Supporting Information

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SI Methods

In Cell Replicon RNA Synthesis. Machupo virus (MACV) replicon assays were performed as described previously (1), except that the pMCm S-segment replicon was modified for quantitative analysis by substituting the eGFP reporter protein sequence with that of gaussia luciferase (humanized gaussia luciferase cDNA was a generous gift from Xandra Breakefield, Harvard Medical School, Boston, MA) (2). Z plasmids were created by cloning the MACV–Carvallo Z (accession no. AY619642), JUNV–Candid#1 Z (accession no. AY746354) (3), and LCMV–Armstrong Z (accession no. AY847351) ORFs into pGEM3 (Promega).

Briefly, confluent 6-cm dishes with 2×10^6 BSR-T7 cells were infected at a multiplicity of infection of three, with vaccinia virus vTF73 expressing T7 RNA polymerase (4) before transfection with 1 μg of pL and 6 μg of pMCm-Gluc and 0.5 μg of Z plasmids as indicated. Cells were incubated at 37 °C for 5 h and then the medium was changed to DMEM supplemented with 2% FBS, 10 mM Hepes at pH 7.7, and penicillin/kanamycin/streptomycin (100 units/mL; 20 μg/mL; 20 μg/mL), and cells were incubated at 34 °C. Supernatant containing secreted gaussia luciferase was harvested at 48 h posttransfection, cell debris was pelleted by centrifugation at $16{,}100 \times g$ for 1 min, and luciferase activity was measured according to manufacturer's protocols (Renilla Luciferase Assay system; Promega) using a 1:125 dilution of clarified cell supernatant. Statistical significance was calculated using an unpaired, two-tailed *t* test.

Recombinant Protein Expression and Purification. Full-length MACV L was expressed in insect cells and purified by Ni-affinity and ion-exchange chromatography (GE Healthcare) as described previously (1). GST-tagged MACV Z and lymphocytic choriomeningitis virus (LCMV) Z constructs were assembled by overlapping extension PCR and cloning into pET16 (HisHisHisHisHisHisGlyGly GST GlySerGlyGly Z), expressed in BL21 Star DH5α *Escherichia coli* (Invitrogen) and purified by Ni-affinity. Likewise, the MBP-tagged MACV Z construct was assembled into pET16 (LysSerSerHisHisHisHisHisHisGlySerSer MBP GluAsnLeuTyrPheGlnSerGlySerGlyGly MACV Z) and the MBP–Z fusion protein was expressed and purified by Ni-affinity. Untagged MACV Z and free MBP were purified by dialyzing MBP–Z fusion in the presence of a 1:20 mass ratio of 6×His-tagged tobacco etch virus (TEV) protease overnight at 4 °C. Following TEV cleavage, untagged Z was separated from free MBP and TEV protease by a second round of Ni-affinity purification. A 6×His-tagged murine eIF4E construct was amplified from cDNA (generous gift from Gerhard Wagner, Harvard Medical School, Boston, MA), assembled in pET16 (LysSerSerHisHisHisHisHisHisGlySerSer eIF4E) and used to express eIF4E in *E. coli*. EIF4E was initially purified by Ni-affinity and then the active fraction was isolated using m7GTP-sepharose (GE Healthcare) as described previously (5). Where indicated, eIF4E was also analyzed by immunoblot and chemiluminescence with a rabbit anti-eIF4E antibody (Cell Signaling) and goat antirabbit HRP-conjugated secondary antibody (Sigma). All recombinant proteins were dialyzed (20 mM Tris-HCl at pH 7.0, 250 mM NaCl, 10% glycerol and 1 mM DTT) and stored as aliquots at −80 °C. Recombinant proteins (~200 μg) were analyzed by gel filtration using a Superdex 200 column (GE Healthcare) and the same binding buffer used for MACV GST-Z and MACV L pull-down assays. Molecular weight estimates were based on 0.5-mL fractions continuously collected, analyzed by 4–12% SDS/PAGE, and compared with the elution of molecular weight standards.

