SUPPLEMENTARY DATA

Positive-sense RNA viruses reveal the complexity and dynamics of the cellular and viral epitranscriptome during infection

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Oligonucleotide name	Sequence (5’–3’)
ZIKV probe 9150-9172	CACCTCCTGAGTTTTCTCTTCC
ZIKV probe 4630-4653	CTCTCCTTTCTTCACTTCTTTGGG
ZIKV probe 2140-2163	TATTGAGTGTCTGATTGCTTGTCA
DENV probe 1441-1465	TGTCAATTCTGCTTCTGTGATGGAA
DENV probe 8934-8961	CCTAGCTTCTTCTCTCTTTTTCCCATCA
DENV probe 9137-9156	GCTCCTCCCTCTTTCTTGCT
HCV probe 339-363	TGAGGTTTAGGATTTGTGCTCATGG
HCV probe 1568-1592	CGTTGGTGTTAATGAGCTGAATGTT
HCV probe 7765-7789	TGTTGTACAGTACACCTTGTTATGG
Polio probe 162-182	GGGTAACAGAAGTGCTTGTTC
Polio probe 34-57	AGAGTACTGGCCACCACGTGGGCC
Polio probe 450-479	CCTCCGGCCCCTGAATGCGGCTAAT
HIV-1 probe 168-195	CTGTTCGGGCGCCACTGCTAGAGATTT
HIV-1 probe 193-214	CCCTTTCGCTTTCAGGTCCCT
HIV-1 probe 412-435	TATGTTTTAATTTATATTTTT

Table S1. Sequences of biotinylated antisense oligonucleotides used in affinity capture procedures.

PTM	**Mock**	**ZIKV**	**DENV**	**HCV**	**PV**	PTM	**Mock**	**ZIKV**	**DENV**	**HCV**	**PV**
No. PTMs	**47**	**44**	**41**	**40**	**47**	cmo^{5}U*	0.00178 ± 0.0028	0.000138 ± 0.0011			0.00371 ± 0.0014
Cm	0.0899 ± 0.0124	0.0976 ± 0.0003	0.146 ± 0.0005	0.0833 ± 0.001	0.134 ± 0.0009	chm^{5}U	0.00178 ± 0.0015	0.000138 ± 0.0001			0.00371 ± 0.003
m^{3}C	0.0995 ± 0.0137	0.108 ± 0.0004	0.162 ± 0.0006	0.0922 ± 0.0011	0.148 ± 0.001	mcm^{5}s^{2}U					0.00359 ± 0.0031
m^{4}C	0.154 ± 0.0213	0.167 ± 0.0006	0.25 ± 0.0009	0.142 ± 0.0017	0.228 ± 0.0015	mcmo^{5}U*	0.0146 ± 0.0118				
m^{5}C	0.145 ± 0.02	0.157 ± 0.0006	0.235 ± 0.0008	0.134 ± 0.0016	0.215 ± 0.0014	mchm^{5}U	0.0146 ± 0.0118				
m$^4_4$C*		0.012 ± 0.0007	0.000149 ± 0.0001	0.0706 ± 0.0001	0.00206 ± 0.0001	Am	0.304 ± 0.016	0.469 ± 0.0006	0.528 ± 0.0048	0.531 ± 0.0082	0.442 ± 0.0027
m^{5}Cm*		0.0722 ± 0.0047	0.000896 ± 0.0001	0.423 ± 0.0008	0.0124 ± 0.0001	m^{1}A	0.0176 ± 0.0009	0.0271 ± 0.0001	0.0305 ± 0.0002	0.0307 ± 0.0004	0.0255 ± 0.0001
m$^4_4$Cm*			0.002 ± 0.0001			m^{2}A*	0.0176 ± 0.0009	0.0271 ± 0.0001	0.0305 ± 0.0002	0.0307 ± 0.0004	0.0255 ± 0.0001
f^{5}C					0.00275 ± 0.0165	m^{6}A	0.0579 ± 0.003	0.0893 ± 0.0001	0.101 ± 0.0009	0.101 ± 0.0015	0.0841 ± 0.0005
hm^{5}C					0.00421 ± 0.0048	m^{8}A	0.00674 ± 0.0003	0.0104 ± 0.0001	0.0117 ± 0.0001	0.0118 ± 0.0001	0.00979 ± 0.0001
ac^{4}C	0.692 ± 0.0508	0.514 ± 0.0003	0.574 ± 0.0101	0.351 ± 0.0056	0.483 ± 0.0047	m^{1}Am*	0.00961 ± 0.0044	0.00987 ± 0.0003	0.0087 ± 0.0003	0.112 ± 0.0002	0.00547 ± 0.001
ac^{4}Cm*	0.0137 ± 0.002	0.00252 ± 0.0023	0.00711 ± 0.0014		0.0065 ± 0.0057	m^{6}Am	0.00961 ± 0.0044	0.00987 ± 0.0003	0.0087 ± 0.0003	0.112 ± 0.0002	0.00547 ± 0.001
Y	2.5851 ± 0.0578	1.8937 ± 0.0338	2.0601 ± 0.073	3.215 ± 0.0082	1.7535 ± 0.0689	m$^2_8$A*	0.00874 ± 0.004	0.00902 ± 0.0003	0.00794 ± 0.0002	0.102 ± 0.0002	0.00499 ± 0.0009
D	0.333 ± 0.066	0.142 ± 0.0013	0.291 ± 0.0061	0.384 ± 0.0029	0.258 ± 0.0013	m$^6_2$A	0.00874 ± 0.004	0.00902 ± 0.0003	0.00794 ± 0.0002	0.102 ± 0.0002	0.00499 ± 0.0009
Um	0.158 ± 0.0023	0.0823 ± 0.0007	0.102 ± 0.0016	0.0699 ± 0.002	0.094 ± 0.0014	m$^6_6$A	0.00874 ± 0.004	0.00902 ± 0.0003	0.00794 ± 0.0002	0.102 ± 0.0002	0.00499 ± 0.0009
m^{3}U	0.0902 ± 0.0013	0.0244 ± 0.0002	0.0374 ± 0.0006	0.0176 ± 0.0005	0.0318 ± 0.0004	i^{6}A	0.000777 ± 0.005		0.00378 ± 0.0001		
m^{5}U	0.0902 ± 0.0013	0.0244 ± 0.0002	0.0374 ± 0.0006	0.0176 ± 0.0005	0.0318 ± 0.0004	I	0.017 ± 0.0024	0.00261 ± 0.0023	0.0313 ± 0.0612	0.0589 ± 0.0008	0.0248 ± 0.0017
Ym	0.245 ± 0.0036	0.127 ± 0.001	0.158 ± 0.0025	0.108 ± 0.0031	0.145 ± 0.0022	m^{1}I	0.00137 ± 0.0508	0.163 ± 0.0003	0.1 ± 0.0378		0.128 ± 0.0109
m^{1}Y	0.0317 ± 0.0004	0.00857 ± 0.0001	0.0131 ± 0.0002	0.00618 ± 0.0001	0.0112 ± 0.0001	Gm	0.0193 ± 0.0009	0.0121 ± 0.0002	0.0184 ± 0.0009	0.0107 ± 0.0007	0.0152 ± 0.0003
m^{3}Y*	0.0317 ± 0.0004	0.00857 ± 0.0001	0.0131 ± 0.0002	0.00618 ± 0.0001	0.0112 ± 0.0001	m^{1}G	0.138 ± 0.0066	0.0869 ± 0.0015	0.132 ± 0.0068	0.0764 ± 0.005	0.109 ± 0.0021
m^{5}D*					0.0183 ± 0.0155	m^{2}G	0.138 ± 0.0066	0.0869 ± 0.0015	0.132 ± 0.0068	0.0764 ± 0.005	0.109 ± 0.0021
m^{3}Um*	0.00712 ± 0.002	0.00336 ± 0.0003	0.00517 ± 0.0011	0.229 ± 0.0001	0.0182 ± 0.0082	m^{7}G	0.138 ± 0.0066	0.0869 ± 0.0015	0.132 ± 0.0068	0.0764 ± 0.005	0.109 ± 0.0021
m^{5}Um*	0.00712 ± 0.002	0.00336 ± 0.0003	0.00517 ± 0.0011	0.229 ± 0.0001	0.0182 ± 0.0082	m^{1}Gm*	0.00395 ± 0.0002	0.00444 ± 0.0001		0.00143 ± 0.0001	
nm^{5}U*					0.00419 ± 0.0013	m^{2}Gm*	0.0208 ± 0.0013	0.00444 ± 0.0001	0.0291 ± 0.0001	0.177 ± 0.0004	0.0154 ± 0.0001
mnm^{5}U*				0.000618 ± 0.0004		m$^2_2$G	0.0208 ± 0.0013	0.0233 ± 0.0005	0.00553 ± 0.0001	0.0336 ± 0.0001	0.00293 ± 0.0001
ho^{5}U	0.0048 ± 0.0101	0.00678 ± 0.0013			0.153 ± 0.0155	m$^2_7$G	0.0208 ± 0.0013	0.0233 ± 0.0005	0.0291 ± 0.0001	0.177 ± 0.0004	0.0154 ± 0.0001
ncm^{5}U	0.0459 ± 0.0093	0.00125 ± 0.0023	0.0124 ± 0.0001	0.00147 ± 0.003	0.0326 ± 0.006	preQ1*	0.0208 ± 0.0013	0.0233 ± 0.0005	0.00553 ± 0.0001	0.0336 ± 0.0001	0.00293 ± 0.0001
ncm^{5}Um*	0.00252 ± 0.0057					m$^2_2$Gm*	0.00551 ± 0.0007	0.00826 ± 0.0012		0.0301 ± 0.0002	
mcm^{5}U	0.0085 ± 0.0028					0 0.5% AvP 1% >5%					

