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Supplemental Material to:

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Evidence for a crucial role of a host non-coding RNA in influenza A virus replication

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Antibodies		
Primary antibodies	**Company**	**Dilution**
mouse monoclonal beta-Actin	Sigma-Aldrich	1:3000
mouse monoclonal viral ion channel protein	Santa Cruz	1:1000
rabbit polyclonal 14-3-3-epsilon (YWHAE) p(Ser58) (ABIN318720)	antibodies-online	1:3000
rabbit polyclonal Lamin A/C (2032)	Cell Signaling	1:3000
rabbit anti-H1N1 Hemagglutinin (ABIN399002)	antibodies-online	1:1000
mouse anti Influenza A Nucleoprotein (MCA0400)	AbD Serotec	1:2500
mouse anti Influenza B Nucleoprotein (BM3149)	Acris Antibody	1:1000
goat polyclonal anti Influenza Non-Structural Protein 1	Santa Cruz	1:1000
mouse monoclonal anti-VSV Glycoprotein (P5D4)	Sigma-Aldrich	1:500
mouse anti-Digoxigenin IgG (11333062910)	Roche	1:200
Secondary antibodies	**Company**	**Dilution**
donkey anti-goat IgG HRP (sc2020)	Santa Cruz	1:3000
sheep anti-mouse IgG HRP (NA931)	Amersham	1:3000
donkey anti-rabbit IgG HRP (NA934)	Amersham	1:3000
Cy3-goat-anti-mouse IgG (115-165-146)	Dianova	1:100
Cy2-rabbit-anti-mouse IgG (315-225-003)	Dianova	1:100

Primer sequences		
Gene	**primer forward 5'-3'**	**primer reverse 5'-3'**
GAPDH	GGTATCGTGGAAGGACTCATGAC	ATGCCAGTGAGCTTCCCGTTCAG
RNU1-1	ATACTTACCTGGCAGGGGAG	CAGGGGAAAGCGCGAACGCA
MX1	GTTTCCGAAGTGGACATCGCA	GAAGGGCAACTCCTGACAGT
VIN	CTAGGAGACACCCGGACAGT	GCCCTGTGAGATGGGTTTAG
IFNβ	CAGCTCTTTCCATGAGCTAC	CAGCCAGTGCTAGATGAATC
ENST00000511543	AACCACCCCATCTACCATCA	TGGCTCAGCTGTACGATTTG
ENST00000499418	TGGAGCTTGCCTTCAACTTT	TTATTCTGCCACCAGGGAAG
ENST00000512341	ACTCAGTGATTTGCCCAAGG	CCAACAGGAAGATGGGACTC

siRNA sequences	
siRNA	**sequence 5'-3'**
IAV Nucleoprotein	AAGGAUCUUAUUUCUUCGGAG
VIN siRNA 1	CTGTGACATGTAGATTGCTAA
VIN siRNA 2	CCGGAGCCGTTTACAGTTTGA
VIN siRNA 3	CGCGCCCTGTCCCGCCATATA

