Supplemental information for:

Quantification of influenza virus mini viral RNAs using Cas13

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Supplementary Figures

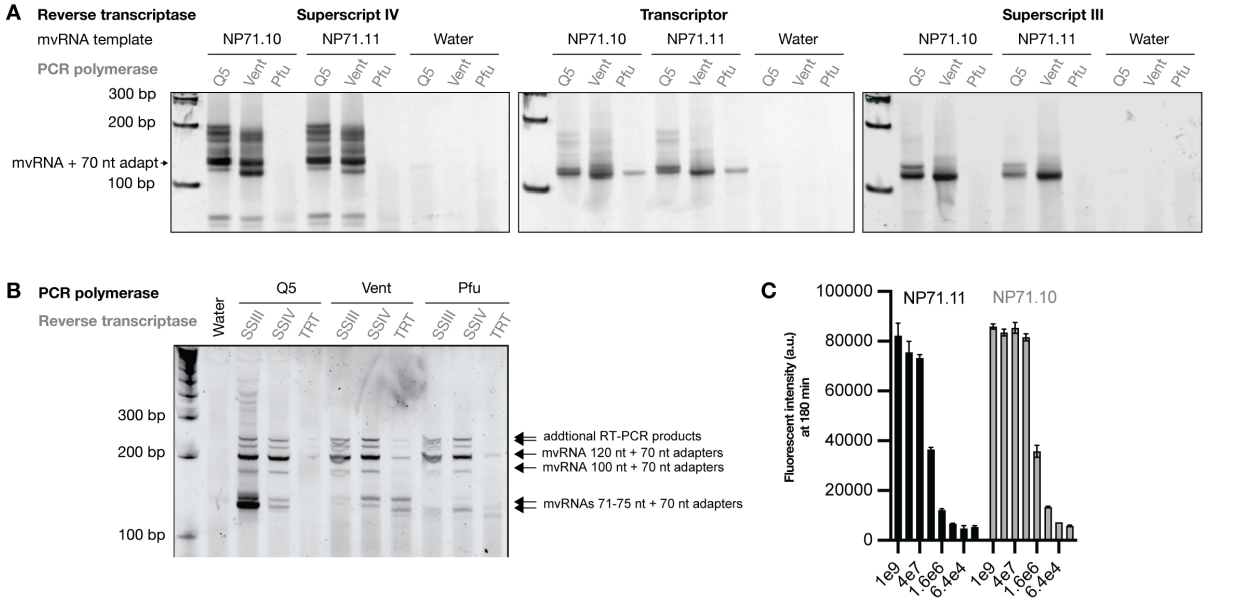

Figure S1. Detection of synthetic mvRNAs by different RT and PCR enzymes. A) Two synthetic mvRNAs were reverse transcribed into cDNA by three different RT enzymes and subsequently amplified by three different PCR enzymes. For sequences used, see Table S1. **B**) RT-PCR amplification of 4 different mvRNAs, with lengths 71, 75, 100 and 120 nt, by three different RT and PCR enzymes. Expected sizes and the length of PCR primers are indicated. RT enzymes used were superscript III (III), superscript IV (IV), Transcriptor (TRT). PCR enzymes used were Pfu polymerase (PFU), Taq polymerase (Taq), and Q5 polymerase (Q5). **C**) Detection synthetic mvRNAs NP71.10 and NP71.11 diluted in HEK293T total RNA using LbuCas13a and crRNAs CL68 and CL69, respectively. Error bars indicate standard deviation of three independent repeats.