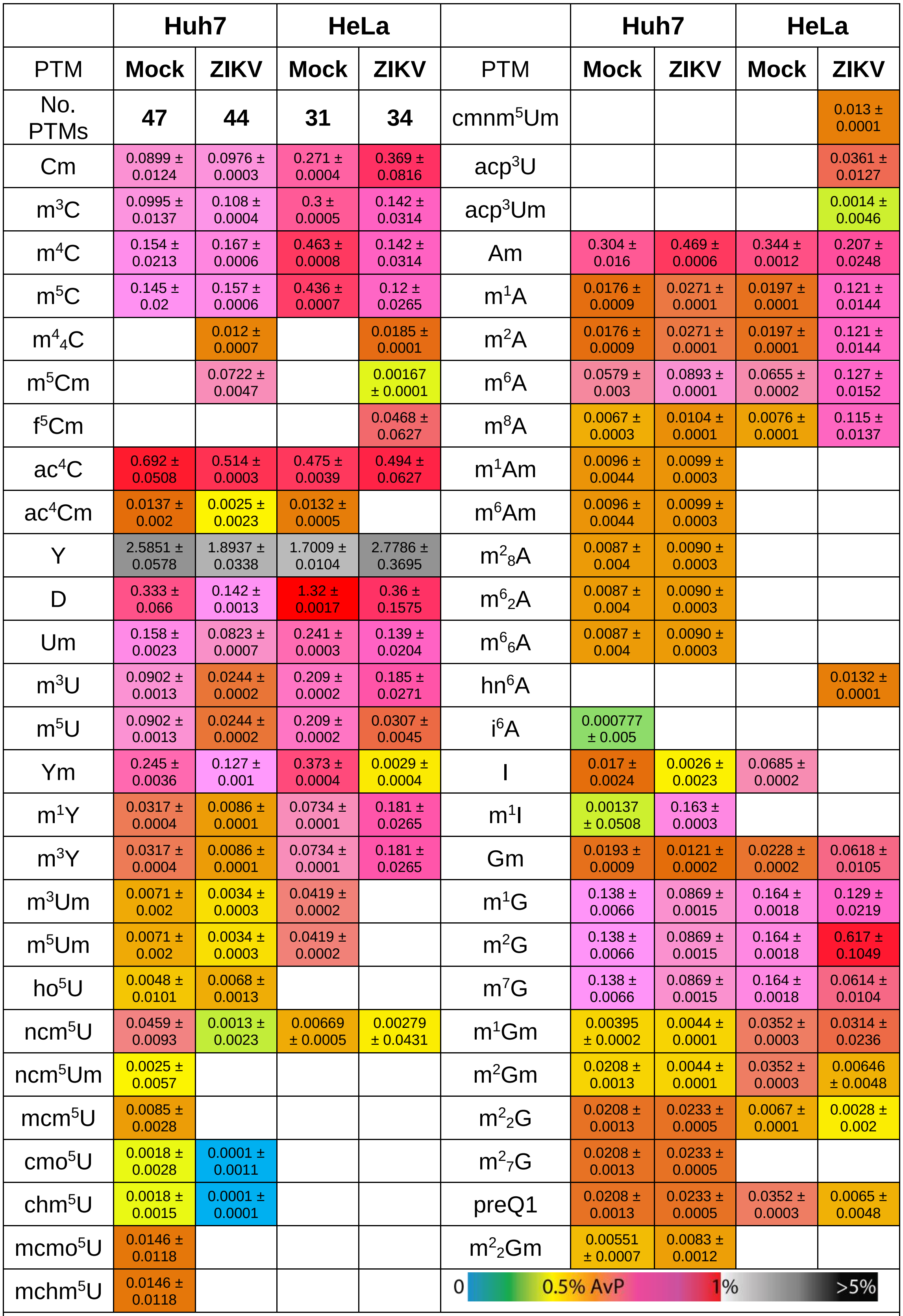
<u>Table S2.</u> PTM profiles obtained from total RNA extracts of mock-infected Huh7 cells (control), and Huh7 cells infected with ZIKV, DENV, HCV, and PV, as described in ***Materials and Methods***. Full PTM names are listed in **Table S3**. The abundance of each PTM relative to the combined abundances of the canonical ribonucleotides is calculated as described in ***Materials and Methods***. Any color change along the gradient carries a $p < 0.05$ statistical significance. An asterisk (*) indicates PTMs that to date have not been reported in mammalian systems.

Symbol	PTM Full name	Symbol	PTM Full name
C	Cytidine	cmo^5U*	uridine 5-oxyacetic acid
Cm	2'-O-methylcytidine	chm^5U	5-carboxyhydroxymethyluridine
m^3C	3-methylcytidine	mcm^5s^2U	5-methoxycarbonylmethyl-2-thiouridine
m^4C	N4-methylcytidine	$mcmo^5U$*	uridine 5-oxyacetic acid methyl ester
m^5C	5-methylcytidine	$mchm^5U$	5-(carboxyhydroxymethyl)uridine methyl ester
m^4_4C*	N4,N4-dimethylcytidine	$cmnm^5Um$*	5-carboxymethylaminomethyl-2′-O-methyluridine
m^5Cm*	5,2'-O-dimethylcytidine	acp^3U	3-(3-amino-3-carboxypropyl)uridine
m^4_4Cm*	N4,N4,2'-O-trimethylcytidine	acp^3Um*	3-(3-amino-3-carboxypropyl)methyluridine
f^5C	5-formylcytidine	A	Adenosine
f^5Cm	5-formyl-2'-O-methylcytidine	Am	2'-O-methyladenosine
hm^5C	5-hydroxymethylcytidine	m^1A	1-methyladenosine
ac^4C	N4-acetylcytidine	m^2A*	2-methyladenosine
ac^4Cm*	N4-acetyl-2'-O-methylcytidine	m^6A	N6-methyladenosine
U	Uridine	m^8A	C8-methyladenosine
Y	Pseudouridine	m^1Am*	1,2'-O-dimethyladenosine
D	Dihydrouridine	m^6Am	N6,2'-O-dimethyladenosine
Um	2'-O-methyluridine	m^2_8A*	2,8-dimethyladenosine
m^3U	3-methyluridine	m^6_2A	N6,N6-dimethyladenosine
m^5U	5-methyluridine	m^6_6A	N6,N6-dimethyladenosine
Ym	2'-O-methylpseudouridine	m^6_6Am*	N6,N6,2'-O-trimethyladenosine
m^1Y	1-methylpseudouridine	hn^6A*	N6-hydroxynorvalylcarbamoyladenosine
m^3Y*	3-methylpseudouridine	i^6A	N6-isopentenyladenosine
m^5D*	5-methyldihydrouridine	I	Inosine
m^3Um*	3,2'-O-dimethyluridine	Im	2'-O-methylinosine
m^5Um*	5,2'-O-dimethyluridine	m^1I	1-methylinosine
s^2Um	2-thio-2'-O-methyluridine	G	Guanosine
m^5s^2U	5-methyl-2-thiouridine	Gm	2'-O-methylguanosine
nm^5U*	5-methylaminouridine	m^1G	1-methylguanosine
mnm^5U*	5-methylaminomethyluridine	m^2G	N2-methylguanosine
f^5U	5-formyl-uridine	m^7G	7-methylguanosine
f^5Um*	5-formyl-O-methyluridine	m^1Gm*	1,2'-O-dimethylguanosine
ho^5U	5-hydroxyuridine	m^2Gm*	N2,2-O-dimethylguanosine
ncm^5U	5-carbamoylmethyluridine	m^2_2G	N2,N2-dimethylguanosine
ncm^5Um*	5-carbamoylmethyl-2′-O-methyluridine	m^2_7G	N2,7-dimethylguanosine
mcm^5U	5-methoxycarbonylmethyluridine	PreQ1*	7-aminomethyl-7-deazaguanosine
mo^5U	5-methoxyuridine	m^2_2Gm*	N2,N2,2-O-trimethylguansine

Table S3. Symbol and name of PTMs identified in this study. An asterisk (*) indicates PTMs that to date have not been reported in mammalian systems.

PTM	**Mock**	**HIV-1**	PTM	**Mock**	**HIV-1**
No. PTMs	**37**	**38**	m^{6}A	0.038 ± 0.002	0.0597 ± 0.0041
Cm	0.0841 ± 0.0171	0.0981 ± 0.0159	m^{8}A	0.0044 ± 0.0109	0.0070 ± 0.0218
m^{3}C	0.093 ± 0.0189	0.109 ± 0.0177	ac^{4}C	0.66 ± 0.0331	0.701 ± 0.0144
m^{5}C	0.135 ± 0.0274	0.158 ± 0.0256	f^{5}Cm		0.0013 ± 0.0144
m^{4}C	0.144 ± 0.0293	0.168 ± 0.0272	m^{6}Am	0.0040 ± 0.0152	0.0029 ± 0.013
Y	1.6964 ± 0.0413	2.0087 ± 0.0338	m^{1}Am	0.0040 ± 0.0152	0.0029 ± 0.013
D	0.334 ± 0.0971	0.36 ± 0.0374	m$^{6}_{2}$A	0.0037 ± 0.0139	0.0027 ± 0.0118
Um	0.148 ± 0.0258	0.118 ± 0.0123	m$^{6}_{6}$A	0.0037 ± 0.0139	0.0027 ± 0.0118
Ym	0.23 ± 0.0401	0.183 ± 0.0191	m$^{2}_{8}$A	0.0037 ± 0.0139	0.0027 ± 0.0118
m^{5}U	0.0793 ± 0.0138	0.0502 ± 0.0052	m^{7}G	0.0798 ± 0.023	0.096 ± 0.0205
m^{1}Y	0.0279 ± 0.0048	0.0176 ± 0.0018	m^{1}G	0.0798 ± 0.023	0.096 ± 0.0205
m^{3}U	0.0793 ± 0.0138	0.0502 ± 0.0052	m^{2}G	0.0798 ± 0.023	0.096 ± 0.0205
m^{3}Y	0.0279 ± 0.0048	0.0176 ± 0.0018	Gm	0.0111 ± 0.0031	0.0134 ± 0.0028
I	0.0111 ± 0.0015	0.0105 ± 0.0013	ac^{4}Cm	0.0048 ± 0.0014	0.0060 ± 0.0017
m^{5}Cm		0.0011 ± 0.0003	ncm^{5}U	0.0302 ± 0.0027	0.0247 ± 0.0024
m$^{4}_{4}$C		0.0007 ± 0.0002	m^{1}Gm	0.0028 ± 0.0003	0.0027 ± 0.0004
m^{5}Um	0.0139 ± 0.0488	0.0088 ± 0.0032	m$^{2}_{2}$G	0.0148 ± 0.0017	0.0144 ± 0.0022
m^{3}Um	0.0139 ± 0.0488	0.0088 ± 0.0032	m^{2}Gm	0.0028 ± 0.0003	0.0027 ± 0.0004
Am	0.2 ± 0.0109	0.314 ± 0.0218	preQ1	0.0148 ± 0.0017	0.0144 ± 0.0022
m^{1}A	0.0114 ± 0.0006	0.018 ± 0.0012	m$^{2}_{7}$G	0.0148 ± 0.0017	0.0144 ± 0.0022
m^{2}A	0.0114 ± 0.0006	0.018 ± 0.0012	0 0.5% AvP 1% >5%		

Table S4. PTM profiles obtained from total RNA extracts from mock- and HIV-infected 293T cells, as described in ***Materials and Methods***. Full PTM names are listed in **Table S3**. The abundance of each PTM relative to the combined abundances of the canonical ribonucleotides is calculated as described in ***Materials and Methods***. Any color change along the gradient carries a $p < 0.05$ statistical significance.



