

Supplementary material for article: Salmikangas et al., 2020. Detection of - and + RNA strands in enterovirus infected cells and tissues.

Supplementary table 1. Antibodies used in IF and bDNA FISH.

Antibody	Type	Final concentration	Origin
CVA9-8863 rabbit monoclonal antibody	Primary (rabbit anti-CVA9 capsid)	1:100	Merja Roivainen, THL, Helsinki
CVA9-861 rabbit monoclonal antibody	Primary (rabbit anti-CVA9 capsid)	1:100	Merja Roivainen, THL, Helsinki
CVA9-K3 rabbit monoclonal antibody	Primary (rabbit anti-CVA9 capsid)	1:100	Merja Roivainen, THL, Helsinki
J-2 mouse mono-clonal antibody	Primary (mouse anti-dsRNA)	1:8000	Scicons (Prod.No. 10010200)
Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 488 (GAR488)	Secondary (goat anti-rabbit)	1:400	Invitrogen/ Molecular Probes, Ref. A-11008
Goat anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 488 (GAM488)	Secondary (goat anti-mouse)	1:400	Invitrogen/ Molecular Probes, Ref. A-11029
Goat anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 633 (GAM633)	Secondary (goat anti-mouse)	1:400	Invitrogen/ Molecular Probes, Ref. A-21052

Supplementary table 2. ViewRNA in-house/custom probe set sequences used for +RNA (EVAB+) and -RNA (EVAB-) detection. Probe set sequence information of EVAB+ has previously been published in [24].

Oligo number	EVAB+	EVAB-
1	gactcatggatctccttcattgg	ccaatgaaggagatccatgagtc
2	gcgtccttagtccaacgaatg	cattcgttgactaaggacgc
3	cataccctgagtaataaaaagcaa	ttgcttttgattactcagggtatg
4	aaccaaacaggactaagactagcat	atgctagtcttagctctgttgggt
5	taaggtaaactgagtcatttaggctac	gtagcctaaatgactcagttacctta
6	acaggtggccaaaagtcattc	gaatgacttttgccacctgt
7	tgtacatcatggttctgtgttagg	cctacacacagaacctgatgtaca
8	tctgcttgggtgggaaat	atttccaaccaaagcagga
9	gattttggcagagatcatccataa	ttatggatgatctctgcaaaatc
10	aatagtacatgtctttccatctg	cagatgggaaagacatgtcacttt
11	tctggaggtagtgagtacacactgg	ccagtggtactcactacctccaga
12	tgtacctcaaaagtgatctggg	cccagatcactttgatgggtaca
13	tatgtcttcatgggcatgaca	tgtcatgccatgaaagacata
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15	gatcttgggtgttcttggatct	agatccaagaacaccaagatc

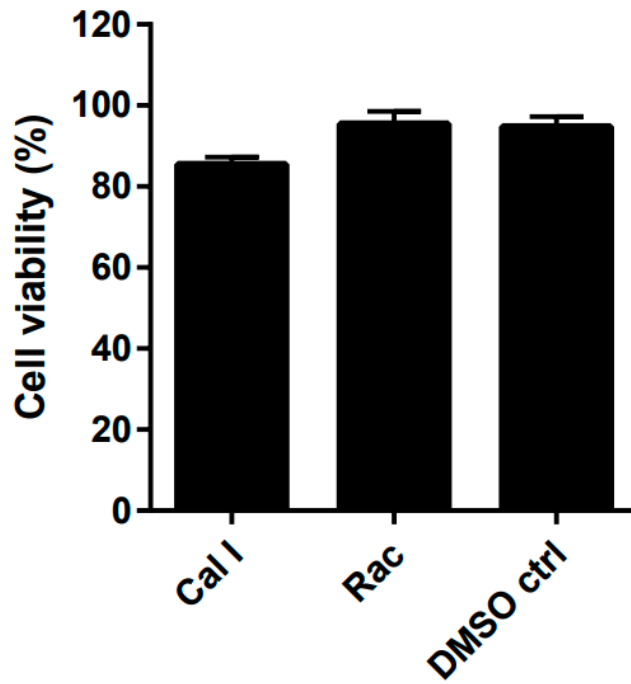
16	taggcacagtgagcgacat	atgtgcgctcactgtgccta
17	cctgttacaccttcagcattagtg	cactaatgctgaagggtgacaagg
18	cctgaattgatccaagtcaatcc	ggattgacttgatcaattcagg
19	catcatcacatgatcaatcat	atgattgcatatggtgatgatgtg
20	ggccatgggtatgatgcaat	attgcatcatacccatggcc
21	ctggtgccagaacaccctg	cagggtgttctggcaccag
22	tgttggatcattgagttgaagatg	catctcaactcaatgatcaacaaca
23	ttgcacaggtagtaatgtagttg	caactacattgactacctgtgcaa
24	tgtctctgtacaggtggggag	ctcccaccactgtacagagaca
25	cagaccatactgtccatgcact	agtgcattggacaagtatggtctg
26	tcacataagtaccattgtagggt	aacctaccaatggtgacttatgtga
27	ccttgttcatcattgaagtagtgct	agcactactcaatgatgaacaagg
28	gagctctcaatgaactcgatttct	agaaatcgagttcattgagagctc
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30	ttgtacatgagcattctcttgggtg	caccaagagaatgctcatgtacaa
31	gctagcacagcctcattgacct	aggtaatgaggctgtgctagc
32	ggggaactgtctggtgtaatt	aattaacaccagcaagttcccc
33	tgtaaaagtggaaatctccttgct	agcaaggagattccactttgaca
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46	caatagctatatgtaacaatcttcaa	ttgaaagattgtaccatatagctattg
47	ggggaacagaagtgttcat	atcaagcacttctgtttcccc
48	ccaacgcagccaccgc	gcggtggctgcgttgg
49	cccatgggcaggccg	cggcctgccatggg
50	gagcgtcccatgggttgc	gcaacctatgggacgctc
51	ctcttcgaccatgtcagtattg	caatactgacatggtgcaagag
52	gtgagcagtctattgatactcagtc	ggactgagtatcaatgactgctcac
53	acgttttctccttcaaccgc	gcggttgaaggagaaaacgt
54	agtagttggccggtaacga	tcgttaccggccaactact
55	tcatggtgttactaggttctcga	tcgagaaacctagtaacaccatga
56	agcgaaacactccgcaactt	aagttgcggagtgtttcgtc
57	cctgatctacactgggtgctg	cagcaccctagtagatcagg
58	ggaatgcggtgactcatcga	tcgatgagtcaccgcattcc
59	cacggtcgcctgtg	ccacgggcgaccgtg
60	ccagagtgtagcggcctg	actgggcgctagcactctgg
61	gcgcacaaaggtaccgtgata	tatcacggtaccttgtgcgc