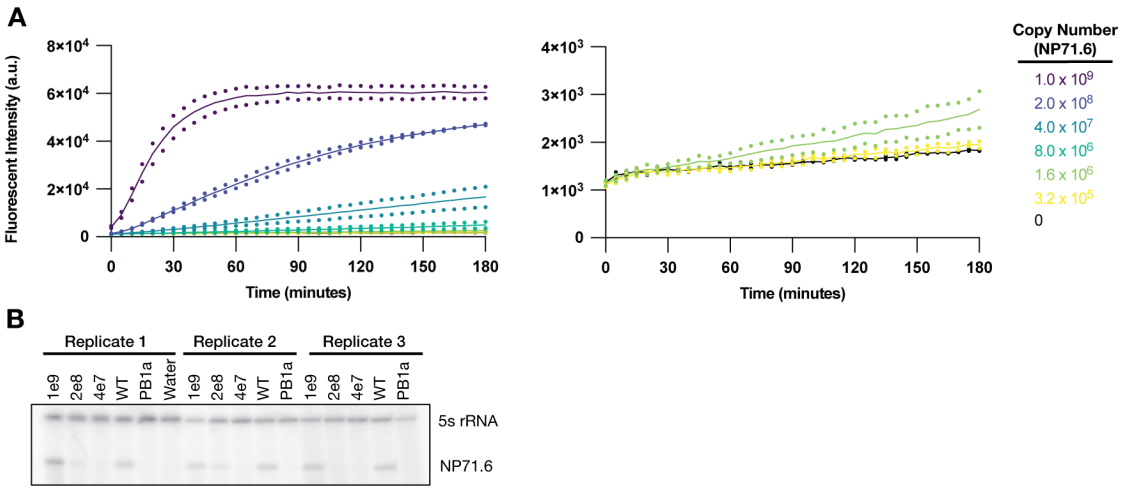

Figure S2. A) LbuCas13a detection of synthetic mvRNA NP71.6 diluted in HEK293T total RNA. Right graph is a close-up of bottom part of left graph. **B**) Detection of mvRNA NP71.6 by primer extension following plasmid-based expression in HEK293T cells in the presence of WT or an inactive (PB1a) WSN RNA polymerase. 5S rRNA was used as loading control.

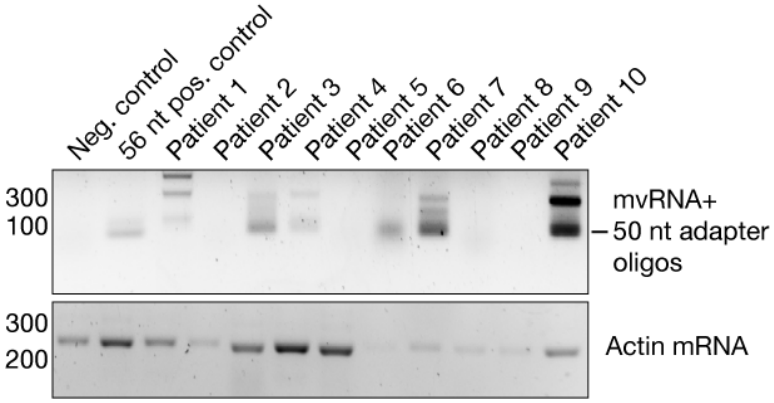

Figure S3. Detection of mvRNAs in influenza A virus positive clinical samples using RT-PCR. A 56 nt long segment 5-based mvRNA was used as positive control. As negative control, a clinical sample was used that had tested negative for influenza A virus genetic material.

Supplementary Tables

Table S1. Sequences of mvRNAs studied.

Name	Lab Reference	Sequence (5′ to 3′)	Figure
NP71.10	GC50_13	AGUAGAAACAAGGGUAUUUUUCUUUACUAGUGGCAGCAAAAGCAGGGUAACUAGUCUACCCUGCUUUUGCU	S1A
NP71.11	GC50_15	AGUAGAAACAAGGGUAUUUUUCUUUACUAGUGGCAGCAAAAGCACCCAUACUAGUCUACCCUGCUUUUGCU	S1A
NP71.6	GC50.9	AGUAGAAACAAGGGUAUUUUUCUUUACUAGUCCGGCCGUUUUGGUUGCCACUAGUCUACCCUGCUUUUGCU	2B-2F, S2
NP61	vNP61	AGUAGAAACAAGGGUAUUUUUCGAUGUCACUCUGUGAGUGAUUAUCUACCCUGCUUUUGCU	3, 4, 5
mvRNA75	mvRNA75	AGTAGAAACAAGGGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCCCTGCTTTTGCT	S1B
mvRNA100	mvRNA100	AGTAGAAACAAGGGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATACAAATAAATGTCCAGACCTGCAGGCATGCAAGCTCCTGCTTTTGCT	S1B
mvRNA120	mvRNA120	AGTAGAAACAAGGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCCTGCTTTTGCT	S1B

Table S2. crRNA sequences used.