	Huh7		HeLa			Huh7		HeLa	
PTM	Mock	ZIKV	Mock	ZIKV	PTM	Mock	ZIKV	Mock	ZIKV
No. PTMs	**47**	**44**	**31**	**34**	cmnm^{5}Um				0.013 ± 0.0001
Cm	0.0899 ± 0.0124	0.0976 ± 0.0003	0.271 ± 0.0004	0.369 ± 0.0816	acp^{3}U				0.0361 ± 0.0127
m^{3}C	0.0995 ± 0.0137	0.108 ± 0.0004	0.3 ± 0.0005	0.142 ± 0.0314	acp^{3}Um				0.0014 ± 0.0046
m^{4}C	0.154 ± 0.0213	0.167 ± 0.0006	0.463 ± 0.0008	0.142 ± 0.0314	Am	0.304 ± 0.016	0.469 ± 0.0006	0.344 ± 0.0012	0.207 ± 0.0248
m^{5}C	0.145 ± 0.02	0.157 ± 0.0006	0.436 ± 0.0007	0.12 ± 0.0265	m^{1}A	0.0176 ± 0.0009	0.0271 ± 0.0001	0.0197 ± 0.0001	0.121 ± 0.0144
m$^{4}_{4}$C		0.012 ± 0.0007		0.0185 ± 0.0001	m^{2}A	0.0176 ± 0.0009	0.0271 ± 0.0001	0.0197 ± 0.0001	0.121 ± 0.0144
m^{5}Cm		0.0722 ± 0.0047		0.00167 ± 0.0001	m^{6}A	0.0579 ± 0.003	0.0893 ± 0.0001	0.0655 ± 0.0002	0.127 ± 0.0152
f^{5}Cm				0.0468 ± 0.0627	m^{8}A	0.0067 ± 0.0003	0.0104 ± 0.0001	0.0076 ± 0.0001	0.115 ± 0.0137
ac^{4}C	0.692 ± 0.0508	0.514 ± 0.0003	0.475 ± 0.0039	0.494 ± 0.0627	m^{1}Am	0.0096 ± 0.0044	0.0099 ± 0.0003		
ac^{4}Cm	0.0137 ± 0.002	0.0025 ± 0.0023	0.0132 ± 0.0005		m^{6}Am	0.0096 ± 0.0044	0.0099 ± 0.0003		
Y	2.5851 ± 0.0578	1.8937 ± 0.0338	1.7009 ± 0.0104	2.7786 ± 0.3695	m$^{2}_{8}$A	0.0087 ± 0.004	0.0090 ± 0.0003		
D	0.333 ± 0.066	0.142 ± 0.0013	1.32 ± 0.0017	0.36 ± 0.1575	m$^{6}_{2}$A	0.0087 ± 0.004	0.0090 ± 0.0003		
Um	0.158 ± 0.0023	0.0823 ± 0.0007	0.241 ± 0.0003	0.139 ± 0.0204	m$^{6}_{6}$A	0.0087 ± 0.004	0.0090 ± 0.0003		
m^{3}U	0.0902 ± 0.0013	0.0244 ± 0.0002	0.209 ± 0.0002	0.185 ± 0.0271	hn^{6}A				0.0132 ± 0.0001
m^{5}U	0.0902 ± 0.0013	0.0244 ± 0.0002	0.209 ± 0.0002	0.0307 ± 0.0045	i^{6}A	0.000777 ± 0.005			
Ym	0.245 ± 0.0036	0.127 ± 0.001	0.373 ± 0.0004	0.0029 ± 0.0004	I	0.017 ± 0.0024	0.0026 ± 0.0023	0.0685 ± 0.0002	
m^{1}Y	0.0317 ± 0.0004	0.0086 ± 0.0001	0.0734 ± 0.0001	0.181 ± 0.0265	m^{1}I	0.00137 ± 0.0508	0.163 ± 0.0003		
m^{3}Y	0.0317 ± 0.0004	0.0086 ± 0.0001	0.0734 ± 0.0001	0.181 ± 0.0265	Gm	0.0193 ± 0.0009	0.0121 ± 0.0002	0.0228 ± 0.0002	0.0618 ± 0.0105
m^{3}Um	0.0071 ± 0.002	0.0034 ± 0.0003	0.0419 ± 0.0002		m^{1}G	0.138 ± 0.0066	0.0869 ± 0.0015	0.164 ± 0.0018	0.129 ± 0.0219
m^{5}Um	0.0071 ± 0.002	0.0034 ± 0.0003	0.0419 ± 0.0002		m^{2}G	0.138 ± 0.0066	0.0869 ± 0.0015	0.164 ± 0.0018	0.617 ± 0.1049
ho^{5}U	0.0048 ± 0.0101	0.0068 ± 0.0013			m^{7}G	0.138 ± 0.0066	0.0869 ± 0.0015	0.164 ± 0.0018	0.0614 ± 0.0104
ncm^{5}U	0.0459 ± 0.0093	0.0013 ± 0.0023	0.00669 ± 0.0005	0.00279 ± 0.0431	m^{1}Gm	0.00395 ± 0.0002	0.0044 ± 0.0001	0.0352 ± 0.0003	0.0314 ± 0.0236
ncm^{5}Um	0.0025 ± 0.0057				m^{2}Gm	0.0208 ± 0.0013	0.0044 ± 0.0001	0.0352 ± 0.0003	0.00646 ± 0.0048
mcm^{5}U	0.0085 ± 0.0028				m$^{2}_{2}$G	0.0208 ± 0.0013	0.0233 ± 0.0005	0.0067 ± 0.0001	0.0028 ± 0.002
cmo^{5}U	0.0018 ± 0.0028	0.0001 ± 0.0011			m$^{2}_{7}$G	0.0208 ± 0.0013	0.0233 ± 0.0005		
chm^{5}U	0.0018 ± 0.0015	0.0001 ± 0.0001			preQ1	0.0208 ± 0.0013	0.0233 ± 0.0005	0.0352 ± 0.0003	0.0065 ± 0.0048
mcmo^{5}U	0.0146 ± 0.0118				m$^{2}_{2}$Gm	0.00551 ± 0.0007	0.0083 ± 0.0012		
mchm^{5}U	0.0146 ± 0.0118				0 0.5% AvP 1% >5%				

Table S5. PTM profiles obtained from total RNA extracts from mock- and ZIKV-infected Huh7 and HeLa cells, as described in ***Materials and Methods***. Full PTM names are listed in **Table S3**. The abundance of each PTM relative to the combined abundances of the canonical ribonucleotides is calculated as described in ***Materials and Method***. Any color change along the gradient carries a $p < 0.05$ statistical significance.

