# PNAS
www.pnas.org

Supplementary Information for

Ebolavirus polymerase uses an unconventional genome replication mechanism

Laure R. Deflubé, Tessa N. Cressey, Adam Hume, Judith Olejnik[2], Elaine Haddock, Friederike Feldmann, Hideki Ebihara, Rachel Fearns, and Elke Mühlberger
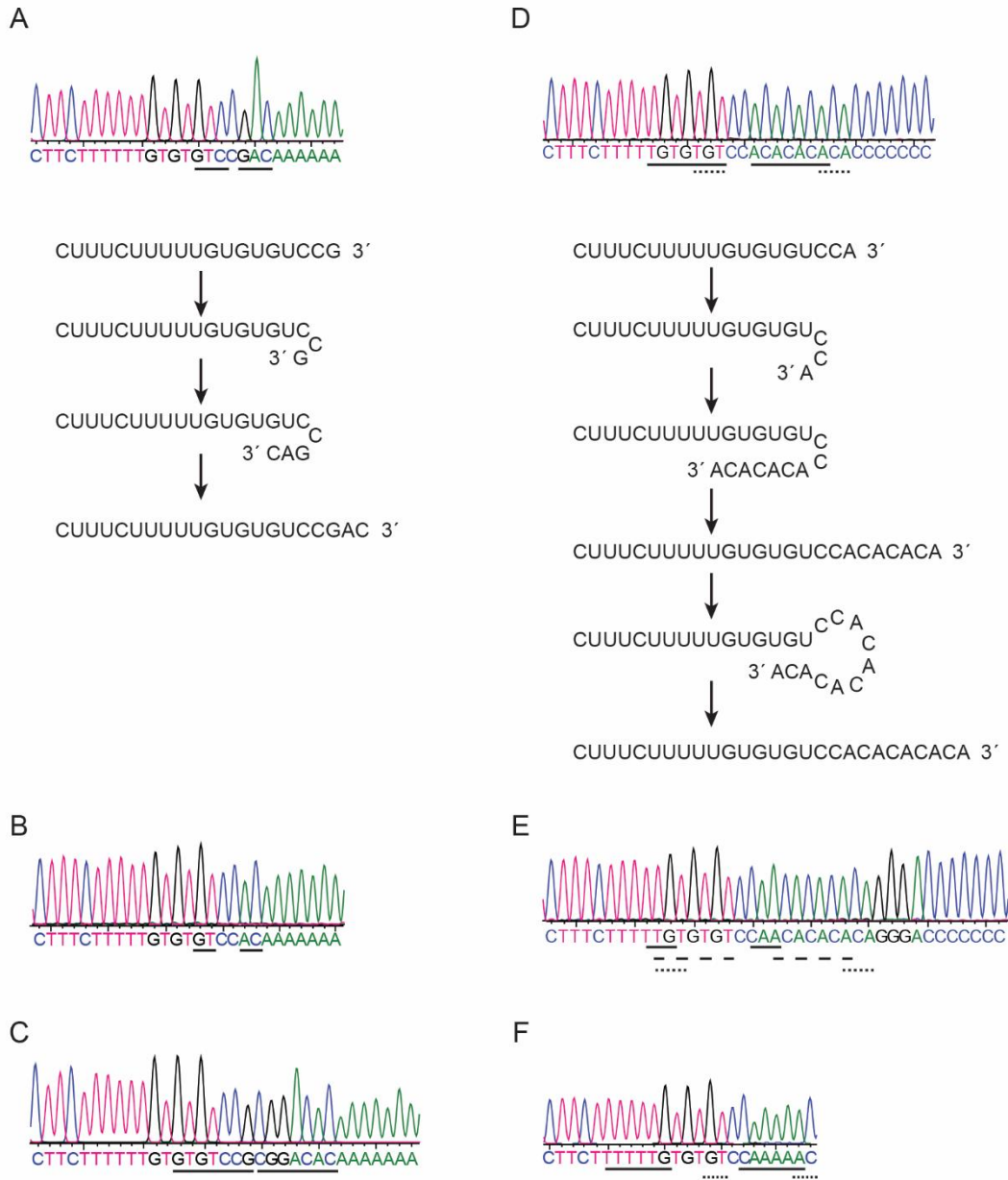
Rachel Fearns and Elke Mühlberger
Email: rfearns@bu.edu, muehlber@bu.edu

This PDF file includes:

Figs. S1 to S5
Tables S1 to S3

1
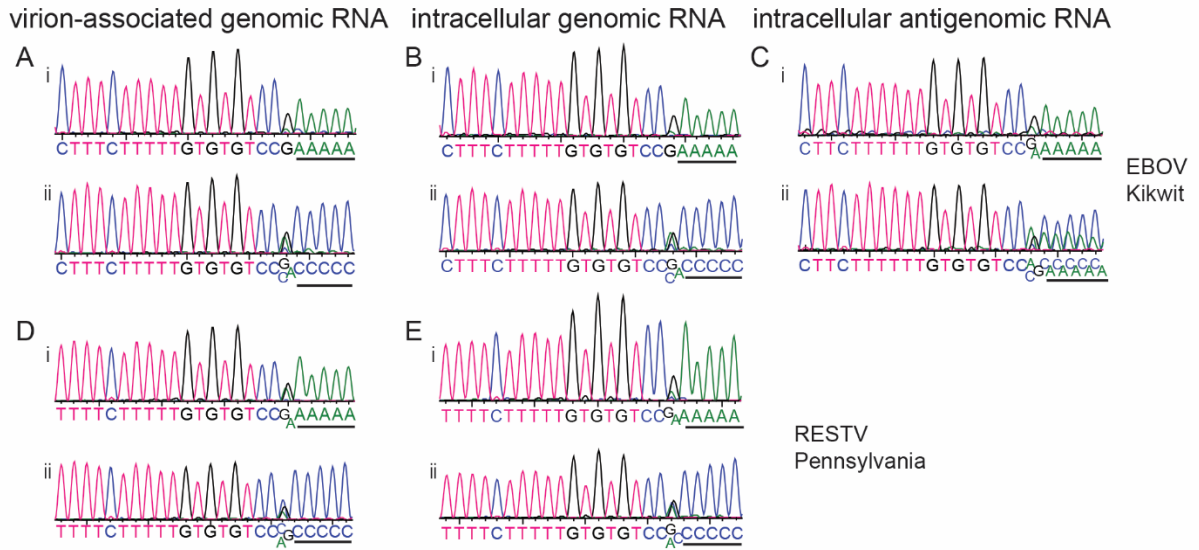
Fig. S1. Sequence analysis of the 3´ ends of EBOV Kikwit and RESTV Pennsylvania genomic and antigenomic RNAs. Vero cells were infected with EBOV Kikwit (A-C) or RESTV Pennsylvania (D, E) and virion-associated or total cellular RNA was used for RACE analysis. The traces show sequences of the 3´ RACE PCR population obtained from virion-associated RNA purified from cell supernatants (A and D), intracellular genome RNA (B and E), or intracellular antigenome RNA (C). In each case, panels i and ii show results from RNA tailed with ATP and CTP, respectively.

