

Ebola virus encodes for two microRNAs in Huh7-infected cells

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Supplementary Tables and Figures

Supplementary Table S1. Primer sequences used in RT-qPCR assays in Huh7 cells.

	Gene	5'-3' Sequence (Forward) 5'-3' Sequence (Reverse)	Amplicons (length; nt)	T _m (°C)	Accession number
1	DUSP16	GTGAAGAAAAGTTGCGAGCGT ACTGGACTGAAAGCTTTTGGGG	269	60.51	NM_030640
2	NAMPT	GGAGCATCTGCTCACTTGGT CCCCAAGCTGTTATGGTACTGT	143	60.04	NM_005746.3
3	FGD1	TCGTCTGGGACTACTGGCTG CCAGGGTCAAGGGACAAACT	995	64.02	NM_004463.3
4	PUM2	TGTGTCTCACCTCGGTACT AAAAGCTCTCCATTCCCCG	278	60	NM_015317.5
5	UNC5D	GGGTGCCCATTGACGCGG TCGCCATTGTCAGTTCCTCG	298	60.62	NM_080872.4
6	PLCB4	CTCCAGGCAAAGTGACAGGAAA TGCAGGTGATGTCCACTTCG	118	60.54	NM_000933.4
7	CDK13	AGCAGCTAACCTACAGTTGAAGA AACAATTTCCCTGTCTCCCA	165	59.32	NM_003718
8	VAPA	TGAAGACTACAGCACCTCGC CCCTGGTGGAGTTATACCCAAT	284	59.49	NM_003574.6
9	P2RY13	CTGAATAATGACTGCCGCC GCTTCCAGTGTCACCTTTGG	70	59.02	NM_176894.3
10	WDR7	TCCGTAGTTCCGCCCTATCC GAAGGCAACTGGCAGGTAGT	983	60	NM_015285.3
11	INPP5E	GGGAAGGACACGTACGACAG GCTGCCAACGGAATGTTGTC	203	60.25	NM_019892.6
12	STAG2	GGGTGTAAAGAAGAGGAAGGGG GAAAAATGTGTTTCTGACTCCTGTA	360	58.89	NM_001042749.2
13	SMURF2	CTGCGCCTGACAGGACTTC TCATTGTCATTTGGCCCCAGT	299	60.51	NM_022739.4
14	CGRRF1	ACATCGCGGTGGTCTTTACC ACATCCAACCAAACCATCCC	70	60.47	NM_006568.3