	Huh7		**HeLa**		**293T**			**Huh7**		**HeLa**		**293T**	
PTM	**Mock**	**PV**	**Mock**	**PV**	**Mock**	**PV**	PTM	**Mock**	**PV**	**Mock**	**PV**	**Mock**	**PV**
No. PTMs	**47**	**47**	**31**	**38**	**37**	**40**	mcm^{5}s^{2}U		0.0036 ± 0.0031				
Cm	0.0899 ± 0.0124	0.134 ± 0.0009	0.271 ± 0.0004	0.125 ± 0.0057	0.0841 ± 0.0171	0.111 ± 0.0093	mcmo^{5}U	0.0146 ± 0.0118					
m^{3}C	0.0995 ± 0.0137	0.148 ± 0.001	0.3 ± 0.0005	0.138 ± 0.0063	0.093 ± 0.0189	0.133 ± 0.0111	mchm^{5}U	0.0146 ± 0.0118					
m^{4}C	0.154 ± 0.0213	0.228 ± 0.0015	0.463 ± 0.0008	0.214 ± 0.0097	0.144 ± 0.0293	0.133 ± 0.0111	cmnm^{5}Um						0.01 ± 0.004
m^{5}C	0.145 ± 0.02	0.215 ± 0.0014	0.436 ± 0.0007	0.201 ± 0.0092	0.135 ± 0.0274	0.344 ± 0.0289	acp^{3}U						0.0379 ± 0.004
m$^4_4$C		0.0021 ± 0.0001		0.0045 ± 0.0001		0.0003 ± 0.0001	Am	0.304 ± 0.016	0.442 ± 0.0027	0.344 ± 0.0012	0.407 ± 0.0302	0.2 ± 0.0109	0.242 ± 0.0116
m^{5}Cm		0.0124 ± 0.0001		0.027 ± 0.0011		0.0021 ± 0.0001	m^{1}A	0.0176 ± 0.0009	0.0255 ± 0.0001	0.0197 ± 0.0001	0.0233 ± 0.0017	0.0114 ± 0.0006	0.075 ± 0.0035
f^{5}C		0.0028 ± 0.0165					m^{2}A	0.0176 ± 0.0009	0.0255 ± 0.0001	0.0197 ± 0.0001	0.0233 ± 0.0017	0.0114 ± 0.0006	0.075 ± 0.0035
hm^{5}C		0.0042 ± 0.0048					m^{6}A	0.0579 ± 0.003	0.0841 ± 0.0005	0.0655 ± 0.0002	0.0775 ± 0.0057	0.038 ± 0.002	0.0889 ± 0.0042
ac^{4}C	0.692 ± 0.0508	0.483 ± 0.0047	0.475 ± 0.0039		0.66 ± 0.0331	0.452 ± 0.0085	m^{8}A	0.0067 ± 0.0003	0.0098 ± 0.0001	0.0076 ± 0.0001	0.0090 ± 0.0006	0.0044 ± 0.0109	0.0612 ± 0.0029
ac^{4}Cm	0.0137 ± 0.002	0.0065 ± 0.0057	0.0132 ± 0.0005	0.0117 ± 0.0008	0.0048 ± 0.0014		m^{1}Am	0.0096 ± 0.0044	0.0055 ± 0.001		0.0088 ± 0.0001	0.0040 ± 0.0152	0.0022 ± 0.0006
Y	2.5851 ± 0.0578	1.7535 ± 0.0689	1.7009 ± 0.0104	2.0926 ± 0.0053	1.6964 ± 0.0413	1.931 ± 0.0442	m^{6}Am	0.0096 ± 0.0044	0.0055 ± 0.001		0.0088 ± 0.0001	0.0040 ± 0.0152	0.0022 ± 0.0006
D	0.333 ± 0.066	0.258 ± 0.0013	1.32 ± 0.0017	0.298 ± 0.0013	0.334 ± 0.0971	0.429 ± 0.0971	m$^2_8$A	0.0087 ± 0.004	0.0050 ± 0.0009		0.008 ± 0.0001	0.0037 ± 0.0139	
Um	0.158 ± 0.0023	0.094 ± 0.0014	0.241 ± 0.0003	0.0926 ± 0.0072	0.148 ± 0.0258	0.121 ± 0.0085	m$^6_2$A	0.0087 ± 0.004	0.0050 ± 0.0009		0.008 ± 0.0001	0.0037 ± 0.0139	0.0146 ± 0.004
m^{3}U	0.0902 ± 0.0013	0.0318 ± 0.0004	0.209 ± 0.0002	0.0308 ± 0.0024	0.0793 ± 0.0138	0.0597 ± 0.0042	m$^6_6$A	0.0087 ± 0.004	0.0050 ± 0.0009		0.008 ± 0.0001	0.0037 ± 0.0139	
m^{5}U	0.0902 ± 0.0013	0.0318 ± 0.0004	0.209 ± 0.0002	0.0308 ± 0.0024	0.0793 ± 0.0138	0.0597 ± 0.0042	hn^{6}A						0.0082 ± 0.0001
Ym	0.245 ± 0.0036	0.145 ± 0.0022	0.373 ± 0.0004	0.143 ± 0.0111	0.23 ± 0.0401	0.121 ± 0.0085	i^{6}A	0.0008 ± 0.0005					
m^{1}Y	0.0317 ± 0.0004	0.0112 ± 0.0001	0.0734 ± 0.0001	0.0108 ± 0.0008	0.0279 ± 0.0048	0.0565 ± 0.0039	I	0.017 ± 0.0024	0.0248 ± 0.0017	0.0685 ± 0.0002	0.0349 ± 0.0004	0.0111 ± 0.0015	0.0346 ± 0.0068
m^{3}Y	0.0317 ± 0.0004	0.0112 ± 0.0001	0.0734 ± 0.0001	0.0108 ± 0.0008	0.0279 ± 0.0048	0.0565 ± 0.0039	Im						0.0273 ± 0.0001
m^{5}D		0.0183 ± 0.0155					m^{1}I	0.0014 ± 0.0508	0.128 ± 0.0109				0.0205 ± 0.0001
m^{3}Um	0.0071 ± 0.002	0.0182 ± 0.0082	0.0419 ± 0.0002		0.0139 ± 0.0488		Gm	0.0193 ± 0.0009	0.0152 ± 0.0003	0.0228 ± 0.0002	0.0156 ± 0.0023	0.0111 ± 0.0031	0.29 ± 0.0272
m^{5}Um	0.0071 ± 0.002	0.0182 ± 0.0082	0.0419 ± 0.0002		0.0139 ± 0.0488		m^{1}G	0.138 ± 0.0066	0.109 ± 0.0021	0.164 ± 0.0018	0.112 ± 0.017	0.0798 ± 0.023	0.211 ± 0.0198
nm^{5}U		0.0042 ± 0.0013					m^{2}G	0.138 ± 0.0066	0.109 ± 0.0021	0.164 ± 0.0018	0.112 ± 0.017	0.0798 ± 0.023	0.33 ± 0.031
f^{5}U				0.0034 ± 0.0001		0.0263 ± 0.0001	m^{7}G	0.138 ± 0.0066	0.109 ± 0.0021	0.164 ± 0.0018	0.112 ± 0.017	0.0798 ± 0.023	0.0022 ± 0.0002
ho^{5}U	0.0048 ± 0.0101	0.153 ± 0.0155		0.269 ± 0.0106			m^{1}Gm	0.0040 ± 0.0002		0.0352 ± 0.0003	0.0341 ± 0.0008	0.0028 ± 0.0003	0.0070 ± 0.0022
ncm^{5}U	0.0459 ± 0.0093	0.0326 ± 0.006	0.0067 ± 0.0005	0.0065 ± 0.0013	0.0302 ± 0.0027	0.0166 ± 0.0044	m^{2}Gm	0.0208 ± 0.0013	0.0154 ± 0.0001	0.0352 ± 0.0003	0.0341 ± 0.0008	0.0028 ± 0.0003	0.0070 ± 0.0022
ncm^{5}Um	0.0025 ± 0.0057						m$^2_2$G	0.0208 ± 0.0013	0.0029 ± 0.0001	0.0067 ± 0.0001	0.0065 ± 0.0001	0.0148 ± 0.0017	0.0122 ± 0.0038
mcm^{5}U	0.0085 ± 0.0028						m$^2_7$G	0.0208 ± 0.0013	0.0154 ± 0.0001		0.0089 ± 0.0002	0.0148 ± 0.0017	0.0631 ± 0.0199
cmo^{5}U	0.0018 ± 0.0028	0.0037 ± 0.0014					preQ1	0.0208 ± 0.0013	0.0029 ± 0.0001	0.0352 ± 0.0003	0.0341 ± 0.0008	0.0148 ± 0.0017	0.0044 ± 0.0013
chm^{5}U	0.0018 ± 0.0015	0.0037 ± 0.003					m$^2_2$Gm	0.0055 ± 0.0007					

0 0.5% AvP 1% >5%

Table S6. PTM profiles obtained from total RNA extracts of mock-infected Huh7, HeLa, and 293T cells, as described in ***Materials and Methods***. Full PTM names are listed in **Table S3**. The abundance of each PTM relative to the combined abundances of the canonical ribonucleotides is calculated as described in ***Materials and Method***. Any color change along the gradient carries a p < 0.05 statistical significance.

PTM	**Huh7**	**HeLa**	**293T**	PTM	**Huh7**	**HeLa**	**293T**
No. PTMs	**47**	**31**	**37**	mchm^{5}U	0.0146 ± 0.0118		
Cm	0.0899 ± 0.0124	0.271 ± 0.0004	0.0841 ± 0.0171	Am	0.304 ± 0.016	0.344 ± 0.0012	0.2 ± 0.0109
m^{3}C	0.0995 ± 0.0137	0.3 ± 0.0005	0.093 ± 0.0189	m^{1}A	0.0176 ± 0.0009	0.0197 ± 0.0001	0.0114 ± 0.0006
m^{4}C	0.154 ± 0.0213	0.463 ± 0.0008	0.144 ± 0.0293	m^{2}A	0.0176 ± 0.0009	0.0197 ± 0.0001	0.0114 ± 0.0006
m^{5}C	0.145 ± 0.02	0.436 ± 0.0007	0.135 ± 0.0274	m^{6}A	0.0579 ± 0.003	0.0655 ± 0.0002	0.038 ± 0.002
ac^{4}C	0.692 ± 0.0508	0.475 ± 0.0039	0.66 ± 0.0331	m^{8}A	0.0067 ± 0.0003	0.0076 ± 0.0001	0.0044 ± 0.0109
ac^{4}Cm	0.0137 ± 0.002	0.0132 ± 0.0005	0.00475 ± 0.0014	m^{1}Am	0.0096 ± 0.0044		0.0040 ± 0.0152
Y	2.5851 ± 0.0578	1.7009 ± 0.0104	1.6964 ± 0.0413	m^{6}Am	0.0096 ± 0.0044		0.0040 ± 0.0152
D	0.333 ± 0.066	1.32 ± 0.0017	0.334 ± 0.0971	m$^2{}_8$A	0.0087 ± 0.004		0.0037 ± 0.0139
Um	0.158 ± 0.0023	0.241 ± 0.0003	0.148 ± 0.0258	m$^6{}_2$A	0.0087 ± 0.004		0.0037 ± 0.0139
m^{3}U	0.0902 ± 0.0013	0.209 ± 0.0002	0.0793 ± 0.0138	m$^6{}_6$A	0.0087 ± 0.004		0.0037 ± 0.0139
m^{5}U	0.0902 ± 0.0013	0.209 ± 0.0002	0.0793 ± 0.0138	i^{6}A	0.00078 ± 0.005		
Ym	0.245 ± 0.0036	0.373 ± 0.0004	0.23 ± 0.0401	I	0.017 ± 0.0024	0.0685 ± 0.0002	0.0111 ± 0.0015
m^{1}Y	0.0317 ± 0.0004	0.0734 ± 0.0001	0.0279 ± 0.0048	m^{1}I	0.0014 ± 0.0508		
m^{3}Y	0.0317 ± 0.0004	0.0734 ± 0.0001	0.0279 ± 0.0048	Gm	0.0193 ± 0.0009	0.0228 ± 0.0002	0.0111 ± 0.0031
m^{3}Um	0.0071 ± 0.002	0.0419 ± 0.0002	0.0139 ± 0.0488	m$_1$G	0.138 ± 0.0066	0.164 ± 0.0018	0.0798 ± 0.023
m^{5}Um	0.0071 ± 0.002	0.0419 ± 0.0002	0.0139 ± 0.0488	m$_2$G	0.138 ± 0.0066	0.164 ± 0.0018	0.0798 ± 0.023
ho^{5}U	0.0048 ± 0.0101			m$_7$G	0.138 ± 0.0066	0.164 ± 0.0018	0.0798 ± 0.023
ncm^{5}U	0.0459 ± 0.0093	0.0067 ± 0.0005	0.0302 ± 0.0027	m^{1}Gm	0.0040 ± 0.0002	0.0352 ± 0.0003	0.0028 ± 0.0003
ncm^{5}Um	0.0025 ± 0.0057			m^{2}Gm	0.0208 ± 0.0013	0.0352 ± 0.0003	0.0028 ± 0.0003
mcm^{5}U	0.0085 ± 0.0028			m$^2{}_2$G	0.0208 ± 0.0013	0.0067 ± 0.0001	0.0148 ± 0.0017
cmo^{5}U	0.0018 ± 0.0028			m$^2{}_7$G	0.0208 ± 0.0013		0.0148 ± 0.0017
chm^{5}U	0.0018 ± 0.0015			preQ1	0.0208 ± 0.0013	0.0352 ± 0.0003	0.0148 ± 0.0017
mcmo^{5}U	0.0146 ± 0.0118			m$^2{}_2$Gm	0.0055 ± 0.0007		

0 0.5% AvP 1% >5%

Table S7. PTM profiles obtained from total RNA extracts of mock-infected Huh7, HeLa, and 293T cells, as described in ***Materials and Methods***. Full PTM names are listed in **Table S3**. The abundance of each PTM relative to the combined abundances of the canonical ribonucleotides is calculated as described in ***Materials and Method***. Any color change along the gradient carries a $p < 0.05$ statistical significance.

