNA # 1 and HBD2 siRNA # 2 are directed against the central portion and C-terminal portion of HBD2, respectively) were subjected to RT-PCR to detect HBD2 and GAPDH (loading control). B. A549 cells transfected with either control or HBD2 specific siRNAs (siRNA # 1 and siRNA # 2) were pre-treated with TNF (20 ng/ml) for 20h, followed by RSV infection for 36h. Viral titer at 36h post-infection was measured by plaque assay analysis. The plaque assay values for the above experiments are expressed as pfu/ml and each value represents the mean ± standard deviation for three determinations.