Z Intravirion Concentration Estimate. Previous approximations of the ratio of arenavirus virion proteins (1 L: 160 NP: 60 GPc: 20 Z) (6) and the number of NP molecules within an arenavirus virion (~1,530 copies of NP) (7) were used to estimate the number of molecules of Z within an average arenavirus virion [(1,530/160) × 20 or ~190 copies of Z]. The Z intravirion concentration of 0.8–6.0×10^{-4} M was then calculated according to the observed size of spherical arenavirus particles (50–100 nm radius) (8) using the following equation: $[Z]^{\text{virion}} = (190 \text{ copies of Z per virion} \times N_A{}^{-1}) / ((4/3)\pi(50\text{–}100 \text{ nm})^3)$.

RNA Capping Reactions. Unlabeled MACV in vitro RNA synthesis reactions were carried out with 1 mM ATP/CTP/UTP/GTP and in the absence of exogenous GpC dinucleotide primer. Following incubation for 2 h at 30 °C, reactions were treated with 2.4 μg of proteinase K (Sigma) at 45 °C for 45 min followed by phenol-chloroform extraction and ethanol precipitation. RNA was washed 3 times with 70% ethanol and resuspended in 10 μL of dH_2O. Five microliters of RNA was denatured by heating at 65 °C for 3 min and then capped in the presence of 20 μCi of [α-^{32}P]-GTP, 100 μM *S*-adenosylmethionine, 0.05 units of yeast inorganic pyrophosphatase (New England Biolabs), and 7.5 units of vaccinia virus guanyltransferase (D1/D12, EpiCentre ScriptCap enzyme) in 20-μL reaction mixtures. After 2 h at 37 °C, reactions were treated with 10 units of calf intestinal alkaline phosphatase (New England Biolabs) for 1 h and then terminated by the addition of equal volume of formamide stop solution (95% deionized formamide, 20 mM EDTA) and incubation at 95–100 °C for 2.5 min. Reactions were analyzed and separated by denaturing gel electrophoresis as with labeled RNA synthesis reactions.

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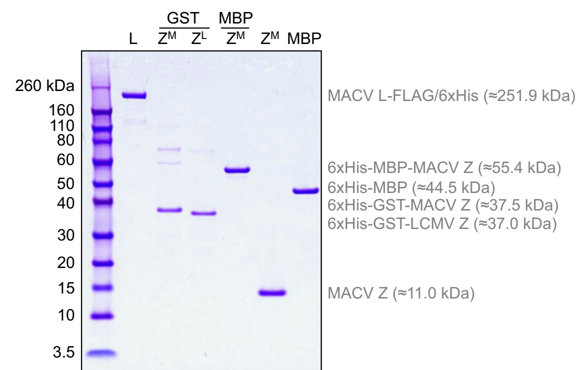

Fig. S1. Recombinant arenavirus protein purification. (*A*) Recombinant arenavirus proteins were purified as described in experimental procedures, and 2.0 μg of each protein was analyzed by 4–12% gradient SDS/PAGE and Coomassie blue staining. The predicted molecular mass of each protein is indicated on the *Right*.