	Intracellular RNA		Virion RNA			Intracellular RNA		Virion RNA	
PTM	Mock	HIV-1	Mock	HIV-1	PTM	Mock	HIV-1	Mock	HIV-1
No. PTMs	0	36	0	33	m^{1}A		0.0223 ± 0.0001		0.024 ± 0.0001
Cm		0.268 ± 0.0003		0.0416 ± 0.0001	m^{2}A		0.0223 ± 0.0001		0.024 ± 0.0001
m^{3}C		0.297 ± 0.0004		0.0461 ± 0.0001	m^{6}A		0.074 ± 0.0001		0.0797 ± 0.0001
m^{4}C		0.459 ± 0.0006		0.0712 ± 0.0002	m^{8}A		0.0086 ± 0.0001		0.0093 ± 0.0001
m^{5}C		0.432 ± 0.0005		0.0671 ± 0.0002	m^{1}Am		0.0108 ± 0.0001		
ac^{4}C		0.563 ± 0.0026		0.487 ± 0.0094	m$_6$Am		0.0108 ± 0.0001		
ac^{4}Cm		0.0176 ± 0.0003			m$^2_8$A		0.0098 ± 0.0001		
Y		2.1613 ± 0.3708		1.7989 ± 0.0204	m$^6_2$A		0.0098 ± 0.0001		
D		2.52 ± 0.001		0.115 ± 0.0001	m$^6_6$A		0.0098 ± 0.0001		
Um		0.326 ± 0.0004		0.0315 ± 0.0221	I		0.152 ± 0.0002		0.0253 ± 0.0007
m^{3}U		0.383 ± 0.0005		0.0036 ± 0.0025	m^{1}I		0.0266 ± 0.0004		0.284 ± 0.0116
m^{5}U		0.383 ± 0.0005		0.0036 ± 0.0025	Gm		0.0278 ± 0.0003		0.0002 ± 0.0001
Ym		0.504 ± 0.0007		0.0487 ± 0.0342	m^{1}G		0.199 ± 0.0021		0.0013 ± 0.0004
m^{1}Y		0.135 ± 0.0001		0.0013 ± 0.0008	m^{2}G		0.199 ± 0.0021		0.0013 ± 0.0004
m^{3}Y		0.135 ± 0.0001		0.0013 ± 0.0008	m^{7}G		0.199 ± 0.0021		0.0013 ± 0.0004
m^{3}Um				0.0068 ± 0.0001	m^{1}Gm		0.0251 ± 0.0001		0.0009 ± 0.0001
m^{5}Um				0.0068 ± 0.0001	m^{2}Gm		0.0251 ± 0.0001		0.0009 ± 0.0001
f^{5}Um				0.0038 ± 0.0012	m$^2_2$G		0.132 ± 0.0003		0.0047 ± 0.0001
ho^{5}U				0.0822 ± 0.0427	m$^2_7$G		0.132 ± 0.0003		0.0047 ± 0.0001
ncm^{5}U		0.0451 ± 0.0002			preQ1		0.132 ± 0.0003		0.0047 ± 0.0001
Am		0.389 ± 0.0007		0.419 ± 0.0003	0 0.5% AvP 1% >5%				

Table S8. PTM profiles of isolated HIV-1 RNA from 293T cell lysates and virions, as described in ***Materials and Methods***. Full PTM names are listed in **Table S3**. The abundance of each PTM relative to the combined abundances of the canonical ribonucleotides is calculated as described in ***Materials and Methods***. Any color change along the gradient carries a $p < 0.05$ statistical significance.

PTM	Mock	ZIKV	DENV	HCV	PV	PTM	Mock	ZIKV	DENV	HCV	PV
Totals	0	32	39	42	41	mo^{5}U				0.0029 ±0.0003	0.0025 ±0.0003
Cm		0.0859 ±0.0212	0.103 ±0.0009	0.126 ±0.0004	0.113 ±0.011	Am		0.325 ±0.1043	0.376 ±0.0032	0.56 ±0.0039	0.663 ±0.0813
m^{3}C		0.095 ±0.0234	0.114 ±0.001	0.14 ±0.0004	0.125 ±0.0121	m^{1}A		0.0187 ±0.006	0.0217 ±0.0001	0.0323 ±0.0002	0.0383 ±0.0046
m^{4}C		0.147 ±0.0363	0.176 ±0.0016	0.216 ±0.0007	0.192 ±0.0187	m^{2}A		0.0187 ±0.006	0.0217 ±0.0001	0.0323 ±0.0002	0.0383 ±0.0046
m^{5}C		0.138 ±0.0341	0.165 ±0.0015	0.203 ±0.0006	0.181 ±0.0176	m^{6}A		0.0619 ±0.0198	0.0716 ±0.0006	0.107 ±0.0007	0.126 ±0.0154
m$^{4}_{4}$C				0.0001 ±0.0001		m^{8}A		0.0072 ±0.0023	0.0083 ±0.0001	0.0124 ±0.0001	0.0147 ±0.0018
m^{5}Cm				0.0005 ±0.0001		m^{1}Am		0.0033 ±0.0011	0.0063 ±0.0001	0.0098 ±0.0002	0.0089 ±0.0111
ac^{4}C		0.532 ±0.1809	0.612 ±0.0051	0.514 ±0.0057	0.446 ±0.0513	m^{6}Am		0.0033 ±0.0011	0.0063 ±0.0001	0.0098 ±0.0002	0.0089 ±0.0111
ac^{4}Cm			0.0026 ±0.0002		0.0045 ±0.0056	m$^{2}_{8}$A		0.003 ±0.001	0.0057 ±0.0001	0.0089 ±0.0002	0.0081 ±0.0101
Y		1.4314 ± 0.0334	1.9815 ± 0.0253	2.0089 ± 0.0195	1.9706 ± 0.0244	m$^{6}_{2}$A		0.003 ±0.001	0.0057 ±0.0001	0.0089 ±0.0002	0.0081 ±0.0101
D		0.0194 ±0.0076	0.115 ±0.0011	0.167 ±0.0003	0.0701 ±0.0832	m$^{6}_{6}$A		0.003 ±0.001	0.0057 ±0.0001	0.0089 ±0.0002	0.0081 ±0.0101
Um		0.0765 ±0.0302	0.0908 ±0.0013	0.109 ±0.0007	0.113 ±0.0143	I		0.001 ±0.0004	0.0076 ±0.0001	0.0095 ±0.0007	0.0066 ±0.0078
m^{3}U		0.0211 ±0.0083	0.0297 ±0.0004	0.0428 ±0.0002	0.0456 ±0.0058	m^{1}I		0.0741 ±0.0313	0.115 ±0.0012	0.0537 ±0.0022	0.0344 ±0.0309
m^{5}U		0.0211 ±0.0083	0.0297 ±0.0004	0.0428 ±0.0002	0.0456 ±0.0058	Gm		0.0081 ±0.0054	0.0126 ±0.0002	0.0124 ±0.0002	0.0134 ±0.0036
Ym		0.118 ±0.0466	0.14 ±0.002	0.169 ±0.0011	0.174 ±0.0221	m^{1}G		0.058 ±0.0392	0.0906 ±0.0021	0.0889 ±0.002	0.096 ±0.0262
m^{1}Y		0.0074 ±0.0029	0.0104 ±0.0001	0.015 ±0.0001	0.016 ±0.002	m^{2}G		0.058 ±0.0392	0.0906 ±0.0021	0.0889 ±0.002	0.096 ±0.0262
m^{3}Y		0.0074 ±0.0029	0.0104 ±0.0001	0.015 ±0.0001	0.016 ±0.002	m^{7}G		0.058 ±0.0392	0.0906 ±0.0021	0.0889 ±0.002	0.096 ±0.0262
m^{5}D		0.0013 ±0.0009		0.0003 ±0.0001		m^{1}Gm			0.002 ±0.0001	0.0023 ±0.0001	0.001 ±0.0012
m^{3}Um			0.0051 ±0.0001	0.01 ±0.0001	0.0017 ±0.0005	m^{2}Gm			0.002 ±0.0001	0.0023 ±0.0001	0.001 ±0.0012
m^{5}Um			0.0051 ±0.0001	0.01 ±0.0001	0.0017 ±0.0005	m$^{2}_{2}$G			0.0103 ±0.0002	0.0122 ±0.0001	0.005 ±0.0061
f^{5}U				0.005 ±0.0002		m$^{2}_{7}$G			0.0103 ±0.0002	0.0122 ±0.0001	0.005 ±0.0061
ho^{5}U		0.0094 ±0.0097			0.0278 ±0.0269	preQ1			0.0103 ±0.0002	0.0122 ±0.0001	0.005 ±0.0061
ncm^{5}U			0.0031 ±0.0001		0.002 ±0.0016	m$^{2}_{2}$Gm		0.0066 ±0.0051	0.001 ±0.0001		0.0183 ±0.0022
ncm^{5}Um				0.0055 ±0.0001		0 0.5% AvP 1% >5%					

Table S9A. PTM profiles of viral RNA isolated from cell lysates of mock-, ZIKV-, DENV-, HCV-, and PV-infected Huh7 cells, as described in ***Materials and Methods***. Full PTM names are listed in **Table S3**. The abundance of each PTM relative to the combined abundances of the canonical ribonucleotides is calculated as described in ***Materials and Methods***. Any color change along the gradient carries a $p < 0.05$ statistical significance.