Table S1
List of antibodies, primer sequences and siRNA sequences used

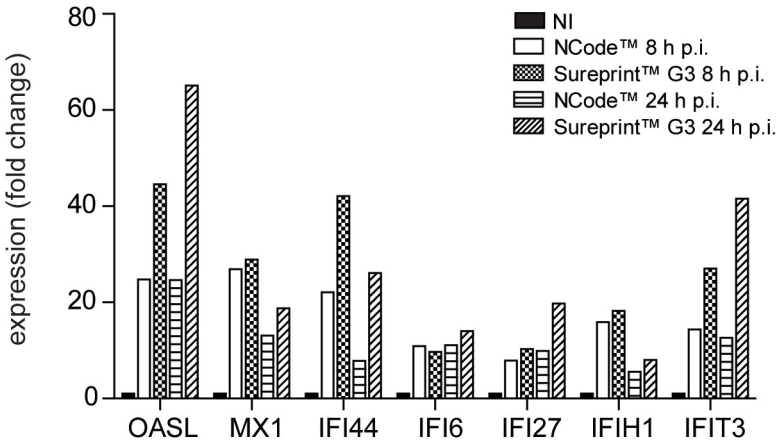

Figure S1: Induction of IFN-inducible genes by influenza A/WSN/33 (H1N1) virus infection. Human lung epithelial A549 cells were infected with influenza A/WSN/33 (H1N1) virus (MOI 1) for either 8 h or 24 h. RNA extracted from infected and uninfected control cells was reverse-transcribed using OligodT priming, labeled with a Cy5 or Cy3 fluorophore, and hybridized to NCode™ and Sureprint™ G3 microarrays. Data from two independent experiments are presented as mean fold-change expression of the indicated genes compared with non-infected (NI) reference.