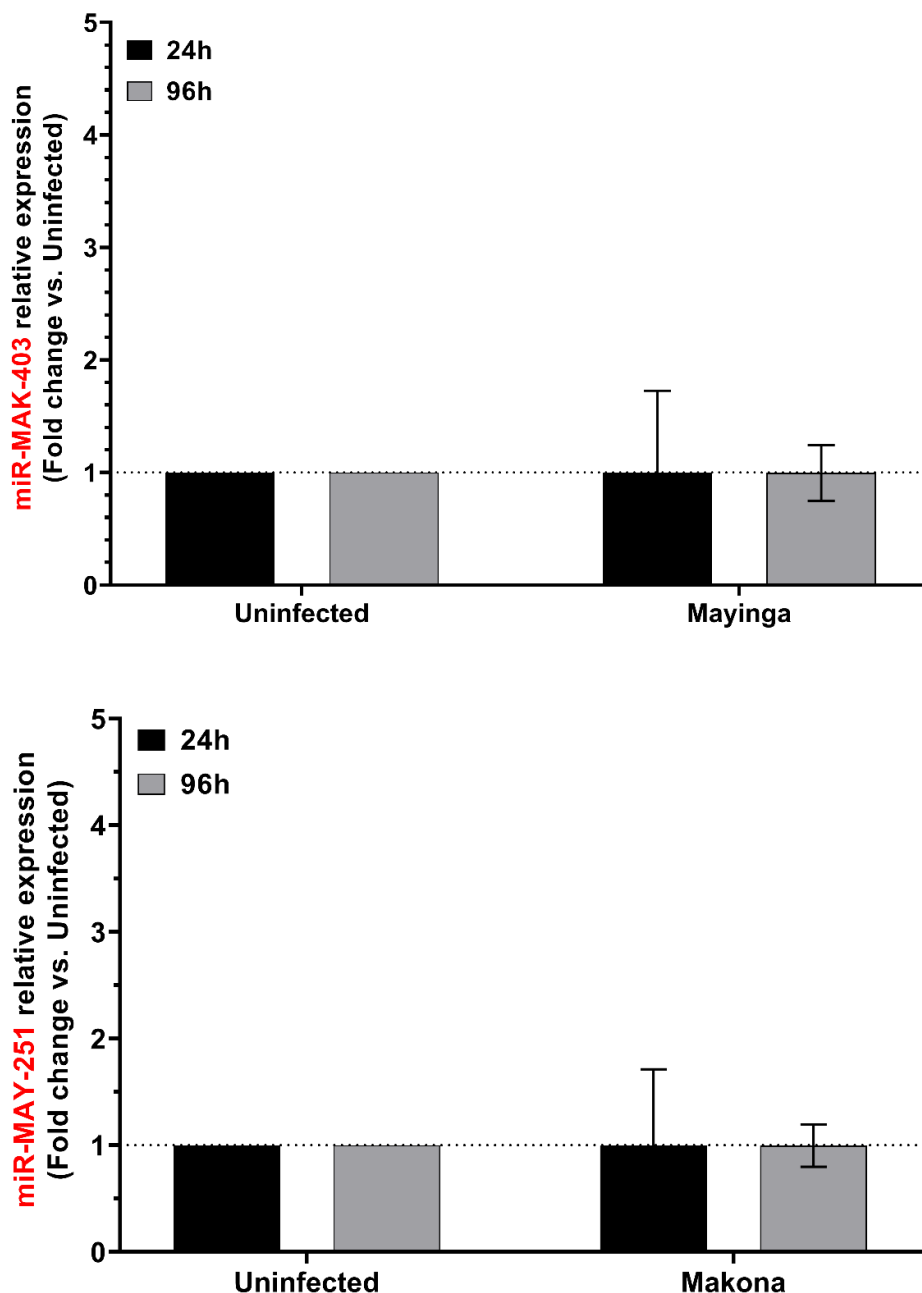
The gene names listed from 1 to 6 are those targeted by EBOV-miR-MAY-251, and those from 7 to 14 by EBOV-miR-MAK-403. T_m, melting temperature; nt, nucleotides.



Supplementary Table S2. Potential mRNA targets and the position of their binding sites for the two EBOV microRNAs, EBOV-miR-MAY-251 and EBOV-miR-MAK-403.

	Gene	Minimum free energy (kcal/mol)	EBOV microRNA binding site position (nt)
1	DUSP16	-25.6 (others are <= -23.9)	2024 (2150, 3023, 6574, 2665)
2	NAMPT	-24.2 (others are <= -21.5)	2986 (46, 577, 339, 458)
3	FGD1	-25.5 (others are <= -23.9)	3481 (1169, 4223, 837, 1517)
4	PUM2	-25.8 (others are <= -22.7)	1269 (1023, 1350, 1650, 1148)
5	UNC5D	-26.0 (others are <= -22.8)	2080 (3437, 1438, 5963, 7041)
6	PLCB4	-26.4 (others are <= -23.1)	1863 (659, 464, 3258, 4653)
7	CDK13	-22.6 (others are <= -21.0)	3927 (5461, 8631, 4925, 8484)
8	VAPA	-22.6 (others are <= -20.6)	5211 (1141, 4661, 1585, 1357)
9	P2RY13	-24.1 (others are <= -18.3)	364 (436, 1187, 2485, 2179)
10	WDR7	-24.6 (others are <= -19.7)	1166 (2733, 902, 5892, 4850)
11	INPP5E	-22.9 (others are <= -18.0)	3285 (2185, 1505, 1666, 2765)
12	STAG2	-22.4 (others are <= -21.2)	6179 (5405, 4360, 5977, 525)
13	SMURF2	-21.0 (others are <= -18.9)	5997 (2447, 4565, 710, 5568)
14	CGRRF1	-20.2 (others are <= -16.7)	1249 (684, 772, 235, 973)