PTM	**Mock**	**ZIKV**	**DENV**	**HCV**	**PV**	PTM	**Mock**	**ZIKV**	**DENV**	**HCV**	**PV**
Totals	**0**	**41**	**37**	**40**	**40**	nm^{5}U				0.0057 ± 0.0001	
Cm		0.111 ± 0.0004	0.183 ± 0.017	0.167 ± 0.0085	0.113 ± 0.0002	ncm^{5}U		0.0077 ± 0.0018	0.0127 ± 0.0036		0.0037 ± 0.0002
m^{3}C		0.122 ± 0.0004	0.203 ± 0.0189	0.185 ± 0.0095	0.125 ± 0.0002	ncm^{5}Um				0.0128 ± 0.0015	
m^{4}C		0.189 ± 0.0007	0.313 ± 0.0291	0.286 ± 0.0147	0.192 ± 0.0004	mcm^{5}U			0.0115 ± 0.0118		0.0074 ± 0.0003
m^{5}C		0.178 ± 0.0007	0.295 ± 0.0275	0.27 ± 0.0138	0.181 ± 0.0003	Am		0.433 ± 0.0053	0.521 ± 0.0765	0.33 ± 0.0208	0.461 ± 0.002
m$^{4}_{4}$C		0.0003 ± 0.0001		0.0014 ± 0.0001		m^{1}A		0.025 ± 0.0003	0.03 ± 0.0044	0.019 ± 0.0012	0.0266 ± 0.0001
m^{5}Cm		0.0016 ± 0.0001		0.0081 ± 0.0001		m^{2}A		0.025 ± 0.0003	0.03 ± 0.0044	0.019 ± 0.0012	0.0266 ± 0.0001
m$^{4}_{4}$Cm				0.07 ± 0.0194		m^{6}A		0.0824 ± 0.001	0.0992 ± 0.0145	0.0627 ± 0.0039	0.0878 ± 0.0003
ac^{4}C		0.532 ± 0.0073	0.46 ± 0.0998	0.49 ± 0.0184	0.441 ± 0.0022	m^{8}A		0.0096 ± 0.0001	0.0115 ± 0.0016	0.0073 ± 0.0004	0.0102 ± 0.0001
ac^{4}Cm		0.001 ± 0.0001			0.0011 ± 0.0001	m^{1}Am		0.0065 ± 0.0001	0.0039 ± 0.0017		0.0081 ± 0.0001
f^{5}C				0.0107 ± 0.0034	0.004 ± 0.0001	m^{6}Am		0.0065 ± 0.0001	0.0039 ± 0.0017		0.0081 ± 0.0001
f^{5}Cm				0.07 ± 0.0184		m$^{2}_{8}$A		0.0059 ± 0.0001	0.0036 ± 0.0015		0.0074 ± 0.0001
hm^{5}C				0.0057 ± 0.0052		m$^{6}_{2}$A		0.0059 ± 0.0001	0.0036 ± 0.0015		0.0074 ± 0.0001
Y		1.38 ± 0.0284	1.6645 ± 0.3268	1.7295 ± 0.1907	1.3306 ± 0.0065	m$^{6}_{6}$A		0.0059 ± 0.0001	0.0036 ± 0.0015		0.0074 ± 0.0001
D		0.132 ± 0.0049	0.371 ± 0.023	0.502 ± 0.0361	0.147 ± 0.0005	I		0.0121 ± 0.0001	0.0438 ± 0.0039	0.037 ± 0.0059	0.0131 ± 0.0007
Um		0.0925 ± 0.0143	0.127 ± 0.0361	0.119 ± 0.0169	0.0836 ± 0.0002	m^{1}I					0.0269 ± 0.0009
m^{3}U		0.0308 ± 0.0047	0.0582 ± 0.0165	0.0509 ± 0.0072	0.0252 ± 0.0001	Gm		0.0114 ± 0.0003	0.0153 ± 0.0051	0.0148 ± 0.0022	0.0109 ± 0.0002
m^{5}U		0.0308 ± 0.0047	0.0582 ± 0.0165	0.0509 ± 0.0072	0.0252 ± 0.0001	m^{1}G		0.082 ± 0.0028	0.11 ± 0.0373	0.106 ± 0.0161	0.078 ± 0.0015
Ym		0.143 ± 0.0222	0.197 ± 0.0561	0.184 ± 0.0262	0.129 ± 0.0003	m^{2}G		0.082 ± 0.0028	0.11 ± 0.0373	0.106 ± 0.0161	0.078 ± 0.0015
m^{1}Y		0.0108 ± 0.0016	0.0205 ± 0.0058	0.0179 ± 0.0025	0.0088 ± 0.0001	m^{7}G		0.082 ± 0.0028	0.11 ± 0.0373	0.106 ± 0.0161	0.078 ± 0.0015
m^{3}Y		0.0108 ± 0.0016	0.0205 ± 0.0058	0.0179 ± 0.0025	0.0088 ± 0.0001	m^{1}Gm		0.002 ± 0.0001	0.0048 ± 0.0006	0.0054 ± 0.0004	0.0019 ± 0.0001
m^{5}D		0.001 ± 0.0005		0.0728 ± 0.0064	0.001 ± 0.0002	m^{2}Gm		0.002 ± 0.0001	0.0048 ± 0.0006	0.0054 ± 0.0004	0.0019 ± 0.0001
m^{3}Um			0.0162 ± 0.0007	0.0081 ± 0.0005		m$^{2}_{2}$G		0.0107 ± 0.0001	0.0254 ± 0.0032	0.0286 ± 0.0025	0.0101 ± 0.0001
m^{5}Um			0.0162 ± 0.0007	0.0081 ± 0.0005		m$^{2}_{7}$G		0.0107 ± 0.0001	0.0254 ± 0.0032	0.0286 ± 0.0025	0.0101 ± 0.0001
f^{5}U		0.006 ± 0.0031				preQ1		0.0107 ± 0.0001	0.0254 ± 0.0032	0.0286 ± 0.0025	0.0101 ± 0.0001
f^{5}Um		0.0056 ± 0.0001			0.0146 ± 0.0011	m$^{2}_{2}$Gm				0.0059 ± 0.0016	
ho^{5}U		0.0236 ± 0.0259				0 0.5% AvP 1% >5%					

Table S9B. PTM profiles of viral RNA isolated from virions of mock-, ZIKV-, DENV-, HCV-, and PV-infected Huh7 cells, as described in ***Materials and Methods***. Full PTM names are listed in **Table S3**. The abundance of each PTM relative to the combined abundances of the canonical ribonucleotides is calculated as described in ***Materials and Methods***. Any color change along the gradient carries a $p < 0.05$ statistical significance.

PTM	**Huh7**	**ZIKV**	**Arsenite**	**Poly(I:C)**	PTM	**Huh7**	**ZIKV**	**Arsenite**	**Poly(I:C)**
No. PTMs	**47**	**44**	**47**	**36**	mcm^5s^2U			0.0247 ± 0.0001	
Cm	0.0899 ± 0.0124	0.0976 ± 0.0003	0.0692 ± 0.0153	0.15 ± 0.0029	$mcmo^5U$	0.0146 ± 0.0118		0.0056 ± 0.0013	
m^3C	0.0995 ± 0.0137	0.108 ± 0.0004	0.0766 ± 0.0169	0.165 ± 0.0032	$mchm^5U$	0.0146 ± 0.0118		0.0056 ± 0.0013	
m^4C	0.154 ± 0.0213	0.167 ± 0.0006	0.118 ± 0.0261	0.256 ± 0.0049	Am	0.304 ± 0.016	0.469 ± 0.0006	0.409 ± 0.0202	0.403 ± 0.1008
m^5C	0.145 ± 0.02	0.157 ± 0.0006	0.111 ± 0.0245	0.241 ± 0.0047	m^1A	0.0176 ± 0.0009	0.0271 ± 0.0001	0.0234 ± 0.0011	0.0231 ± 0.0057
m^4_4C		0.012 ± 0.0007	0.000531 ± 0.0002		m^2A	0.0176 ± 0.0009	0.0271 ± 0.0001	0.0234 ± 0.0011	0.0231 ± 0.0057
m^5Cm		0.0722 ± 0.0047	0.00319 ± 0.0013		m^6A	0.0579 ± 0.003	0.0893 ± 0.0001	0.0779 ± 0.0038	0.0768 ± 0.0192
ac^4C	0.692 ± 0.0508	0.514 ± 0.0003	0.549 ± 0.0269	0.483 ± 0.0124	m^8A	0.0067 ± 0.0003	0.0104 ± 0.0001	0.0091 ± 0.0004	0.0089 ± 0.0022
ac^4Cm	0.0137 ± 0.002	0.0025 ± 0.0023	0.00489 ± 0.0025		m^1Am	0.0096 ± 0.0044	0.0099 ± 0.0003	0.0055 ± 0.0026	0.0052 ± 0.0019
Y	2.5851 ± 0.0578	1.8937 ± 0.0338	2.1475 ± 0.0963	1.6601 ± 0.0244	m^6Am	0.0096 ± 0.0044	0.0099 ± 0.0003	0.0055 ± 0.0026	0.0052 ± 0.0019
D	0.333 ± 0.066	0.142 ± 0.0013	0.244 ± 0.0567	0.349 ± 0.0057	m^2_8A	0.0087 ± 0.004	0.0090 ± 0.0003	0.0050 ± 0.0024	0.0047 ± 0.0017
Um	0.158 ± 0.0023	0.0823 ± 0.0007	0.0452 ± 0.002	0.105 ± 0.0029	m^6_2A	0.0087 ± 0.004	0.0090 ± 0.0003	0.0050 ± 0.0024	0.0048 ± 0.0017
m^3U	0.0902 ± 0.0013	0.0244 ± 0.0002	0.00734 ± 0.0003	0.0398 ± 0.0011	m^6_6A	0.0087 ± 0.004	0.0090 ± 0.0003	0.0050 ± 0.0024	0.0048 ± 0.0017
m^5U	0.0902 ± 0.0013	0.0244 ± 0.0002	0.00734 ± 0.0003	0.0398 ± 0.0011	i^6A	0.0008 ± 0.005		0.0022 ± 0.0002	
Ym	0.245 ± 0.0036	0.127 ± 0.001	0.0698 ± 0.0031	0.163 ± 0.0045	I	0.017 ± 0.0024	0.0026 ± 0.0023	0.0893 ± 0.0061	0.745 ± 0.0104
m^1Y	0.0317 ± 0.0004	0.0086 ± 0.0001	0.00258 ± 0.0001	0.014 ± 0.0003	m^1I	0.0014 ± 0.0508	0.163 ± 0.0003		0.0938 ± 0.0012
m^3Y	0.0317 ± 0.0004	0.0086 ± 0.0001	0.00258 ± 0.0001	0.014 ± 0.0003	Gm	0.0193 ± 0.0009	0.0121 ± 0.0002	0.0139 ± 0.0005	0.0154 ± 0.0019
m^3Um	0.0071 ± 0.002	0.0034 ± 0.0003	0.00349 ± 0.0007	0.0128 ± 0.0036	m^1G	0.138 ± 0.0066	0.0869 ± 0.0015	0.0997 ± 0.0037	0.11 ± 0.0138
m^5Um	0.0071 ± 0.002	0.0034 ± 0.0003	0.00349 ± 0.0007	0.0128 ± 0.0036	m^2G	0.138 ± 0.0066	0.0869 ± 0.0015	0.0997 ± 0.0037	0.11 ± 0.0138
f^5Um			0.00768 ± 0.0163		m^7G	0.138 ± 0.0066	0.0869 ± 0.0015	0.0997 ± 0.0037	0.11 ± 0.0138
ho^5U	0.0048 ± 0.0101	0.0068 ± 0.0013	0.0274 ± 0.0044		m^1Gm	0.0040 ± 0.0003	0.0044 ± 0.0001	0.0023 ± 0.0008	0.0049 ± 0.0003
ncm^5U	0.0459 ± 0.0093	0.0013 ± 0.0023	0.0339 ± 0.0251		m^2Gm	0.0040 ± 0.0003	0.0044 ± 0.0001	0.0023 ± 0.0008	0.0049 ± 0.0003
ncm^5Um	0.0025 ± 0.0057				m^2_2G	0.0208 ± 0.0017	0.0233 ± 0.0006	0.0119 ± 0.0044	0.0257 ± 0.0019
mcm^5U	0.0085 ± 0.0028				m^2_7G	0.0208 ± 0.0017	0.0233 ± 0.0006	0.0119 ± 0.0044	0.0257 ± 0.0019
cmo^5U	0.0018 ± 0.0028	0.0001 ± 0.0001	0.0147 ± 0.0132		preQ1	0.0208 ± 0.0017	0.0233 ± 0.0006	0.0119 ± 0.0044	0.0257 ± 0.0019
chm^5U	0.0018 ± 0.0029	0.0001 ± 0.0001	0.0147 ± 0.0132		m^2_2Gm	0.0055 ± 0.0007	0.0083 ± 0.0012		

<u>Table S10.</u> PTM profiles obtained from total RNA extracts of mock-, ZIKV-infected, arsenite-treated, and poly(I:C)-transfected Huh7 cells, as described in ***Materials and Methods***. Full PTM names are listed in **Table S3**. The abundance of each PTM relative to the combined abundances of the canonical ribonucleotides is calculated as described in ***Materials and Methods***. Any color change along the gradient carries a $p < 0.05$ statistical significance.