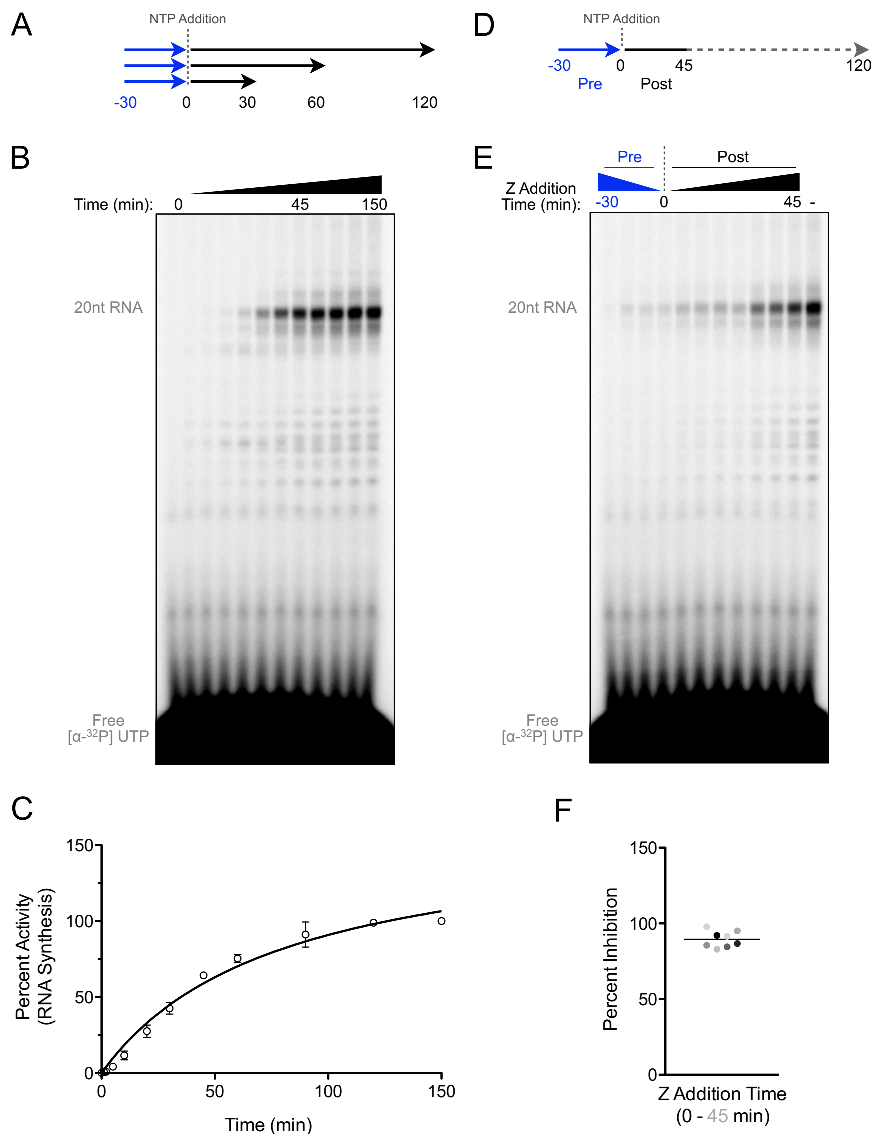

Fig. S2. Kinetics of MACV RNA synthesis and Z inhibition. (*A*) Schematic representation of MACV in vitro RNA synthesis time-course reactions. All reactions included GpC primer and were performed by first preincubating L and the RNA template at 25 °C for 30 min. Subsequently, NTPs and labeled [α-^{32}P]-UTP were added and reactions were shifted to 30 °C. Reactions were terminated at the designated time points, (*B*) analyzed, and (*C*) quantified as in Fig. 1. (*D*) Schematic representation of MACV in vitro RNA synthesis Z inhibition time-course reactions. RNA synthesis reactions were carried out as in *A* except reactions were supplemented with purified MACV Z at the designated time points before or after NTP addition. All reactions were terminated after a 120-min incubation at 30 °C and (*E*) analyzed as in Fig. 1. (*F*) The percentage of Z inhibition for each time point was calculated by determining the percentage of RNA synthesis following Z addition relative to the expected amount of RNA synthesis using the data from *B*. Each point represents an individual time point of Z addition from 0 to 45 min post-NTP addition and is color coded so that lighter shades represent later time points of Z addition.

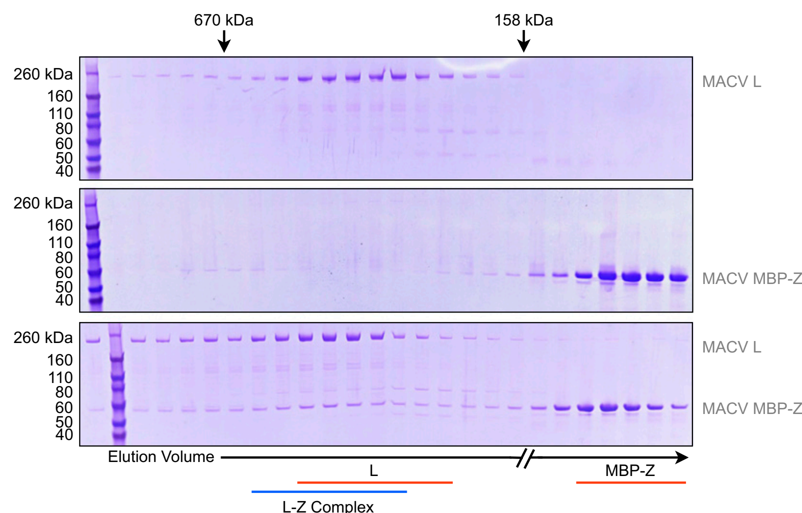

Fig. S3. L and Z form a heterodimeric complex. The molecular weight and stoichiometry of the Z–L complex was estimated by preincubating L and MBP-tagged Z and comparing the Superdex 200 gel-filtration elution profile of the complexed proteins with purified MACV L (L), MBP-tagged MACV Z (MBP-Z), and known molecular weight standards. Consecutive 0.5-mL fractions were collected and analyzed by gradient 4–12% SDS/PAGE and Coomassie stain.