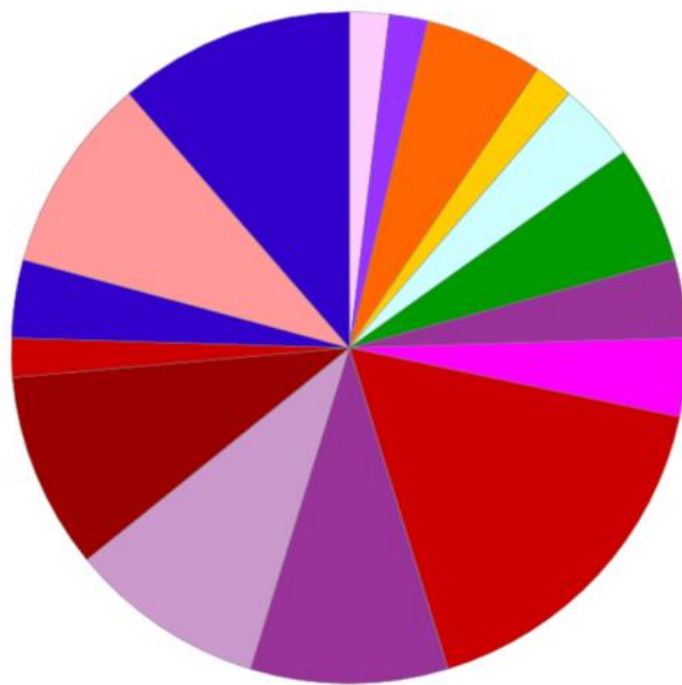
RNAhybrid was used to calculate minimum free energy of each EBOV microRNA-mRNA interaction. The position (nt, nucleotide) for the highest interactions is shown in the table. The gene names listed from 1 to 6 are those targeted by EBOV-miR-MAY-251, and those from 7 to 14 by EBOV-miR-MAK-403.



Supplementary Figure S1. Expression of EBOV-miR-MAY-251 and EBOV-miR-MAK-403 is specific to their viral genome of origin. RT-qPCR data showing relative expression of EBOV-miR-MAY-251 and EBOV-miR-MAK-403 in Huh7 cells infected for 24 h and 96 h with Makona or Mayinga EBOV strain, respectively. qPCR data were normalized with Unisp6 spike-in, reported to control (uninfected), and expressed with a relative quantitation method (ddCT). Data were calculated from three biological replicates and expressed as mean \pm SD. **Statistical analysis.** Two-way analysis of variance (ANOVA) and Sidak multiple comparisons were used. No statistically significant difference (fold change vs uninfected) was observed.