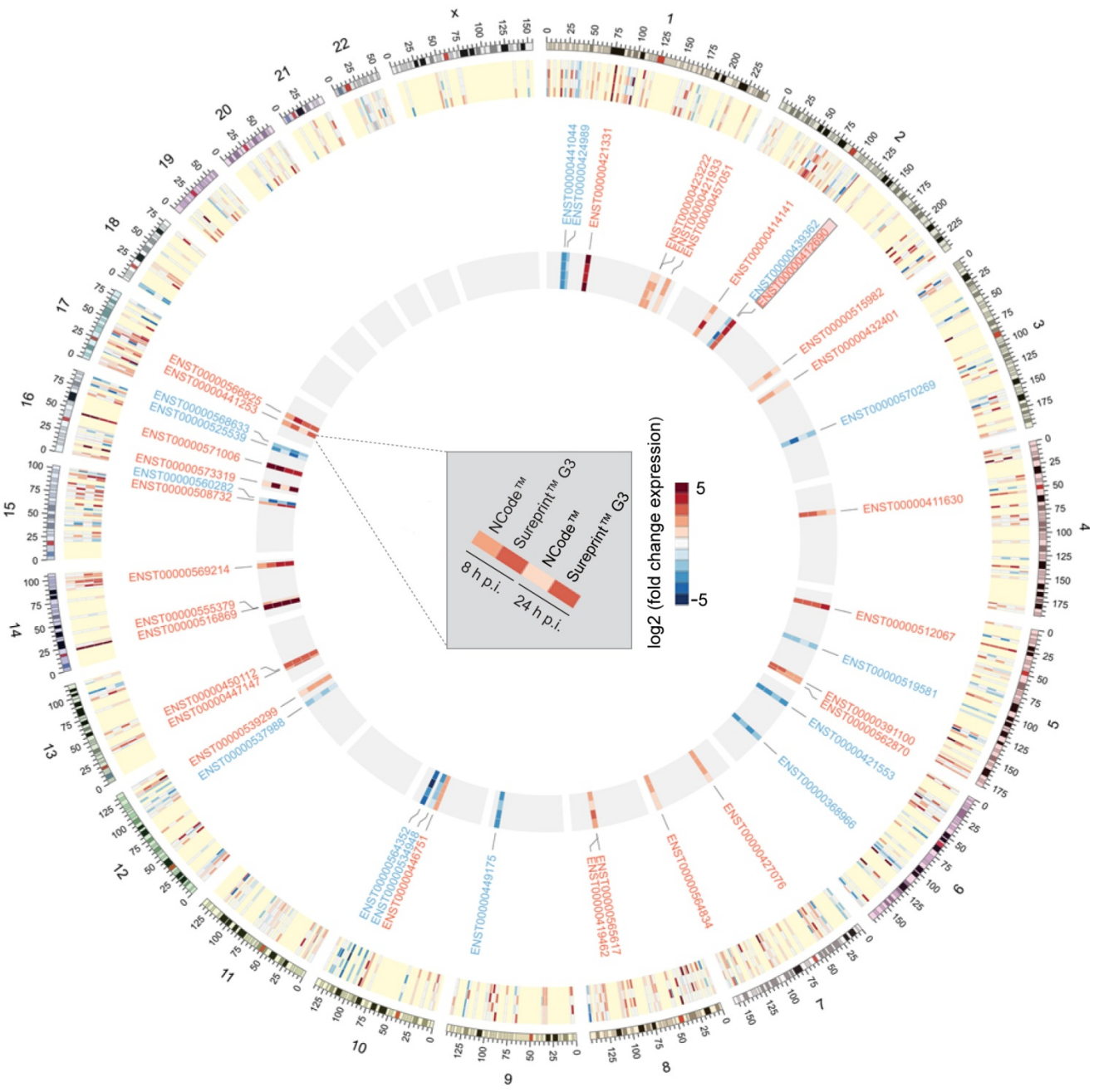

Figure S2: Human genome-wide map of ncRNAs represented by NCode™ and Sureprint G3™ microarrays. *Circos* map[1] showing log2-fold changes in expression in infected samples (compared with uninfected control cells) at 8 h and 24 h p.i. for 1297 ncRNAs (outer ring) represented by probes on NCode™ and Sureprint G3™ microarrays. ncRNAs are shown according to their mapped location on the human genome (by chromosome number). The inner ring shows 42 ncRNA transcripts (labeled by Ensembl transcript ID) that were differentially regulated at least 2-fold upon infection at both time points and on both microarrays (log2 scale). Data shown are from two independent infection experiments, at both time points.

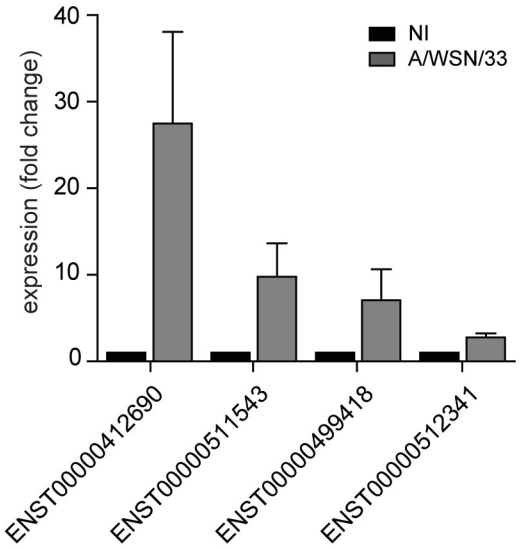

Figure S3: qRT-PCR validation. Several highly induced transcripts were randomly selected for qRT-PCR validation according to a preliminary re-annotation of Sureprint™ G3 probe sequences to Ensembl human genome annotation Release 60. A549 cells were infected with influenza A/WSN/33 (H1N1) virus (MOI 5, 8 h) followed by RNA isolation and qRT-PCR. Data are shown as mean (+/- SD) fold change expression of transcripts for three independent replicates in infected and non-infected (NI) control cells.