A

CTTCTTTTTTGTGTGTCCGACAAAAAA

CUUUCUUUUUGUGUGUCCG 3′

↓

CUUUCUUUUUGUGUGUC
3′ G^C

↓

CUUUCUUUUUGUGUGUC
3′ CAG ^{C}

↓

CUUUCUUUUUGUGUGUCCGAC 3′

D

CTTTCTTTTTTGTGTGTCCACACACACACCCCCCC

CUUUCUUUUUGUGUGUCCA 3′

↓

CUUUCUUUUUGUGUGU$_C$
3′ A^C

↓

CUUUCUUUUUGUGUGU$_C$
3′ ACACACA ^{C}

↓

CUUUCUUUUUGUGUGUCCACACACA 3′

↓

CUUUCUUUUUGUGUGU$^{C\,C\,A}_{\quad C}$
3′ ACA$_{C\,A\,C}$^{A}

↓

CUUUCUUUUUGUGUGUCCACACACACA 3′

B

CTTTCTTTTTTGTGTGTCCACAAAAAAA

C

CTTCTTTTTTGTGTGTCCGCGGACACAAAAAAA

E

CTTTCTTTTTTGTGTGTCCAACACACACAGGGACCCCCCC

F

CTTCTTTTTTGTGTGTCCAAAAAC

Fig. S2. Schematic diagram illustrating how longer 3´ terminal sequences could have been added by back-priming. (A-C) Sequence traces that show evidence of nucleotide additions that could have occurred by virtue of a single back-priming event. (D-F) Sequence traces that show evidence of nucleotide additions that could have occurred by virtue of multiple back-priming events. The palindromic sequences are underlined. In D-F palindromes that could have arisen as a consequence of the second or third back-priming event are underlined with dotted and dashed lines. Panels A and D illustrate the steps that could have occurred for nucleotides to have been added. Note that in most cases, there are other back-

priming possibilities and that in panel E, there is some sequence that cannot be explained by back-priming within the terminal region of the genome.
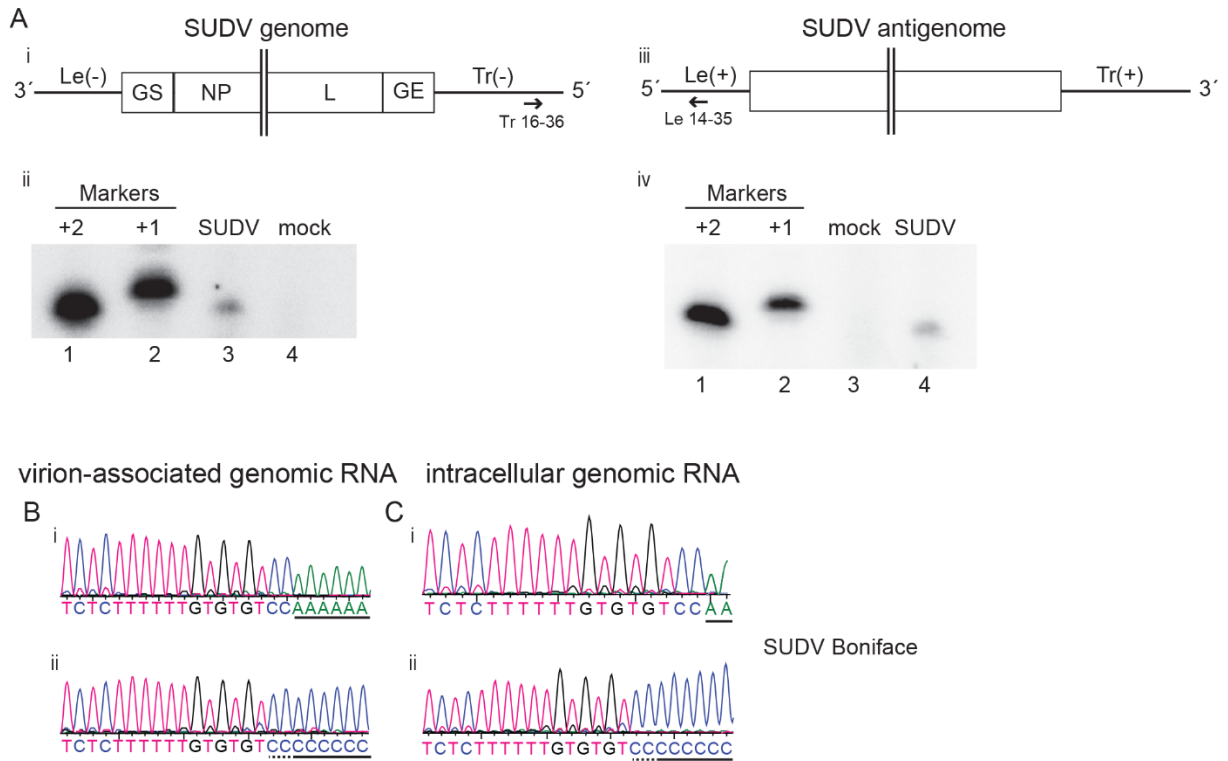
Fig. S3. Analysis of the 5´ ends of SUDV RNAs. (A) Primer extension mapping of the 5´ ends of SUDV genome and antigenome RNAs. (i and iii) Schematic diagrams (not to scale) of the SUDV genome and antigenome showing the annealing positions of the positive (i) and negative (iii) sense primers used for primer extension analysis. (ii and iv) Primer extension analysis of genomic and antigenomic SUDV RNA (SUDV) isolated from infected Vero cells, analyzed with a positive or negative sense primer (ii and iv, respectively). RNA from mock-infected cells (mock) was used as a negative control. [γ-^{32}P]ATP end-labeled DNA oligonucleotides corresponding in length and sequence to the respective cDNA representing initiation from positions +1 and +2, relative to the published sequence were used as markers. (B and C) Vero cells were infected with SUDV isolate Boniface and the 5´ ends of virion-associated (B) or total intracellular genome RNA (C) were amplified by 5´ RACE. The cDNA was tailed with dATP (i) or dCTP (ii). The black line below the sequence traces indicates poly(A) or poly(C) tail sequences that were added to the virus-specific sequence during 5´ RACE. This may include some virus-specific sequence that cannot be distinguished from the poly(A) or poly(C) tail. The first two C