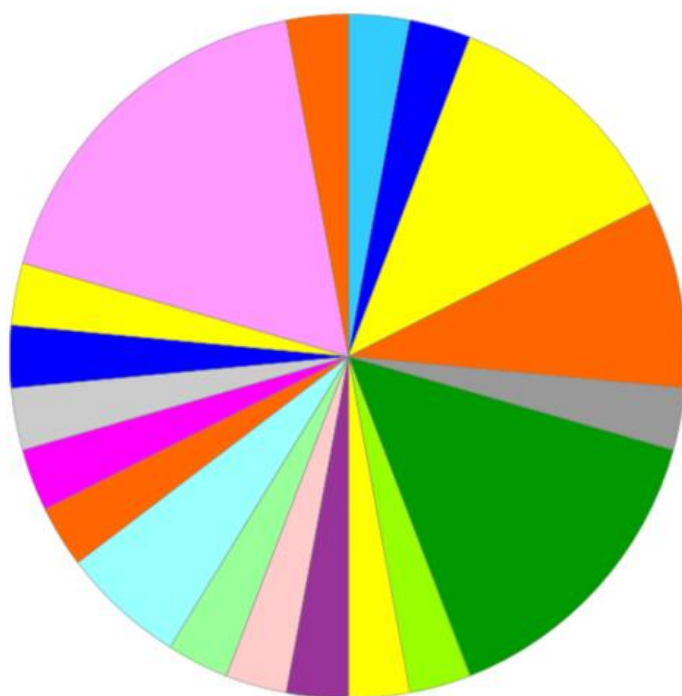


A) miR-MAY-251



PANTHER GO-Slim Protein Class
Total # Genes: 73 ; Total # protein class hits: 53

- cell adhesion molecule (PC00069)
- chaperone (PC00072)
- chromatin/chromatin-binding, or -regulatory protein (PC00077)
- cytoskeletal protein (PC00085)
- extracellular matrix protein (PC00102)
- gene-specific transcriptional regulator (PC00264)
- intercellular signal molecule (PC00207)
- membrane traffic protein (PC00150)
- metabolite interconversion enzyme (PC00262)
- nucleic acid metabolism protein (PC00171)
- protein modifying enzyme (PC00260)
- protein-binding activity modulator (PC00095)
- scaffold/adaptor protein (PC00226)
- translational protein (PC00263)
- transmembrane signal receptor (PC00197)
- transporter (PC00227)

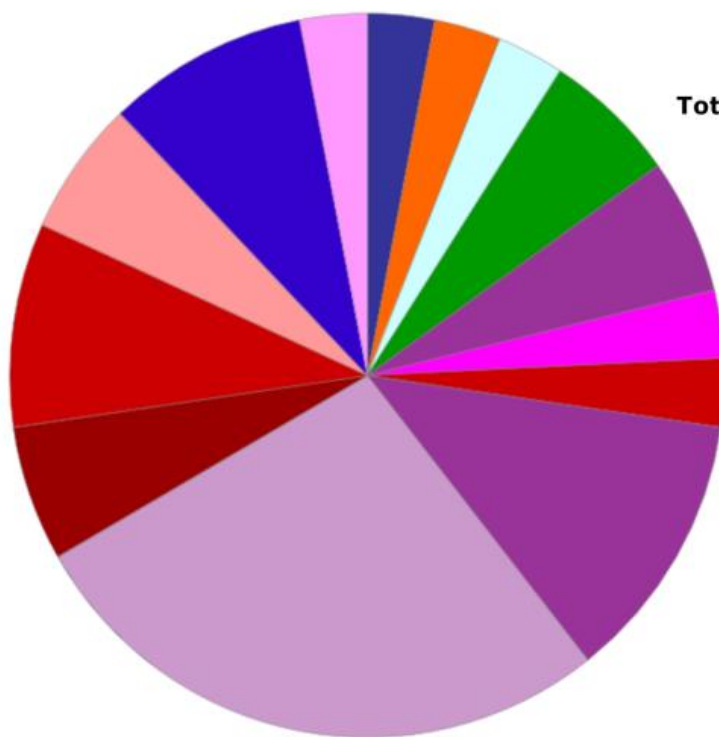


PANTHER Pathway
Total # Genes: 73 ; Total # pathway hits: 34

- 5HT2 type receptor mediated signaling pathway (P04374)
- Alpha adrenergic receptor signaling pathway (P00002)
- Alzheimer disease-presenilin pathway (P00004)
- Angiogenesis (P00005)
- Axon guidance mediated by netrin (P00009)
- Cadherin signaling pathway (P00012)
- Endothelin signaling pathway (P00019)
- Gonadotropin-releasing hormone receptor pathway (P06664)
- Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway (P00027)
- Histamine H1 receptor mediated signaling pathway (P04385)
- Inflammation mediated by chemokine and cytokine signaling pathway (P00031)
- Integrin signalling pathway (P00034)
- Metabotropic glutamate receptor group I pathway (P00041)
- Muscarinic acetylcholine receptor 1 and 3 signaling pathway (P00042)
- Oxytocin receptor mediated signaling pathway (P04391)
- Thyrotropin-releasing hormone receptor signaling pathway (P04394)
- Vitamin D metabolism and pathway (P04396)
- Wnt signaling pathway (P00057)
- p53 pathway by glucose deprivation (P04397)

