Supporting Information

Hastie et al. 10.1073/pnas.1016404108

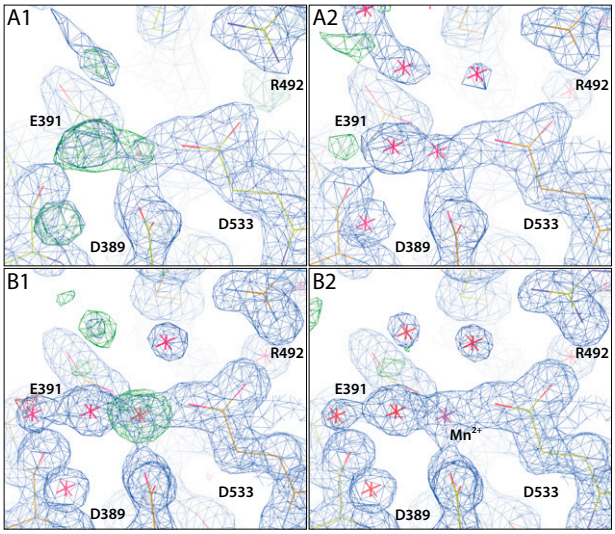

Fig. S1. Electron density in the active site of native and Mn^{2+}-containing NPΔ340 crystals. (*A1*) Electron density of native NPΔ340 without waters or ions modeled. (*A2*) Although two water molecules satisfy positive $F_o - F_c$ density in the map of the native structure, bond distances of ~2 Å between the water molecule and carboxylate oxygens of D389, E391, and D533 indicate the presence of an ion rather than water. We cannot unambiguously deduce the identity of this ion because it was likely obtained from the expression media. (*B1*) Electron density in the active site of Mn^{2+}-soaked NPΔ340. Strong positive density remains after modeling water into the active site, indicating that the bound moiety is likely heavier than water. (*B2*) Positive density in Mn^{2+}-soaked NPΔ340 is satisfied by a partially occupied Mn^{2+} ion. Water molecules are colored red. The Mn^{2+} ion is colored magenta. $2F_o - F_c$ maps are colored blue (contour level 1.5σ), and $F_o - F_c$ difference maps are colored green (contour level 4σ).

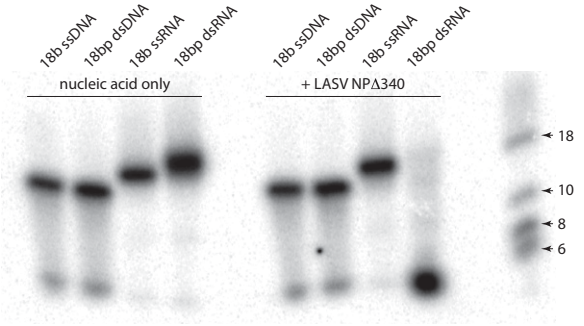

Fig. S2. LASV NPΔ340 digests dsRNA to <6 bases. NPΔ340 was incubated with different 5′ ^{32}P-labeled substrates, and the reaction products were analyzed by denaturing PAGE and autoradiography.

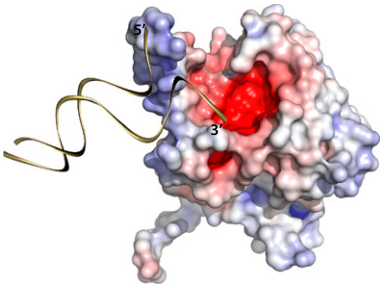

Fig. S3. Model of dsRNA (gold ribbon) entering the LASV NPΔ340 exonuclease active site (electrostatic surface model with limits ± 10 kT/e.) The 3′ end of the dsRNA is digested by NPΔ340. The 5′ end could be coordinated by the basic arm.

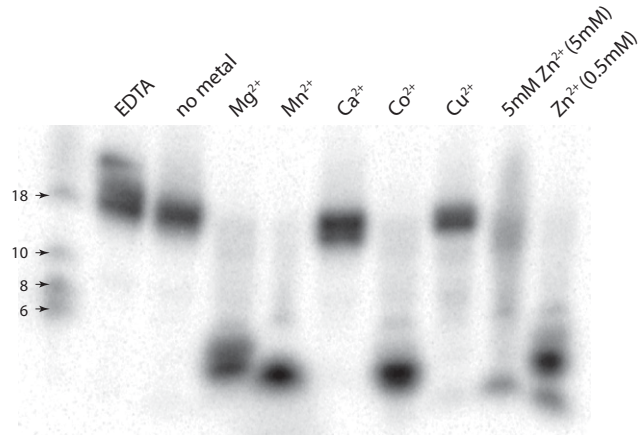

Fig. S4. Metal specificity of LASV NPΔ340. 5′ ^{32}P-labeled dsRNA was incubated with LASV NPΔ340 in the presence of 5 mM EDTA, no added metal, Mg^{2+}, Mn^{2+}, Ca^{2+}, Co^{2+}, or Zn^{2+}. All metals were added at 5 mM unless specified otherwise. Reaction products were analyzed by denaturing PAGE and autoradiography.