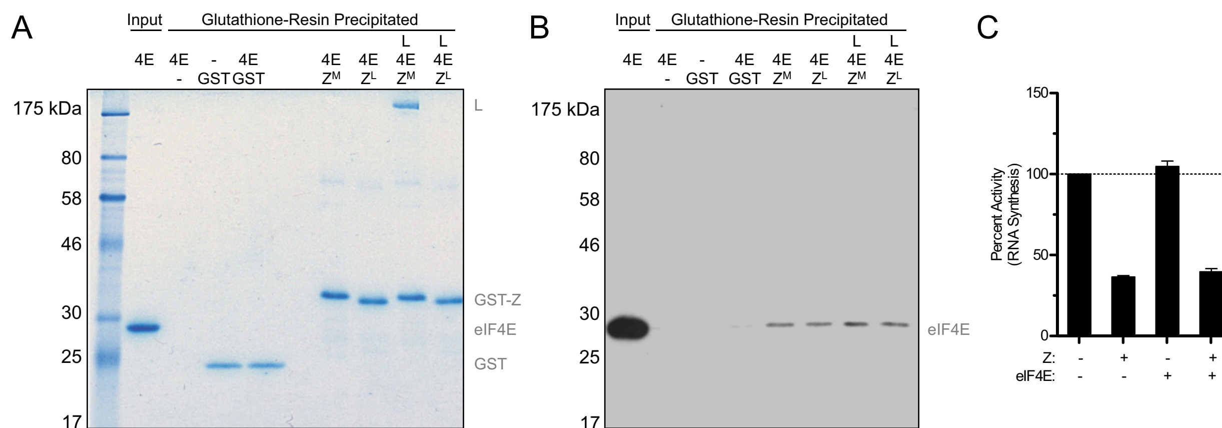

Fig. S4. MACV and LCMV Z-eIF4E interaction. (*A*) GST pull-down assay using purified components. Purified free GST (GST), GST-tagged MACV Z (Z^M) or GST-tagged LCMV Z (Z^L) were incubated alone or with eIF4E (4E) and/or MACV L (L) in the designated combinations before purification with glutathione resin. Pelleted resin was washed three times, and bound proteins were eluted and analyzed by 12% SDS/PAGE and colloidal Coomassie stain or (*B*) by α-eIF4E immunoblot (5-min exposure). Input eIF4E corresponds to one-third of the total input protein in *A* or 10% of the total input protein in *B*. (*C*) In vitro RNA synthesis reactions supplemented with a recombinant GST-tagged MACV Z and eIF4E as indicated. Reactions contained a concentration of MACV Z (1.6 μM) that results in ~60% inhibition of total RNA synthesis, and a threefold molar excess of eIF4E to maximize potential for eIF4E interference with Z–L complex formation.

Fig. S5. Z inhibits L catalytic function. (*A*) RNA synthesis reactions performed in the absence or presence of GpC primer (+GpC) and increasing concentrations of GTP (0, 0.1, 1.0, 10, 50, and 100 μM) or CTP (0; 0.1; 1; 10; 100; 1,000; and 10,000 μM) and [α-^{32}P]-UTP were analyzed as in Fig. 1. (*B*) RNA synthesis reactions in the absence of GpC were conducted with (+) or without CTP (no CTP) and labeled with [α-^{32}P]-GTP. Reactions contained either wild-type (WT) or catalytically inactive (SDD) L, wild-type (WT), or 3′ dideoxy (dd) RNA template and MACV Z (Z^{M}) as indicated. RNA synthesis or terminal-transferase labeled RNA products were analyzed and (*C*) quantified as in Fig. 1 (**P* < 0.001 or Z^{M} *P* = 0.0016).