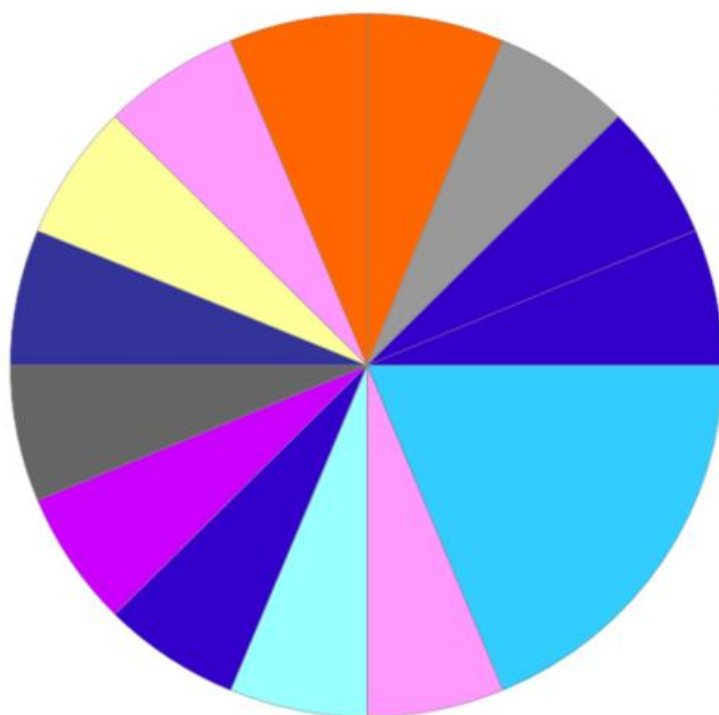


B) miR-MAK-403



PANTHER GO-Slim Protein Class
Total # Genes: 46 ; Total # protein class hits: 33

- [calcium-binding protein \(PC00060\)](#)
- [chromatin/chromatin-binding, or -regulatory protein \(PC00077\)](#)
- [extracellular matrix protein \(PC00102\)](#)
- [gene-specific transcriptional regulator \(PC00264\)](#)
- [intercellular signal molecule \(PC00207\)](#)
- [membrane traffic protein \(PC00150\)](#)
- [metabolite interconversion enzyme \(PC00262\)](#)
- [nucleic acid metabolism protein \(PC00171\)](#)
- [protein modifying enzyme \(PC00260\)](#)
- [protein-binding activity modulator \(PC00095\)](#)
- [scaffold/adaptor protein \(PC00226\)](#)
- [transmembrane signal receptor \(PC00197\)](#)
- [transporter \(PC00227\)](#)
- [viral or transposable element protein \(PC00237\)](#)



PANTHER Pathway
Total # Genes: 46 ; Total # pathway hits: 16

- [Angiogenesis \(P00005\)](#)
- [Axon guidance mediated by netrin \(P00009\)](#)
- [Blood coagulation \(P00011\)](#)
- [CCKR signaling map \(P06959\)](#)
- [EGF receptor signaling pathway \(P00018\)](#)
- [FGF signaling pathway \(P00021\)](#)
- [Integrin signalling pathway \(P00034\)](#)
- [PDGF signaling pathway \(P00047\)](#)
- [Plasminogen activating cascade \(P00050\)](#)
- [TGF-beta signaling pathway \(P00052\)](#)
- [Ubiquitin proteasome pathway \(P00060\)](#)
- [VEGF signaling pathway \(P00056\)](#)
- [Wnt signaling pathway \(P00057\)](#)
- [p53 pathway by glucose deprivation \(P04397\)](#)



Supplementary Figure S2. Pie charts of GO-Slim analysis based on EBOV-miR-MAK-403 and EBOV-miR-MAY-251 mRNA targets. EBOV-miR-MAK-403 (**A**) and EBOV-miR-MAY-251 (**B**) mRNA targets with score >80 were subjected to the publicly available database PANTHER for gene function analysis. The “panther pathway” and “protein class” ontologies are represented here and complete the data set shown in **Figure 3**.