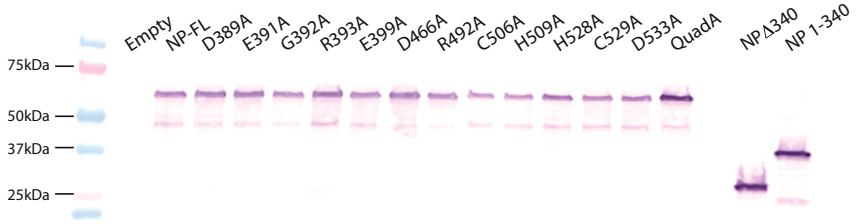

Fig. S5. Protein expression levels of wild-type and mutant NP. Cell lysates from each triplicate set used in the IRF-3 activation assays were pooled and analyzed by Western blotting using an anti-HA monoclonal antibody.

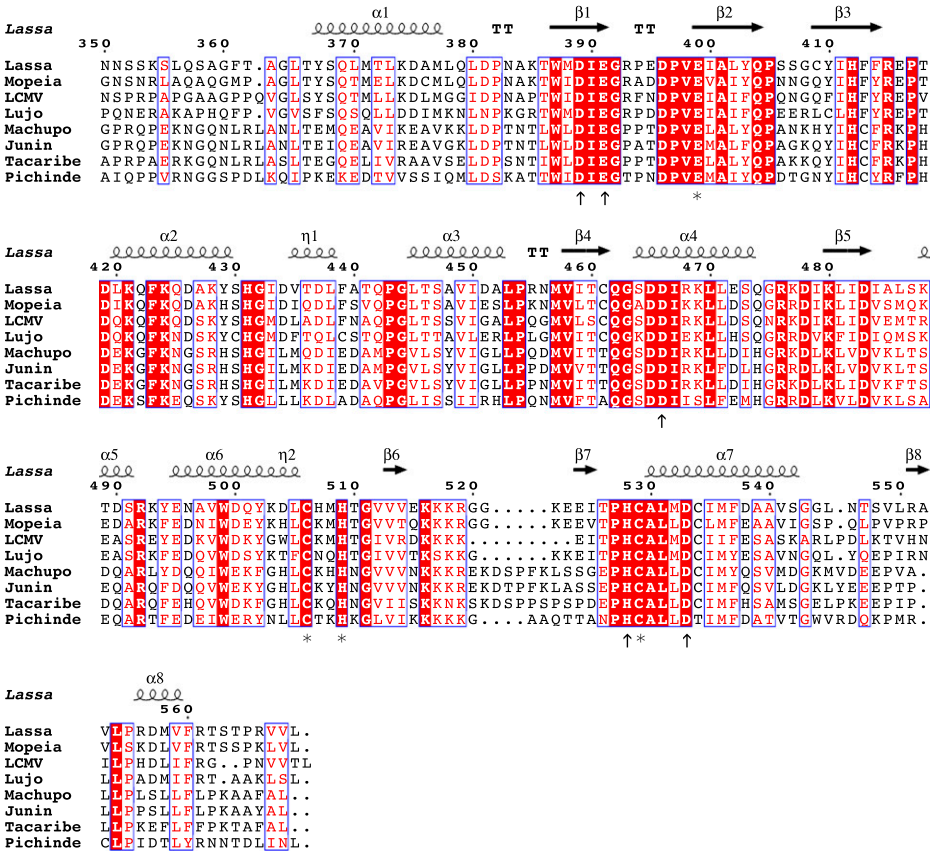

Fig. S6. Sequence comparison of arenaviral NPs. Arenavirus NP sequences were aligned with Clustal W and ESPript (1, 2). DEDDh exonuclease active site residues (indicated by arrows) and Zn^{2+} coordination residues (indicated by asterisks) are completely conserved across the arenavirus family. Note: The TACV sequence presented here reflects the sequences described in Martínez-Sobrido et al. (21) rather than the deposited sequence (accession no. AAA47903).