N6-methyl adenosine counts per genome							
ZIKV		DENV		HCV		PV	
Lysate¥	Virion¥	Lysate¥	Virion	Lysate¥	Virion	Lysate	Virion
7.4	11.4	13.6	9.7	27.6	21.5	10.8	5.4

<u>Table S11:</u> N6-methyl adenosine (m^6A) modifications per genome of each virus. Incidence of m^6A modification was calculated by using the relative abundances of all adenosine species in the sample, which corresponded to the total number of adenosines present in the genome's sequence. The ratio between the abundance of m^6A and the total abundance of adenosine species provided the incidence of m^6A modification. ¥ indicates a sample in which the number of m^6A modifications has previously been reported (27,28).

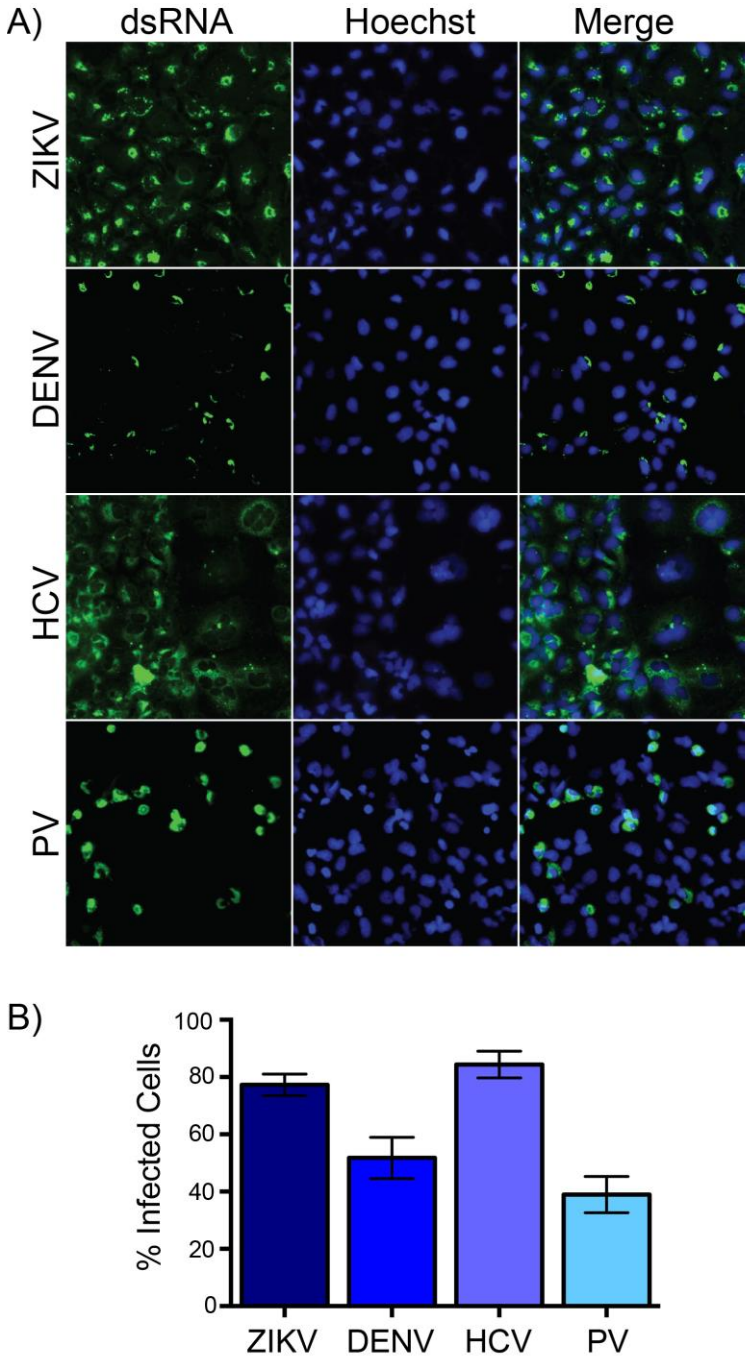


Figure S1. ZIKV, DENV, HCV and PV Infectivity Assay. Huh7 cells were infected with virus at the MOI described in the ***Materials and Methods***. At specific days post-infection the cells were fixed, permeabilized and prepared for immunofluorescence analysis. Virus-infected cells were detected with an antibody to double-stranded RNA and Alexa Fluor 488 conjugated goat anti-mouse antibody, and the nucleus of each cell was stained with Hoechst 33258. B) Quantification of infected cells. Percent infected cells are from three independent experiments, where at least 100 cells/field-of-view were counted. SD of the mean is shown.

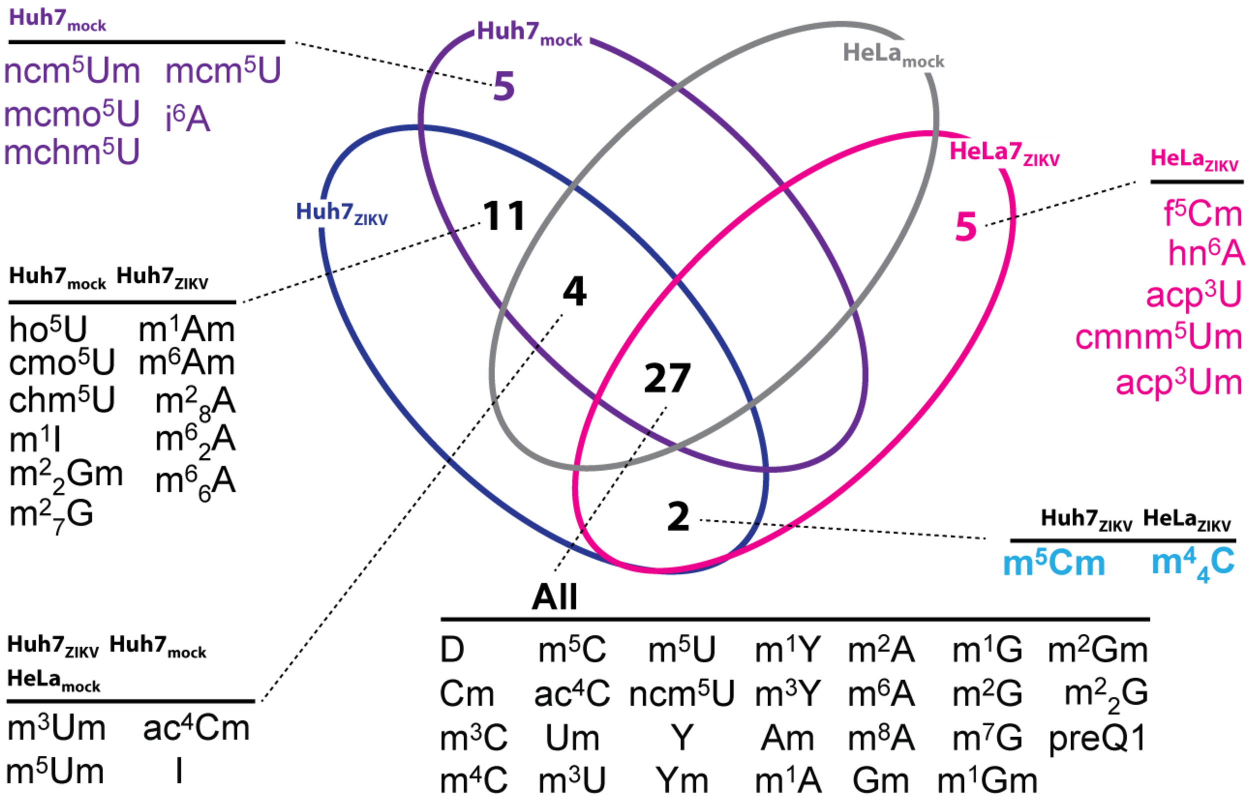


Figure S2. Venn diagram generated from the PTM profiles of total RNA extracts obtained from mock- and ZIKV-infected Huh7 and HeLa cells. These diagrams were based exclusively on the presence/absence of each PTM, while comprehensive data displaying abundance variations are reported separately in **Table S5**. Full PTM names are listed in **Table S3**.