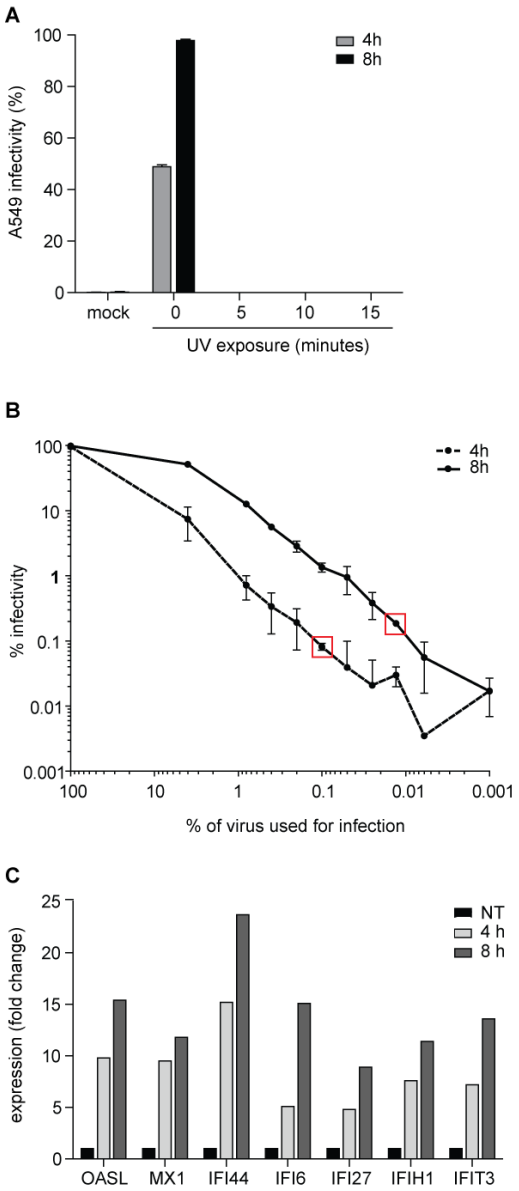

Figure S4: Effects of UV exposure on infectivity and gene expression. (A) UV exposure inactivates influenza A/WSN/33 virions. Human lung epithelial A549 cells were infected with influenza A/WSN/33 virus (MOI 5) for 4 h or 8 h. Supernatants of infected cells were exposed to UV light for the indicated times and then used to infect fresh A549 cells for 4 h and 8 h, respectively. Infectivity of supernatants was calculated according to NP-positive cells (mean +/- SD of two independent experiments). For microarray experiments supernatants exposed with the minimal dose of UV eliminating infectivity (5 min) were used. (B) Sensitivity of infectivity analysis. Titration of A/WSN/33 on A549 cells reveals a reliable detection limit of maximal 0.1% of initially applied virus 4h p.i. and 0.01% virus 8h p.i. (red rectangles). Shown is the mean +/- SD of two independent experiments. (C) Induction of IFN-inducible genes by IAV infected cell supernatants after UV exposure. A549 cells were treated with the supernatants from (A) for 4 h or 8 h and RNA extracted following infection was reverse- transcribed using OligodT priming and labeled with Cy5 or Cy3. Microarray hybridizations were performed using RNA from two independent infections and UV treatments at each time point. Data are presented as mean fold change in expression of the indicated genes compared with a mock-infected and non-UV treated (NT) reference.