Name	Target	Sequence (5′ to 3′)	Limit of Detection (copies) (lowest standard that was 3 standard deviations above mock)	Figure
CL06	Human 5s rRNA*	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGGGCGCGUUCAGGGUGGUAUGGCCGUAG	Not determined	2A
CL11	Human 5s rRNA	GACCACCCCAAAAAUGAAGGGGACUAAAACGGGCGCGUUCAGGGUGGUAUGGCCGUAG	Not determined	2A
CL09	NP71.6	GACCACCCCAAAAAUGAAGGGGACUAAAACUAGACUAGUGGCAACCAAAACGGCCGGA	3e5	2B-F, S2A
CL63/A	NP-61	GACCACCCCAAAAAUGAAGGGGACUAAAACATCACTCACAGAGTGACATCGAAAAATA	8e6	4
CL51/B	NP-61	GACCACCCCAAAAAUGAAGGGGACUAAAACAGAUAAUCACUCACAGAGUGACAUCGAA	3e5	4, 5, 6
CL66/C	NP-61	GACCACCCCAAAAAUGAAGGGGACUAAAACACATCGAAAAATACCCTTGT	4e7	4
CL67/D	NP-61	GACCACCCCAAAAAUGAAGGGGACUAAAACACAGAGTGACATCGAAAAAT	4e7	4
CL60/E	NP-61	GACCACCCCAAAAAUGAAGGGGACUAAAACAGAGTGACATCGAAAAATACCCTTGTTT	1e6	4
CL61/F	NP-61	GACCACCCCAAAAAUGAAGGGGACUAAAACACAGAGTGACATCGAAAAATACCCTTGT	1e6	4
CL62/G	NP-61	GACCACCCCAAAAAUGAAGGGGACUAAAACAGTGACATCGAAAAATACCCTTGTTTCT	8e6	4
CL65/H	NP-61	GACCACCCCAAAAAUGAAGGGGACUAAAACCAGAGTGACATCGAAAAATA	8e6	4

CL64/I	NP-61	GACCACCCCAAAAAUGAAG GGGACUAAAACTGACATCG AAAAATACCCTT	8e6	4
CL68	NP71.11	GACCACCCCAAAAAUGAAG GGGACUAAAACTAGTTACCC TGCTTTTGCTGCCACTAGT	8e6	S1C
CL69	NP71.11	GACCACCCCAAAAAUGAAG GGGACUAAAACTAGTATGG GTGCTTTTGCTGCCACTAGT	8e6	S1C

*LwaCas13a enzyme rather than LbuCas13a enzyme used for the other crRNAs.

Table S3. Other oligonucleotides used.

Name	Description	Sequence (5′ to 3′)	Figure
6UFAM	RNA reporter	/FAM/UUUUUU/3IABKFQ/	2, 3, 4, 5, S2, S4
GC-	Targets NP segment	AGCAAAAGCAGGGTAGACTAGT	2D, 2F, S2B
NP 5′	Targets NP segment	AGTAGAAACAAGGGTATTTTTC	2D, 2F, S2B
GC50.9 probe	Targets NP71.6	/FAM/TTACTAGTC/ZEN/CGGCCGTTTTGGTTGC/3IABKFQ/	2D-F
Lv3ga	vRNA RT primer (contains LNA)	GTTCAGACGTGTGCTCTTCCGATCTAGCG+AAAGCAGG	1A, S1, S5
Lv3aa	vRNA RT primer (contains LNA)	GTTCAGACGTGTGCTCTTCCGATCTAGC+A+AAAGCAGG	1A, S1, S5
Lv5	vRNA forward primer (contains LNA)	CACGACGCTCTTCCGATCTHNNNNNNNAGTAGAA+A+CAAGG	1A, S1, S5
P5short	vDNA fw	GAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	1A, S1, S5
I7short	vDNA rv	GAGATACTGGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	1A, S1, S5

Table S4. Results of influenza patient clinical sample analysis.