residues that belong to the viral sequence are underlined with a dotted line in the poly(C)-tailed sequence traces.
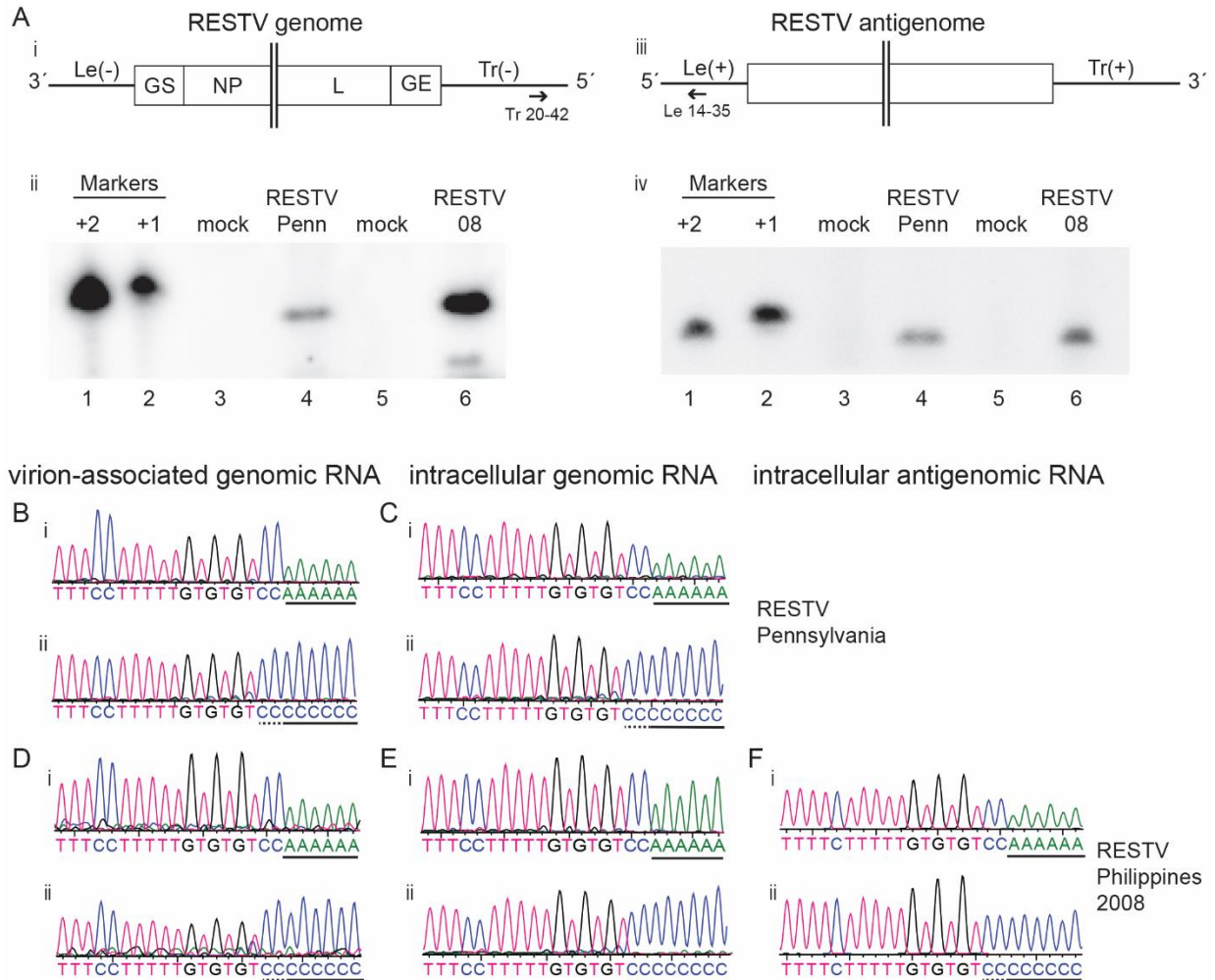
Fig. S4. Analysis of the 5´ ends of RESTV RNAs. (A) Primer extension mapping of the 5´ ends of RESTV genome and antigenome RNAs. (i and iii) Schematic diagrams (not to scale) of the RESTV genome and antigenome showing the annealing positions of the positive (i) and negative (iii) sense primers used for primer extension analysis. (ii and iv) Primer extension analysis of genomic and antigenomic RNA from RESTV isolates Pennsylvania (RESTV Penn) and Philippines 08 (RESTV 08). Total cellular RNA was isolated from infected Vero cells and analyzed with a positive (ii) or negative (iv) sense primer. RNA from mock-infected cells (mock) was used as a negative control. [γ-^{32}P]ATP end-labeled DNA oligonucleotides corresponding in length and sequence to the respective cDNA representing initiation from positions +1 and +2 relative to the published sequence were used as markers. (B-F) Vero cells were infected with RESTV isolate Pennsylvania