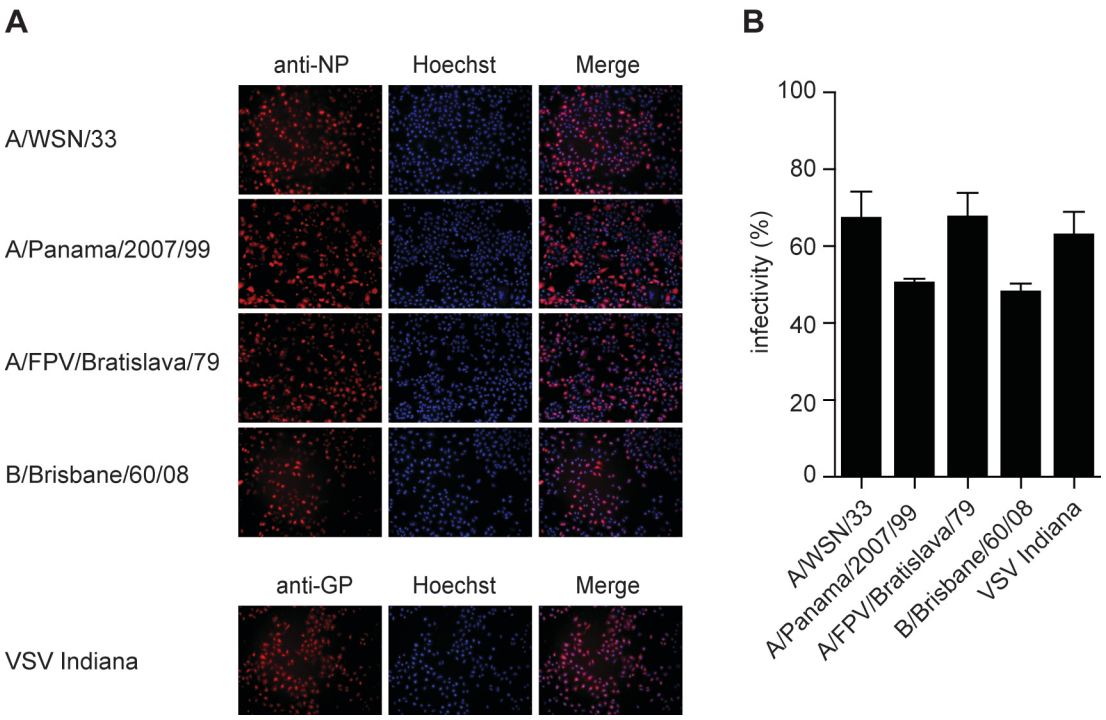

Figure S5: Infection with different RNA viruses. (A) A549 cells were infected with the indicated viruses (MOI 1, 6 h), fixed and stained for influenza A or B virus NP or VSV Glycoprotein (GP) and DNA (Hoechst). Microscopic images were acquired using automated software (10 x magnification, Olympus Soft Imaging Solutions). (B) NP- or GP-positive cells were automatically quantified using ScanR software. Data are represented as mean +/- SD of three technical replicates.

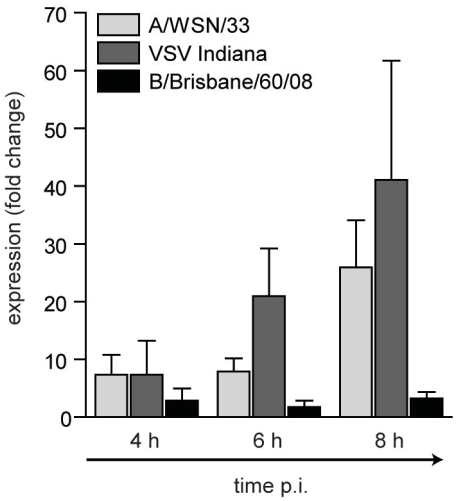

Figure S6: Infection time course with different RNA viruses. A549 cells were infected with viruses (MOI 1) for the indicated times and the expression of VIN was quantified by qRT-PCR. Data are presented as mean fold-change expression (+/- SD) from three independent experiments.

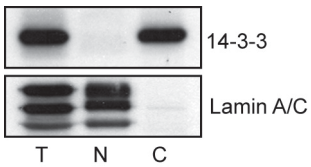

Figure S7: RNA fractionation. Nuclear and cytoplasmic RNA and protein fractions were prepared from A/WSN/33 (H1N1) virus infected A549 cells (MOI 5, 8 h). Western blot analysis of total (T), nuclear (N) and cytoplasmic (C) fractions for 14-3-3 proteins and Lamin A/C.

