

Supporting Information

Baum et al. 10.1073/pnas.1005077107

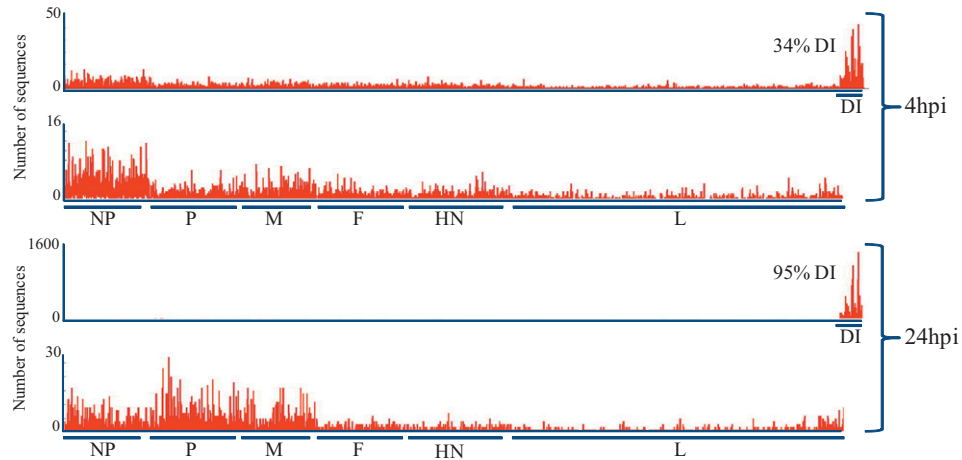


Fig. S1. Deep sequencing of total RNA from SeV-infected cells at 4 and 24 hpi. As in other experiments the obtained sequencing reads were mapped to the SeV genome and individual peaks correspond to the number of reads that begin at a particular nucleotide position in the genome. For both 4 and 24 h time points the number of reads that map to the DI RNA genome was used to calculate the approximate fraction of viral RNA that corresponds to the DI particle (this number is shown as %DI on the graph). (Lower) For both 4- and 24-h time points the same sequencing data are shown as in the Upper graph but the sequences mapping to the DI region are omitted to allow easier visualization of the rest of the genome.

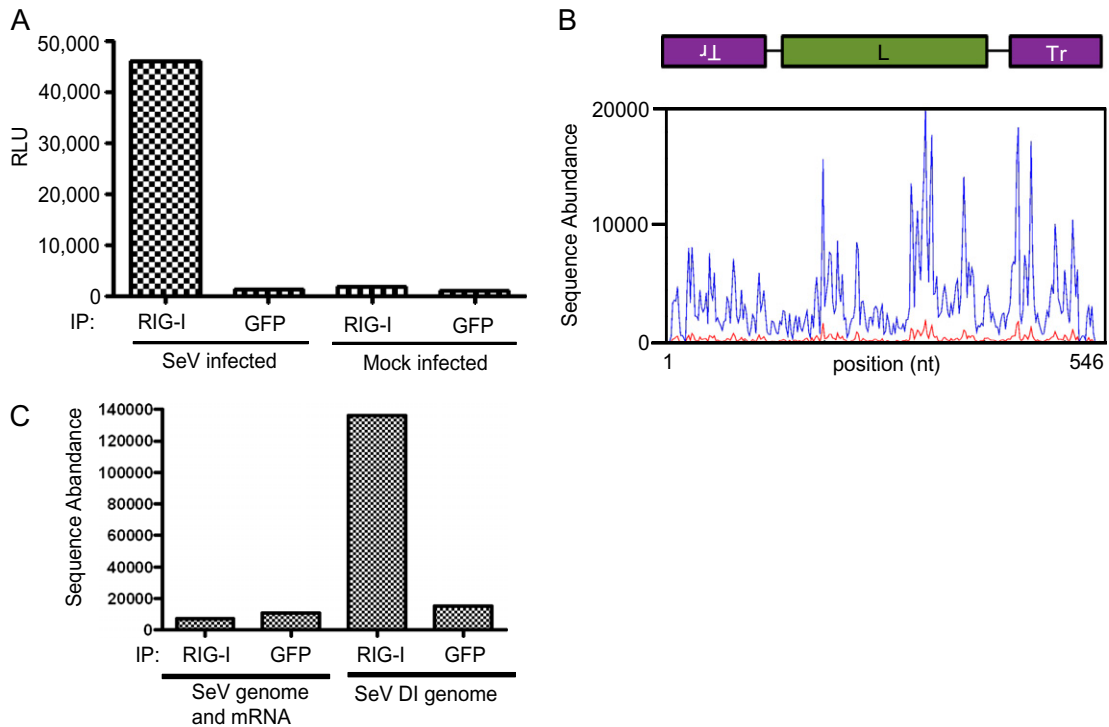


Fig. S2. Immunoprecipitation and deep sequencing analysis of RIG-I-associated RNA from 4-h SeV infections. (A) Transfection of RIG-I-associated RNA into the ISRE-FF reporter cells shows high immunostimulatory activity of this RNA compared with the control (GFP) IP or IPs from mock infected cells. (B) Deep sequencing of RIG-I and control (GFP) IP RNA identifies RNA sequences belonging to the DI particle as being specifically enriched in RIG-I pulldowns. (C) Sequence abundance between the RIG-I IP and control (GFP) IP of reads that map to the DI genome versus the rest of SeV RNA.