7

(B, C) or RESTV isolate Philippines 2008 (D-F), and the 5´ ends of virion-associated (B, D), intracellular genomic viral RNA (C, E) or intracellular antigenomic viral RNA (F) were amplified by 5´ RACE. The cDNA was tailed with dATP (i) or dCTP (ii). The black line below the sequence traces indicates poly(A) or poly(C) tail sequences that were added to the virus-specific sequence during 5´ RACE. This may include some virus-specific sequence that cannot be distinguished from the poly(A) or poly(C) tail. The first two C residues that belong to the viral sequence are underlined with a dotted line in the poly(C)-tailed sequence traces.
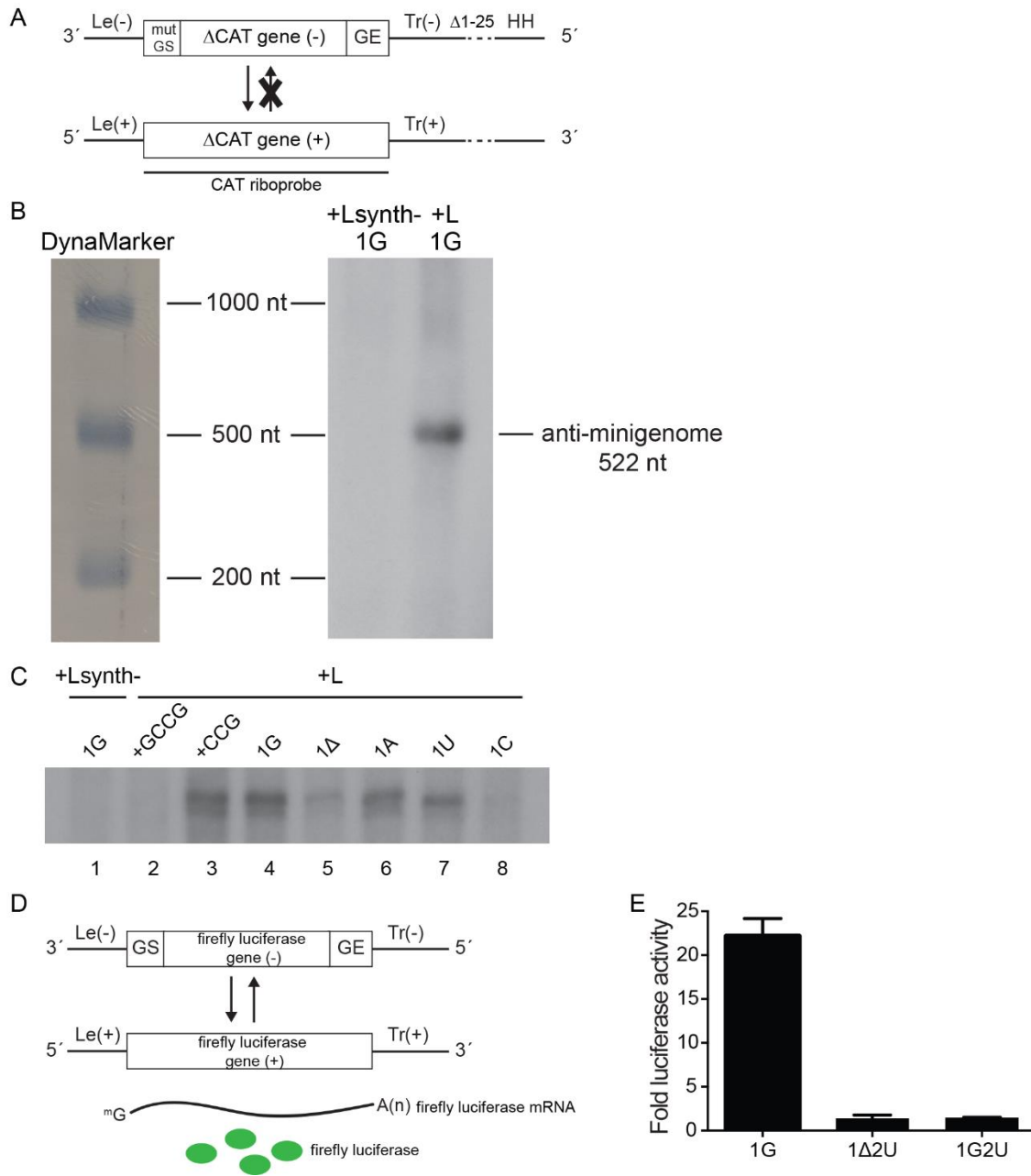
Fig. S5. Analysis of mutant EBOV minigenomes with different 3′ ends. (A) Schematic diagram (not to scale) showing the organization of the single-cycle replicating, non-transcribing EBOV minigenome and the anti-minigenome product. The terminal 25 nucleotides of the trailer were deleted (Δ1-25). The trailer is flanked by an inactive hammerhead ribozyme (HH). The annealing position of the negative sense primer used for primer extension is indicated; mut GS, mutated gene start signal; GE, gene end signal.

Annealing position of riboprobe indicated. (B) Northern blot analysis of anti-minigenome RNA products using a negative sense riboprobe specific to the ΔCAT sequence. DynaMarker Prestain Marker for RNA High was run alongside RNA products on the same gel. Left panel, image of membrane following transfer of RNA. Right panel, autoradiography image. (C) Northern blot analysis of anti-minigenome RNA products using a negative sense riboprobe specific to the ΔCAT sequence. The experiment was performed three times and a representative blot is shown. (D) Schematic diagram (not to scale) showing the replication- and transcription-competent EBOV minigenome expressing a luciferase reporter gene (3E5E-Luc), the corresponding anti-minigenome product, firefly luciferase mRNA, and firefly luciferase. GS, gene start signal; GE, gene end signal. (E) HEK 293T cells were infected with minigenome plasmid 3E5E-Luc along with plasmids encoding the EBOV proteins required for replication and transcription (NP, VP35, VP30, and L), the T7 RNA polymerase, and a plasmid encoding β-galactosidase. As a negative control, L was replaced with the replication-deficient L mutant, L_{synth-}. Two days post transfection, cell lysates were harvested and luciferase and β-galactosidase activities were measured. Luciferase values were normalized to account for differences in transfection efficiency by dividing by the corresponding β-galactosidase values. Fold luciferase activity was calculated by dividing the luciferase value of the wild-type L samples by the values of the corresponding samples with L_{synth-}. The experiment was performed three times and the mean of the results is shown. Standard error of the mean (SEM) for this figure was calculated using GraphPad Prism software.