Fig. Code	Internal Code	Cambridge Code	H1 or H3	Age (years)	Clinical Ct Value	Sex	Total RNA input (ng)	Copies/ uL
H3-1	C1	1193	H3	7	11	F	83.8	3521682.513
H3-2	C2	1392	H3	62	15	F	31.5	Not Detected
H1-1	C3	1109	H1	18	12	F	64.5	6162069.391
H1-2	C4	1275	H1	28	16	M	39.1	954004.4947
H3-3	C5	1196	H3	83	17	F	29.7	2238521.406
H1-3	C6	1163	H1	1	17	M	143.2	786978.3698
H1-4	C7	1268	H1	1	16	M	131.2	1182889.721
H3-4	C8	1375	H3	76	13	M	52.3	718879.6206
H3-5	C9	1497	H3	18	12	F	241	763487.979
H1-5	C10	1127	H1	76	17	M	86.7	2406890.514
H3-6	C11	1134	H3	88	14	F	45.8	674301.8301
H1-6	C12	1135	H1	57	17	F	31.9	838687.1017
H3-7	C13	1217	H3	71	10	F	24.4	4498742.275
H3-8	C14	1169	H3	41(days)	10	M	45.3	1279877.109
H1-7	C15	1175	H1	15	17	M	35.3	857500.4898
H1-8	C16	1139	H1	35	16	F	113.1	8142113.409
H1-9	C17	1248	H1	58	16	F	56	737658.3474
H1-10	C18	1219	H1	79	17	M	43.2	794027.1421
H3-9	C19	1229	H3	2	13	M	118.1	2219306.633
H1-11	C20	1448	H1	42	13	F	153.1	3399464.279
H1-12	C21	1126	H1	84	29	F	67.0	Not detected
H3-10	C22	1588	H3	82	10	F	39.9	4215913.226
H1-13	C23	1464	H1	87	14	F	80.6	2535752.682
H1-14	C24	1403	H1	34	15	F	74.1	545581.3123
H3-11	C25	1608	H3	87	12	F	148.0	564835.1674
H1-15	C26	1633	H1	29	15	F	75.0	4014859.47
H3-12	C27	1673	H3	32	13	F	173.1	531682.0197
H1-16	C28	1314	H1	23	17	F	162.2	640879.3779
H1-17	C29	1435	H1	3	16	F	45.2	6224905.957
H1-18	C30	1689	H1	81	15	F	80.3	1378330.248

Table S5. Results of after fractionation of influenza patient clinical samples.

	copies of NP61	concentration (ng/ul)	Male or Female:	H1 or H3	Age	Ct
h1-8	973170.914	12	F	H1	35	16
h1-11	2098500.93	28.5	F	H1	42	13
h1-17	842701.615	3.9	F	H1	3	16
h1-5	1314884.17	16.3	M	H1	76	17
h1-7	1118396.05	10.6	M	H1	15	17
h1-15	3770601.06	25.7	F	H1	29	15
h3-9	1709364.15	41.8	M	H3	2	13
h3-11	857187.307	34.4	F	H3	87	12
h1-18	1212939.07	31.2	F	H1	81	15
h3-10	3438550.42	13.2	F	H3	82	10
h3-7	2700716.94	5.3	F	H3	71	10
h1-2	935094.563	16.2	M	H1	28	16
h1-3	587837.325	19.4	M	H1	1	17
h1-14	833649.44	7.5	F	H1	34	15
h1-01	5298694.87	9.3	F	H1	18	12
h1-13	1251152.73	3.3	F	H1	87	14
h3-3	2041476.96	6.9	F	H3	83	17