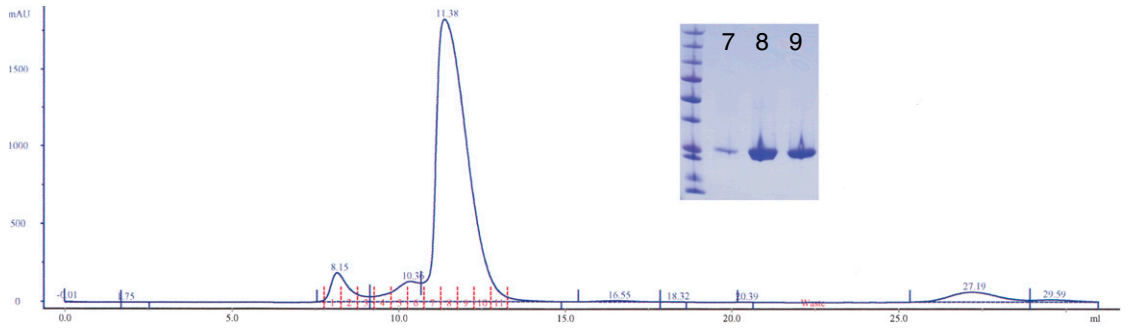

Fig. S7. Purification of NPΔ340. LASV NPΔ340 was expressed in *E. coli* Rosetta 2 cells and purified over Ni-NTA beads. The resulting eluate was purified further over a Superdex 75 size-exclusion column, shown here. Fractions 8 and 9 were pooled and used for crystallization and exonuclease experiments.

1. Gouet P, Courcelle E, Stuart DI, Métoz F (1999) ESPript: Analysis of multiple sequence alignments in PostScript. *Bioinformatics* 15:305–308.
2. Larkin MA, et al. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947–2948.

Table S1. Data collection, phasing, and refinement statistics

Parameter	Native, unbound, moderate resolution	Selenomethionine, unbound	Native, unbound, high resolution	Native, UMP/Mn^{2+} cocrystallization
Data collection				
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions				
a, *b*, *c* (Å)	44.85, 67.17, 80.04	44.97, 67.59, 79.40	44.50, 68.23, 79.01	44.89, 67.98, 78.74
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Wavelength (Å)	1.5418	1.5418	1.1159	1.1159
Resolution (Å)	51.46–2.2 (2.28–2.2)	51.47–2.2 (2.27–2.2)	34.05–1.70 (1.76–1.70)	33.84–1.50 (1.55–1.50)
R_{merge}	0.098 (0.412)	0.073 (0.259)	0.083 (0.471)	0.067 (0.258)
$I/\sigma(I)$	10.4 (2.7)	10.7 (4.0)	6.9 (1.6)	10.1 (2.9)
Completeness (%)	99.4 (94.3)	97.3 (87.4)	97.0 (99.7)	97.1 (93.3)
Redundancy	6.24 (5.21)	4.46 (3.97)	3.32 (3.25)	3.57 (2.84)
Refinement				
Resolution (Å)			34.05–1.70 (1.76–1.70)	33.84–1.50 (1.55–1.50)
No. of unique reflections			26320	38157
R_{work}/R_{free}			0.1917, 0.2490	0.1915, 0.2157
No. of atoms			1840	1952
Protein			1627	1639
Ligand/ion			6	7
Water			207	306
B values				
Protein			21.26	19.17
Ligand/ion			49.10	22.10
Water			30.38	30.62
rmsd				
Bond lengths (Å)			0.007	0.006
Bond angles (°)			0.997	1.099

Values in parentheses are for highest-resolution shell.

Table S2. DNA and RNA oligomers used in exonuclease experiments

Oligomer	Sequence
18-bp ssDNA	5′-CAGGATGTTGATGCTGCT-3′
18-bp dsDNA	5′-CAGGATGTTGATGCTGCT-3′ 3′-GTCCTACAACTACGACGA-5′
18-bp ssRNA	5′-AGAAGGAGGGAGGGAGGA-3′
18-bp dsRNA, blunt ends	5′-AGAAGGAGGGAGGGAGGA-3′ 3′-UCUUCCUCCCUCCCUCCU-5′
21-bp dsRNA, 5′ 3-nt overhang	5′-GAGAGAAGGAGGGAGGGAGGA-3′ 3′-UCUUCCUCCCUCCCUCCUCUC-5′
21-bp dsRNA, 3′ 3-nt overhang	5′-AGAAGGAGGGAGGGAGGAGAG-3′ 3′-UCUUCUUCCUCCCUCCCUCCU-5′
20-bp dsRNA, 5′ PPP, blunt ends	5′-PPP-GGCACAUAUCGAGGUGGACATCACTTACGCTGAGT ACTTCGAAATGTCCC-3′ 3′-CCGUGUAUAGCUCCACCUGU-5′
20-bp dsRNA, 5′ PPP, 1-nt overhang	5′-PPP-GGCACAUAUCGAGGUGGACATCACTTACGCTGAGTACT TCGAAATGTCCC-3′ 3′-CGUGUAUAGCUCCACCUGUA-5′

ss, single-stranded; ds, double-stranded; 5′PPP, 5′ triphosphate.