Table S1. Terminal nucleotides of ebolavirus genomes deposited in GenBank. Sequences highlighted in yellow are representative for more than one sequence as indicated below.

[a] KU182909: Ebola virus Kikwit. 7 out of 12 sequences with 3' GCC and GGU 5' genome ends (9510622, 9510627, 9510628, 9510629, 9510630, 9510631, 9510632).

[b] U182904: Ebola virus Kikwit. 5 out of 12 sequenced isolates with 3' GCC and GGG 5' genome ends (9510621, 9510623, 9510624, 9510625, 9510626).

[c] KY426733: Ebola virus Makona. 50 sequences with 3' GCC and GGU 5' genome ends (J0176 – J0225).

[d] KU143831: Ebola virus Makona. 60 sequences with 3' GCC and GGU 5' genome ends (S1-S60).

[e] KR074996: Ebola virus Makona. 8 sequences with 3' GCC and GG 5' genome ends (DQE1, DQE3, DQE4, DQE5, DQE6, DQE12, DQE13, DQE14).

[f] KP260799: Ebola virus Makona. 4 sequences with 3' GCC and GG 5' genome ends (DPR1-DPR4).

GenBank	Isolate	3' ends	5' ends
	Ebola virus		
MH121168	Zaire ebolavirus isolate Ebola virus/H.sapiens-tc/Zaire/1976/Mayinga-R3816, complete genome	3' GCC	GG 5'
MH121166	Zaire ebolavirus isolate Ebola virus/H.sapiens-tc/COD/1976/Yambuku-Mayinga, complete genome	3' CC	GG 5'
NC_002549	Zaire ebolavirus isolate Ebola virus/H.sapiens-tc/COD/1976/Yambuku-Mayinga, complete genome	3' GCC	GGU 5'
EU224440	Zaire ebolavirus strain Mayinga, complete genome	3' GCC	GGU 5'
AY142960	Zaire Ebola virus strain Mayinga subtype Zaire, complete genome	3' GCC	GGU 5'
AF272001	Zaire Ebola virus strain Mayinga, complete genome	3' GCC	GGU 5'
AF086833	Ebola virus - Mayinga, Zaire, 1976, complete genome	3' GCC	GGU 5'
KR063671	Ebola virus isolate Ebola virus/H.sapiens-tc/COD/1976/Yambuku-Mayinga, complete genome	3' CC	GG 5'
KC242801	Zaire ebolavirus isolate EBOV/H.sapiens-tc/COD/1976/deRoover, complete genome	3' GCC	GGU 5'
KC242791	Zaire ebolavirus isolate EBOV/H.sapiens-tc/COD/1977/Bonduni, complete genome	3' GCC	GGU 5'
KC242792	Zaire ebolavirus isolate EBOV/H.sapiens-tc/GAB/1994/Gabon, complete genome	3' GCC	GGU 5'
AY354458	Zaire ebolavirus strain Zaire 1995, complete genome	3' GCC	GGUU 5'
KR867676	Zaire ebolavirus isolate Ebola virus/H.sapiens-tc/COD/1995/Kikwit-9510621, complete genome	3' CC	GG 5'
MH121165	Zaire ebolavirus isolate Ebola virus/H.sapiens-tc/COD/1995/Kikwit-9510621, complete genome	3' GCC	GGU 5'

MH121164	Zaire ebolavirus isolate Ebola virus/H.sapiens-tc/COD/1995/Kikwit-9510621, complete genome	3' GCC	GG 5'
KT762962	Zaire ebolavirus isolate Ebola virus/H.sapiens-tc/COD/1995/Kikwit-9510621 clone R4415, complete genome	3' GCC	GG 5'
KT762961	Zaire ebolavirus isolate Ebola virus/H.sapiens-tc/COD/1995/Kikwit-9510621 clone R4414, complete genome	3' GCC	GG 5'
KC242799	Zaire ebolavirus isolate EBOV/H.sapiens-tc/COD/1995/13709 Kikwit, complete genome	3' GCC	GGU 5'
KC242796	Zaire ebolavirus isolate EBOV/H.sapiens-tc/COD/1995/13625 Kikwit, complete genome	3' GCC	GGU 5'
KU182909[a]	Ebola virus isolate Ebola virus/H. sapiens-tc/COD/1995/Kikwit-9510622, complete genome	3' GCC	GGU 5'
KU182904[b]	Ebola virus isolate Ebola virus/H. sapiens-tc/COD/1995/Kikwit-9510626, complete genome	3' GCC	GGG 5'
KC242798	Zaire ebolavirus isolate EBOV/H.sapiens-tc/GAB/1996/1Ikot, complete genome	3' GCC	GGU 5'
KC242797	Zaire ebolavirus isolate EBOV/H.sapiens-tc/GAB/1996/1Oba, complete genome	3' GCC	GGU 5'
KC242795	Zaire ebolavirus isolate EBOV/H.sapiens-tc/GAB/1996/1Mbie, complete genome	3' GCC	GGU 5'
KC242794	Zaire ebolavirus isolate EBOV/H.sapiens-tc/GAB/1996/2Nza, complete genome	3' GCC	GGU 5'
KC242793	Zaire ebolavirus isolate EBOV/H.sapiens-tc/GAB/1996/1Eko, complete genome	3' GCC	GGU 5'
KC242800	Zaire ebolavirus isolate EBOV/H.sapiens-tc/GAB/2002/Ilembe, complete genome	3' GCC	GGU 5'
KC242790	Zaire ebolavirus isolate EBOV/H.sapiens-tc/COD/2007/5 Luebo, complete genome	3' GCC	GGU 5'
KC242785	Zaire ebolavirus isolate EBOV/H.sapiens-tc/COD/2007/0 Luebo, complete genome	3' GCC	GGU 5'
KC242789	Zaire ebolavirus isolate EBOV/H.sapiens-tc/COD/2007/4 Luebo, complete genome	3' GCC	GGU 5'
KC242788	Zaire ebolavirus isolate EBOV/H.sapiens-tc/COD/2007/43 Luebo, complete genome	3' GCC	GGU 5'
KC242787	Zaire ebolavirus isolate EBOV/H.sapiens-tc/COD/2007/23 Luebo, complete genome	3' GCC	GGU 5'
KC242786	Zaire ebolavirus isolate EBOV/H.sapiens-tc/COD/2007/1 Luebo, complete genome	3' GCC	GGU 5'
KC242784	Zaire ebolavirus isolate EBOV/H.sapiens-tc/COD/2007/9 Luebo, complete genome	3' GCC	GGU 5'