Supplementary table 3. Components of the RT reaction.

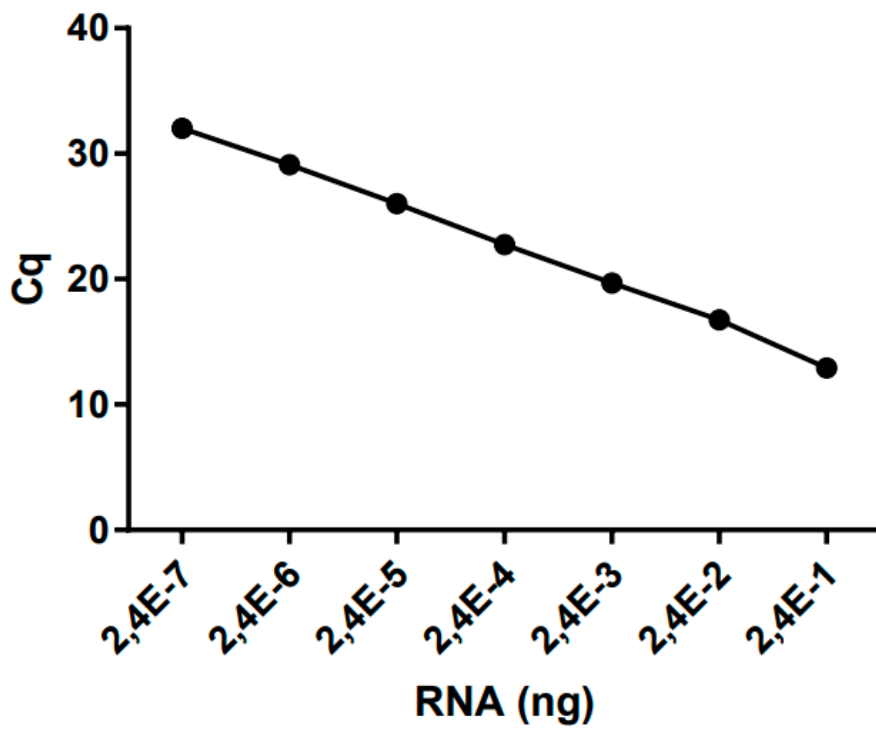
Reagent	Amount (μl) per sample	Final concentration	Origin
Nuclease free water	9.0	-	Alfa Aesar (Ref. J71786)
5x RT-buffer	8.0	1x	Promega (Ref. M5301)
2.5 mM dNTP mix with dATP, dTTP, dCTP and dGTP	8.0	0.5 mM each	Promega (Ref. U1330)
10 μ M ENRI (3+ or 4-) primer	4.8	1.2 μ M	Thermo Scientific Custom Standard DNA Oligos
RNase inhibitor	0.1	4 units	Promega (Ref. N2518)
RT-enzyme (M-MLV RT [H-])	0.1	20 units	Promega (Ref. M530A)
RNA template	10.0	-	Viral RNA extraction
Total	40.0	-	-

Supplementary table 4. Components of the qPCR reaction.

Reagent	Amount (μl) per sample	Final concentration	Origin
Nuclease free water	4.5	-	Alfa Aesar (Ref. J71786)
iQ SYBR Green Supermix	12.5	1x	Bio-Rad (Ref. 1708886)
10 μ M ENRI 4- primer	1.5	0.6 μ M	Thermo Scientific Custom Standard DNA Oligos
10 μ M ENRI 3+ primer	1.5	0.6 μ M	Thermo Scientific Custom Standard DNA Oligos
cDNA template	5.0	1:500	RT-reaction
Total	25.0	-	-



Supplementary figure 1. Cell viability assay for the antiviral drugs Cal-I1 and Rac1-I. After a 5-hour incubation, the antiviral drug Cal-I1 lowers cell viability by ~10%, and the antiviral drug Rac1-I doesn't lower cell viability, compared to a DMSO control.



Supplementary figure 2. A concentration series of RNA isolated from purified CVA9 and detected by qPCR. 10-fold dilutions were used, triplicate samples were measured and the average Cq-values from the triplicates were plotted against the RNA amounts.