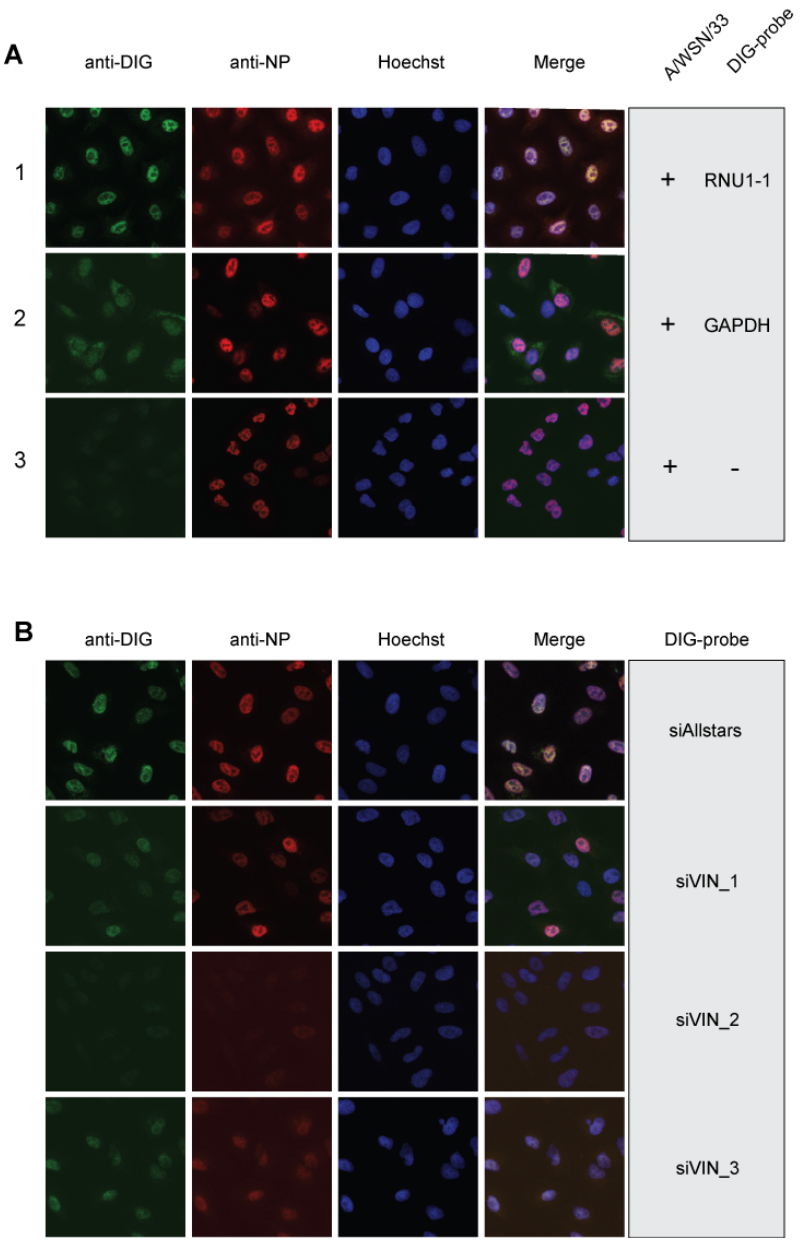

Figure S8: RNA-FISH. (A-B) VIN, *GAPDH* or *RNU1-1* sequence-specific RNA oligonucleotide probes were generated by in vitro transcription incorporating digoxigenin (DIG). Probes were hybridized to A549 wildtype (A) and siRNA VIN knockdown cells (B) infected with A/WSN/33 (H1N1) virus ((A) MOI 5, 4 h p.i. (RNU1-1, GAPDH) or 6 h p.i. (no probe) (B) MOI 1, 6 h p.i.), followed by immunofluorescence staining of DIG (green) and viral NP protein. Nuclei were visualized using Draq5 (blue). Images shown are representatives from two independent experiments.