KP096422	Zaire ebolavirus isolate H.sapiens-tc/GIN/14/WPG-C15, complete genome (Makona)	3' GCC	GG 5'
KP096421	Zaire ebolavirus isolate H.sapiens-tc/GIN/14/WPG-C07, complete genome (Makona)	3' GCC	GG 5'
KP096420	Zaire ebolavirus isolate H.sapiens-tc/GIN/14/WPG-C05, complete genome (Makona)	3' GCC	GG 5'
KJ660348	Zaire ebolavirus isolate H.sapiens-wt/GIN/2014/Makona-Gueckedou-C05, complete genome	3' GCC	GGU 5'
KJ660347	Zaire ebolavirus isolate H.sapiens-wt/GIN/2014/Makona-Gueckedou-C07, complete genome	3' GCC	GGU 5'
KJ660346	Zaire ebolavirus isolate H.sapiens-wt/GIN/2014/Makona-Kissidougou-C15, complete genome	3' GCC	GGU 5'
KT589389	Zaire ebolavirus isolate Ebola virus/H. sapiens-wt/SLE/2014/Makona-201409581, complete genome	3' GCC	G 5'
KT589390	Zaire ebolavirus isolate Ebola virus/H.sapiens-wt/SLE/2014/Makona-201403164, complete genome	3' GCC	G 5'
KP178538	Zaire ebolavirus isolate Ebolavirus/H.sapiens-wt/LIB/2014/Makona-201403007, complete genome	3' GCC	GG 5'
KP240935	Zaire ebolavirus isolate Ebola virus/H.sapiens-wt/USA/2014/Makona-201403305, complete genome	3' GCC	GG 5'
KP240934	Zaire ebolavirus isolate Ebola virus/H.sapiens-wt/USA/2014/Makona-201403293, complete genome	3' GCC	GG 5'
KP240933	Zaire ebolavirus isolate Ebola virus/H.sapiens-wt/LBR/2014/Makona-201403275, complete genome	3' GCC	GG 5'
KP240932	Zaire ebolavirus isolate Ebola virus/H.sapiens-wt/LBR/2014/Makona-201403261, complete genome	3' GCC	GG 5'
KP240931	Zaire ebolavirus isolate Ebola virus/H.sapiens-wt/SLE/2014/Makona-201403147, complete genome	3' GCC	GG 5'
KP342330	Zaire ebolavirus isolate H.sapiens-wt/GIN/2014/Conacry-192, complete genome	3' GCC	GG 5'
MF599505	Zaire ebolavirus isolate Ebola virus/H.sapiens-wt/CCDC/2015/sierra leone/IVDC4_Patient_4_Day7, complete genome	3' CC	GG 5'
KY426733[c]	Zaire ebolavirus isolate Ebola virus/H.sapiens-wt/SLE/2015/Makona-J0225, complete genome	3' GCC	GGU 5'
KU143831[d]	Zaire ebolavirus isolate Ebola virus H.sapiens-wt/SLE/2014/Makona-S60, complete genome	3' GCC	GGU 5'
KR074996[e]	Zaire ebolavirus isolate Ebola virus H.sapiens-wt/LBR/2014/Makona-Liberia-DQE1, complete genome	3' GCC	GG 5'
KR781608	Zaire ebolavirus strain Ebola virus/H.sapiens-rec/LBR/2014/Makona-L2014, complete genome	3' CC	GG 5'
KP260799[f]	Zaire ebolavirus isolate Ebola virus/H.sapiens-wt/MLI/2014/Makona-Mali-DPR1, complete genome	3' GCC	GG 5'