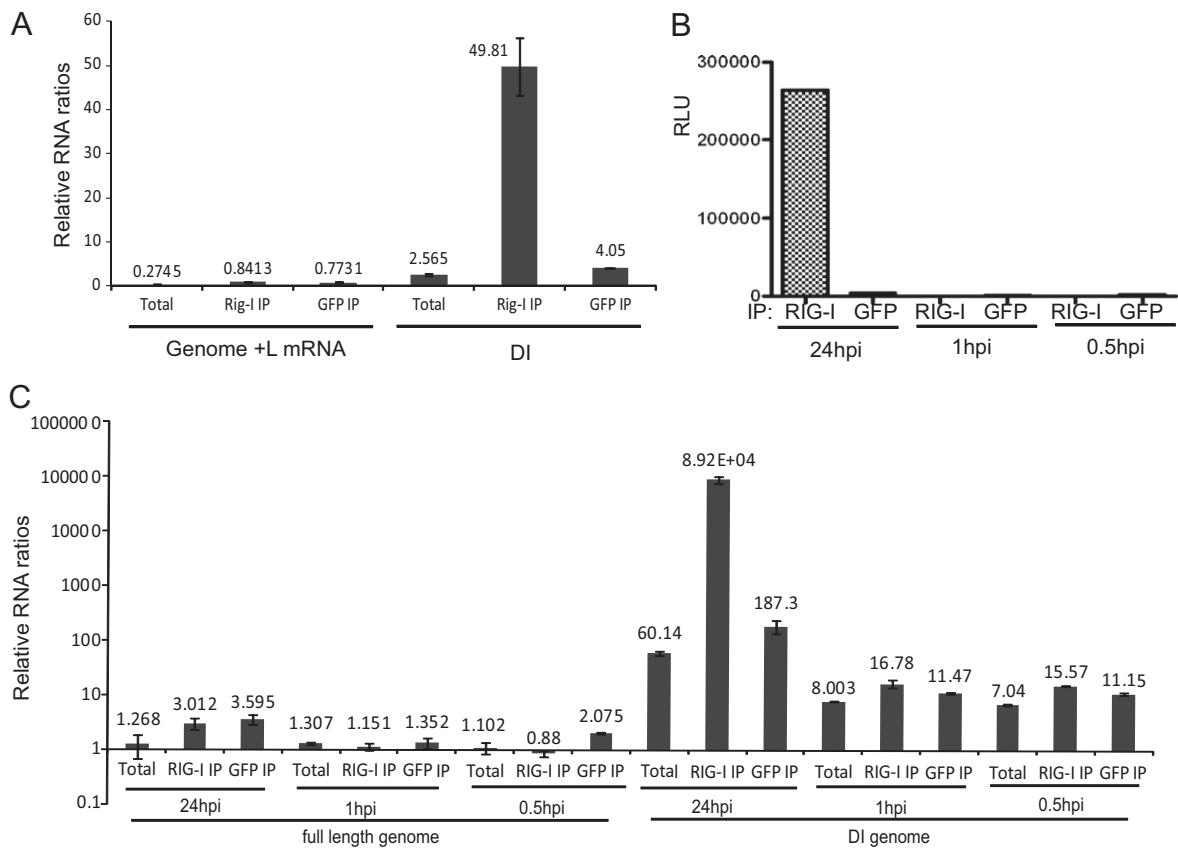


Fig. S3. Quantitative PCR analysis of RIG-I-associated RNA from SeV-infected cells. (A) Comparison of full-length genome/L mRNA levels and DI genome levels between total RNA, RIG-I IP RNA, and GFP IP RNA at 24 hpi. (B) Immunostimulatory activity of RIG-I-associated RNA at 24, 1, and 0.5 hpi. (C) Sense-specific Q-PCR comparison of full-length genome levels and DI genome levels between total RNA, RIG-I IP RNA, and GFP IP RNA at 24, 1, and 0.5 hpi. Data are shown on a log scale.

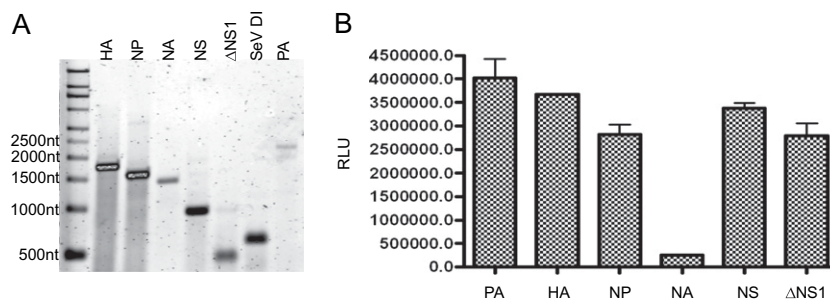


Fig. S4. Immunostimulatory activity of in vitro transcribed influenza PR8 RNAs. (A) Denaturing agarose gel stained with SybrGold (Invitrogen) and visualized on a Typhoon scanner (GE Healthcare) of T7 transcribed influenza RNAs as well as SeV DI RNA. (B) Relative ISRE-FF reporter activation by transfection of equimolar amounts of individual influenza RNAs.

Table S1. Average ratios of eight randomly picked cellular mRNAs between the RIG-I IP and GFP IP samples

mRNA	RIG-I IP/GFP IP
NM_002985	1.2
NM_001402	1.3
NM_001547	1.5
NM_002046	0.9
NM_001002	1.1
NM_172140	1.2
NM_001614	0.9
NM_021009	1.1

Sequences were picked at random and relative abundances from two samples were calculated. The average ratio for the eight mRNAs is 1.1.