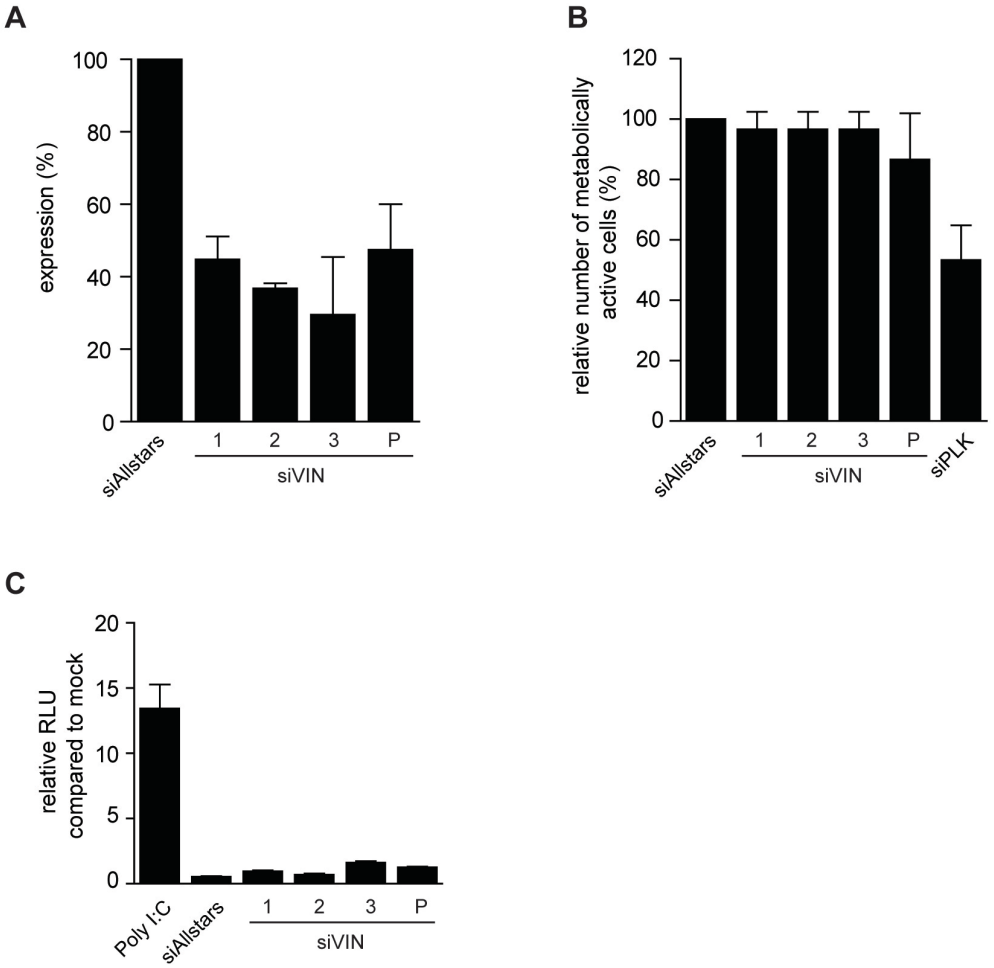

Figure S9: VIN knockdown. (A) SiRNA knockdown efficiency. A549 cells were transfected with 20 nM of each VIN siRNA (1, 2 and 3) individually and 20 nM of all three siRNAs collectively. 48 h later cells were infected with influenza A/WSN/33 virus (MOI 5, 8 h). RNA was isolated and qRT-PCR performed. Data represent mean (+/- SD) VIN expression normalized to Allstars control of three independent experiments. (B) Analysis of siRNA toxicity. Cells were transfected with 20 nM of each siRNA for 48 h and conversion of water-soluble tetrazolium salt (WST-1) reagent was measured to quantify numbers of metabolically active cells. Data are presented as percent of siAllstars treated cells (100% metabolically active). siPLK (Polo-like kinase 1) was used as a positive control (mean of three independent experiments; +/- SD). (C) Induction of IFN by VIN siRNAs. A549 cells stably expressing a firefly luciferase reporter construct under control of the interferon stimulated response element (ISRE) were transfected for 24 h with 20 nM of the indicated siRNAs or treated with 1 pg poly I:C. Subsequently, luciferase expression was measured. Data are represented as relative RLU compared to mock treated cells from three independent experiments (mean +/- SD).

Supplementary References

[1]Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA Circos: an information aesthetic for comparative genomics. Genome Res 2009; 19: 1639-1645