KM034562	Zaire ebolavirus isolate Ebola virus/H.sapiens-wt/SLE/2014/Makona-G3686.1, complete genome	3' GCC	G 5'
KM233113	Zaire ebolavirus isolate Ebola virus/H.sapiens-wt/SLE/2014/Makona-G3856.1, complete genome	3' GCC	GG 5'
KM233109	Zaire ebolavirus isolate Ebola virus/H.sapiens-wt/SLE/2014/Makona-G3846, complete genome	3' GCC	GG 5'
KM233070	Zaire ebolavirus isolate Ebola virus/H.sapiens-wt/SLE/2014/Makona-G3770.2, complete genome	3' GCC	GGU 5'
KM233069	Zaire ebolavirus isolate Ebola virus/H.sapiens-wt/SLE/2014/Makona-G3770.1, complete genome	3' GCC	GG 5'
KM233056	Zaire ebolavirus isolate Ebola virus/H.sapiens-wt/SLE/2014/Makona-G3735.1, complete genome	3' CC	GG 5'
KM233053	Zaire ebolavirus isolate Ebola virus/H.sapiens-wt/SLE/2014/Makona-G3724, complete genome	3' CC	GG 5'
LT605058	Ebola virus isolate Ebola virus Makona isolate Frankfurt genome assembly, complete genome: monopartite	3' GCC	GGU 5'
Sudan virus			
KT878488	Sudan ebolavirus isolate Sudan virus/C.porcellus-lab/SSD/1976/Nzara-Boneface, complete genome	3' GCC	GGU 5'
FJ968794	Sudan ebolavirus strain Boniface, complete genome	3' GCC	GGU 5'
KT750754	Sudan ebolavirus isolate Boniface, complete genome	3' GCC	GGU 5'
KR063670	Sudan ebolavirus isolate Ebola virus/H.sapiens-tc/UGA/2000/Gulu-808892, complete genome	3' GCC	GGG 5'
NC_006432	Sudan ebolavirus isolate Sudan virus/H.sapiens-tc/UGA/2000/Gulu-808892, complete genome	3' GCC	GGU 5'
KU182912	Sudan ebolavirus isolate Sudan virus/H. sapiens-tc/SDN/2000/Gulu-200011676, complete genome	3' GCC	GGG 5'
AY729654	Sudan ebolavirus strain Gulu, complete genome	3' GCC	GGU 5'
KC545392	Sudan ebolavirus isolate EboSud-682 2012, complete genome	3' GCC	GG 5'
KC545391	Sudan ebolavirus isolate EboSud-609 2012, complete genome	3' GCC	GG 5'
KC545390	Sudan ebolavirus isolate EboSud-603 2012, complete genome	3' GCC	GG 5'
KC545389	Sudan ebolavirus isolate EboSud-602 2012, complete genome	3' GCC	GG 5'
KC589025	Sudan ebolavirus isolate EboSud-639, complete genome	3' GCC	GGU 5'
KC242783	Sudan ebolavirus isolate SUDV/H.sapiens-tc/SSD/1979/Maleo, complete genome	3' GCC	GGU 5'
JN638998	Sudan ebolavirus - Nakisamata, complete genome	3' GCC	GGU 5'
EU338380	Sudan ebolavirus isolate EBOV-S-2004 from Sudan, complete genome	3' GCC	GGU 5'

	Reston virus		
KY798004	Reston ebolavirus isolate USA_PA_1989_(813159), complete genome	3' GCC	GG 5'
JX477166	Reston ebolavirus isolate RESTV/M.fascicularis-tc/PHL-USA/1996/Ferlite, Philippines/Alice, TX, complete genome	3' GCC	GG 5'
NC_004161	Reston ebolavirus isolate Reston virus/M.fascicularis-tc/USA/1989/Philippines89-Pennsylvania, complete genome	3' GCC	GG 5'
KY008770	Reston ebolavirus strain Reston virus/M.fascicularis-tc/USA/1989/Philippines89-AZ-1435, complete genome	3' GCC	GG 5'
AY769362	Reston ebolavirus strain Pennsylvania, complete genome	3' GCC	GGU 5'
AB050936	Reston ebolavirus - Reston genomic RNA, complete genome	3' CCC	GGG 5'
AF522874	Reston Ebola virus strain Pennsylvania, complete genome	3' GCC	GG 5'
	Bundibugyo virus		
KC545396	Bundibugyo ebolavirus isolate EboBund-14 2012, complete genome	3' GCC	GG 5'
KC545395	Bundibugyo ebolavirus isolate EboBund-122 2012, complete genome	3' GCC	GG 5'
KC545394	Bundibugyo ebolavirus isolate EboBund-120 2012, complete genome	3' GCC	GG 5'
KC545393	Bundibugyo ebolavirus isolate EboBund-112 2012, complete genome	3' GCC	GG 5'
FJ217161	Bundibugyo ebolavirus, complete genome	3' GCC	GGU 5'
KR063673	Bundibugyo virus isolate Bundibugyo virus/H.sapiens-tc/UGA/2007/Butalya-811250, complete genome	3' GCC	GGU 5'
KU182911	Bundibugyo virus isolate Bundibugyo virus/H. sapiens-tc/UGA/2007/Bundibugyo-200706291, complete genome	3' GCC	GG 5'
	Tai Forest virus		
KU182910	Tai Forest ebolavirus isolate Tai Forest virus/H. sapiens-tc/CIV/1994/Tai Forest-CDC807212, complete genome	3' GCC	GGU 5'
NC_014372	Tai Forest ebolavirus isolate Tai Forest virus/H.sapiens-tc/CIV/1994/Pauleoula-CI, complete genome	3' GCC	GGU 5'
FJ217162	Cote d'Ivoire ebolavirus, complete genome	3' GCC	GGU 5'
	Bombali virus		
MF319185	Bombali ebolavirus isolate Bombali ebolavirus/Mops condylurus/SLE/2016/PREDICT_SLAB000156, complete genome	3' GCC	GGA 5'
MF319186	Bombali ebolavirus isolate Bombali ebolavirus/ Chaerephon pumilus/SLE/2016/PREDICT_SLAB000047, complete genome	3' GCC	GGA 5'

Table S2. Sequence analysis of 3´ and 5´ ends of EBOV Mayinga genomic and antigenomic RNAs. Additional nucleotides upstream of the 3´ CCUGU sequence or downstream of the ACAGG 5´ terminus are underlined. * Also could be C(n)CCUGU. ‡ Also could be ACAGGG(n).

sequence	source of RNA	strand sense	tailing	number of clones
3´ GCCUGU 3´ ACCUGU or 3´ CCUGU 3´ UCCUGU	virion	genome	A	3/12 8/12 1/12
3´ GCCUGU 3´ ACCUGU 3´ CCUGU* 3´ UACCUGU 3´ ACACACACACCUGU	virion	genome	C	1/10 5/10 2/10 1/10 1/10
3´ GCCUGU 3´ ACCUGU or 3´ CCUGU 3´ CACCUGU 3´ CAACACCUGU	cellular	genome	A	6/11 3/11 1/11 1/11
3´ GCCUGU 3´ ACCUGU 3´ CCUGU* 3´AGGGACACACACAACCUGU	cellular	genome	C	3/9 4/9 1/9 1/9
3´ GCCUGU 3´ ACCUGU or 3´ CCUGU 3´ CUGU 3´ CAGCCUGU 3´ CACAGGCGCCUGU	cellular	antigenome	A	4/14 7/14 1/14 1/14 1/14
3´ GCCUGU 3´ ACCUGU 3´ CCUGU* 3´ CAAAAACCUGU	cellular	antigenome	C	1/6 1/6 1/6 3/6
ACAGG 5´ ACAGGUC 5´ ACAGGC 5´	virion	genome	A	5/7 1/7 1/7
ACAGG 5´‡	virion	genome	C	4/4
ACAGG 5´	cellular	genome	A	5/5
ACAGG 5´‡	cellular	genome	C	6/6

Table S3. Primers used in this study. RT, reverse transcription.

Experiment	Primer purpose	Sequence (5' - 3')	Primer #
3' RACE genome	RT primer – adaptor + tail	GAGGACTCGAGCTCAAGCATGCATTTTTTTTTTTTTTT or GAGGACTCGAGCTCAAGCATGCATGGGGGGGGGGGGGGG	2720 or 2721
	PCR primer 1 – tail adaptor	GAGGACTCGAGCTCAAGC	2722
	PCR primer 2 – EBOV-specific	GCTTGTTTGACTGTGAACTAATGCTG	2742
	PCR primer 2 – SUDV-specific	CACTTATCAAGAAGGTTAGAATTG	2847
	PCR primer 2 – RESTV-specific	GAACTTCTCGCGAAATGGATTC	2768
5' RACE genome	RT primer – EBOV-specific	GATAAAATTAAAAGAAAAGGCAGGAC	2745
	RT primer – SUDV-specific	ACAGCCAAAATATTTCCTAGGGCCG	2850
	RT primer – RESTV-specific	TTAAATGATAATGATCTTGGGAACTCG	2771
	PCR primer 1 - tail	GAGGACTCGAGCTCAAGCATGCATTTTTTTTTTTTTTT or GAGGACTCGAGCTCAAGCATGCATGGGGGGGGGGGGGGG	2720 or 2721
	PCR primer 2 – EBOV-specific	GATAAAATTAAAAGAAAAGGCAGGAC	2745
	PCR primer 2 – SUDV-specific	ACAGCCAAAATATTTCCTAGGGCCG	2850
	PCR primer 2 – RESTV-specific	TTAAATGATAATGATCTTGGGAACTCG	2771
3' RACE antigenome	RT primer – adaptor + tail	GAGGACTCGAGCTCAAGCATGCATTTTTTTTTTTTTTT or GAGGACTCGAGCTCAAGCATGCATGGGGGGGGGGGGGGG	2720 or 2721
	PCR primer 1: tail adaptor	GAGGACTCGAGCTCAAGC	2722
	PCR primer 2 – EBOV-specific	AATGGTAATAGCCTTAATCTTTGTGTA	2744
	PCR primer 2 – SUDV-specific	TTACATAAGTATTAAGAAAAAATTAC	2849
	PCR primer 2 – RESTV-specific	GTCGCATTTAAACATGCTATCTTCAG	2770
5' RACE antigenome	RT primer – EBOV Le NP-VP35 IGR	GGTTTTAATCTTCATCATTAG	2881
	RT primer – SUDV Le NP-VP35 IGR	ATGAAGGTTTTAATCTTCATCATGGA	2885

	RT primer – RESTV Le NP-VP35 IGR	ATGAAGGTTTTAATCTTCATCAAATG	2883
	PCR primer 1 – tail	GAGGACTCGAGCTCAAGCATGCATTTTTTTTTTTTTT or GAGGACTCGAGCTCAAGCATGCATGGGGGGGGGGGGGGG	2720 or 2721
	PCR primer 2 – EBOV-specific nested	GTCTGAGGTGGGACTTGTGATG	2743
	PCR primer 2 – SUDV-specific nested	GTGGACTAAAGGGATACCTCGGG	2848
	PCR primer 2 – RESTV-specific nested	GAAGAGTCCCAAGATCACTAGACATACC	2769
EBOV genome primer extension	RT primer – Tr 17-41	AATTTAAAAATAAATCTATTTCTTC	2739
	Tr 1-41 +1 marker	AATTTAAAAATAAATCTATTTCTTCTTTTTTGTGTGTCCAA	2741
	Tr 2-41 +2 marker	AATTTAAAAATAAATCTATTTCTTCTTTTTTGTGTGTCCG	2852
EBOV antigenome and anti-minigenome primer extension	RT primer – Le 14-35 n	GATCCTAAAAATTCTTCTTTCT	2735
	Le 1-35 +1 marker	GATCCTAAAAATTCTTCTTTCTTTTTGTGTGTCCG	2737
	Le 2-35 +2 marker	GATCCTAAAAATTCTTCTTTCTTTTTGTGTGTCC	2851
SUDV genome primer extension	RT primer – Tr 16-36	TTAAAAAATCTGTATTTTCTC	2963
	Tr 1-36 +1 marker	TTAAAAAATCTGTATTTTCTCTTTTTTGTGTGTCCG	2964
	Tr 2-36 +2 marker	TTAAAAAATCTGTATTTTCTCTTTTTTGTGTGTCC	3015
SUDV antigenome primer extension	RT primer – Le 14-35	AATCTTAAAAACTTTTCTTTCT	2961
	Le 1-35 +1 marker	AATCTTAAAAACTTTTCTTTCTTTTTGTGTGTCCG	2962
	Le 2-35 +2 marker	AATCTTAAAAACTTTTCTTTCTTTTTGTGTGTCC	3014
RESTV genome primer extension	RT primer – Tr 20-42	AAAACATTATAAAAAACCAATTT	2766
	Tr 1-42 +1 marker	CACAAAAAGTCTTAAAAAACCTTTTTTCTTTTTGTGTGTCCG	2765
	Tr 2-42 +2 marker	AAAACATTATAAAAAACCAATTTTTTCCTTTTTGTGTGTCC	2854
RESTV antigenome	RT primer – Le 14-35	AGTCTTAAAAAACCTTTTTTCT	2959

primer extension	Le 1-35 +1 marker	**AGTCTTAAAAAACCTTTTTTCTTTTTGTGTGTCCG**	2960
	Le 2-35 +2 marker	**AGTCTTAAAAAACCTTTTTTCTTTTTGTGTGTCC**	3013