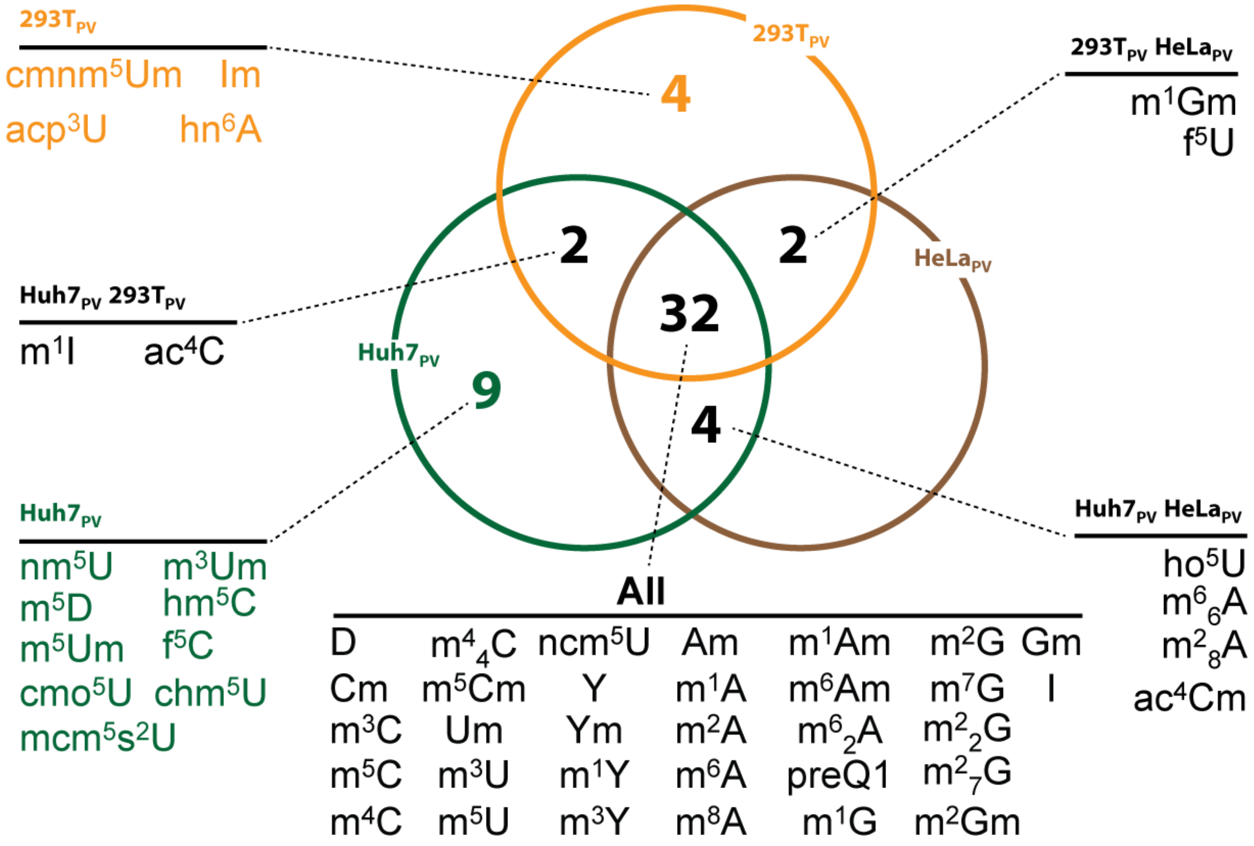


Figure S3. Venn diagram generated from the PTM profiles of total RNA extracts obtained from Huh7, HeLa, and 293T cells infected with PV. These diagrams were based exclusively on the presence/absence of each PTM, while comprehensive data displaying abundance variations are reported separately in **Table S6**. Full PTM names are listed in **Table S3**.

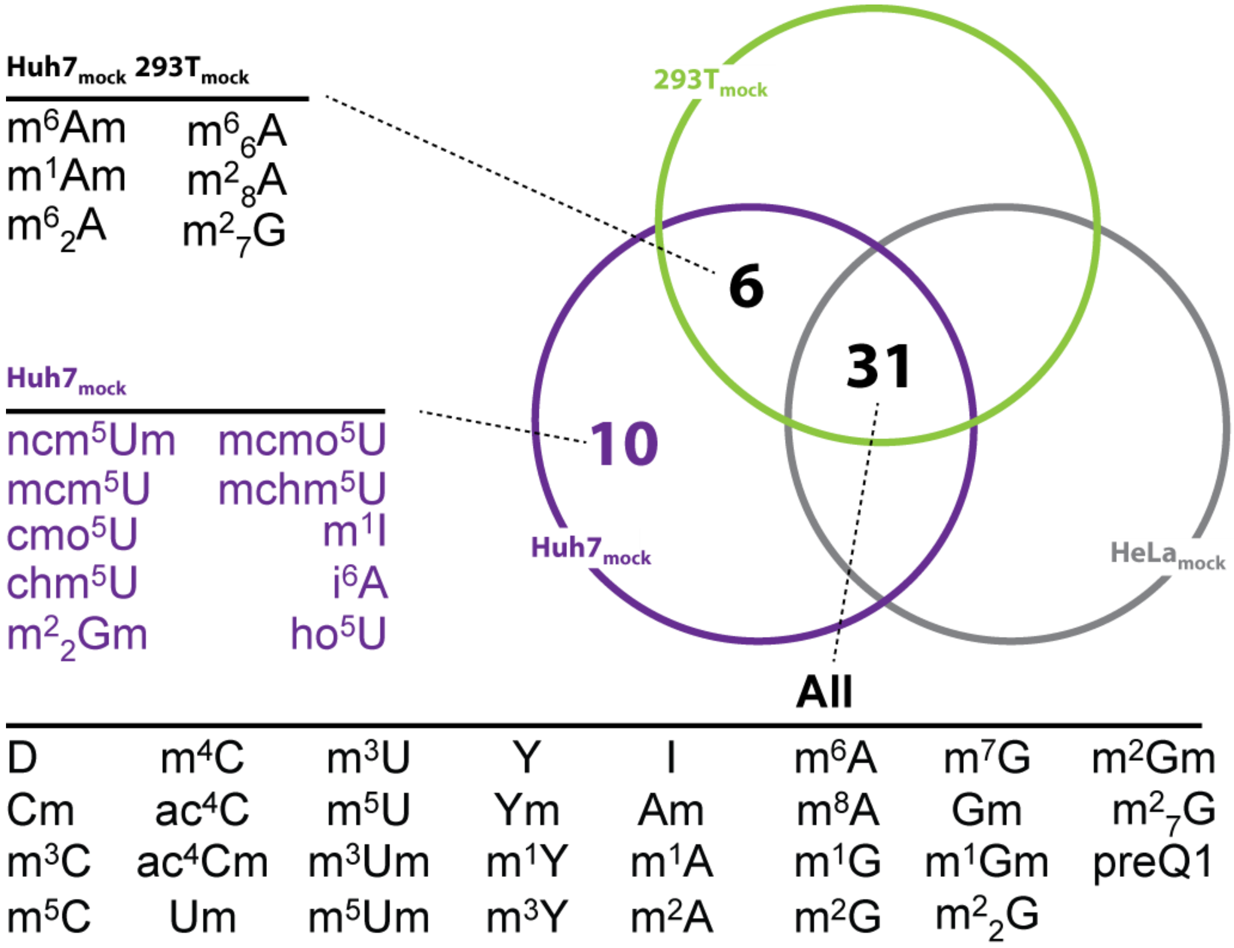


Figure S4. Venn diagram generated from the PTM profiles of total RNA extracts obtained from mock-infected Huh7, HeLa, and 293T cells. These diagrams were based exclusively on the presence/absence of each PTM, while comprehensive data displaying abundance variations are reported separately in **Table S7**. Full PTM names are listed in **Table S3**.

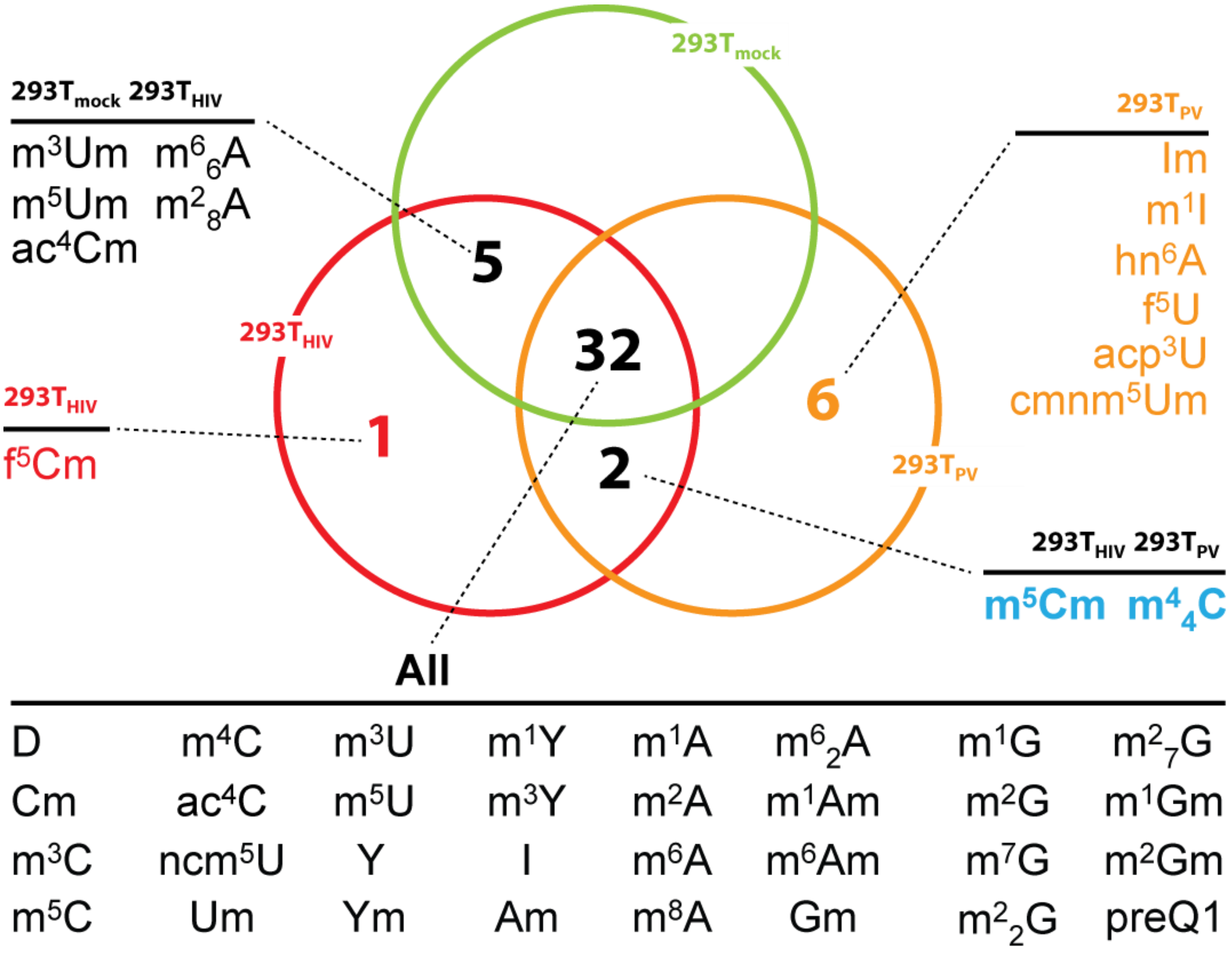


Figure S5. Venn diagram generated from the PTM profiles of total RNA extracts obtained from mock-, PV-, and HIV-infected 293T cells. These diagrams were based exclusively on the presence/absence of each PTM, while comprehensive data displaying abundance variations are reported separately in **Table S4** and **S6**. Full PTM names are listed in **Table S3**.

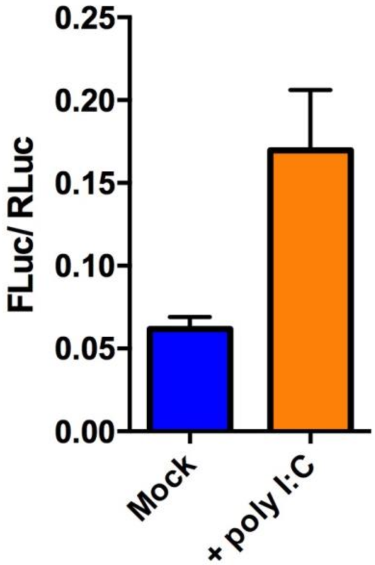
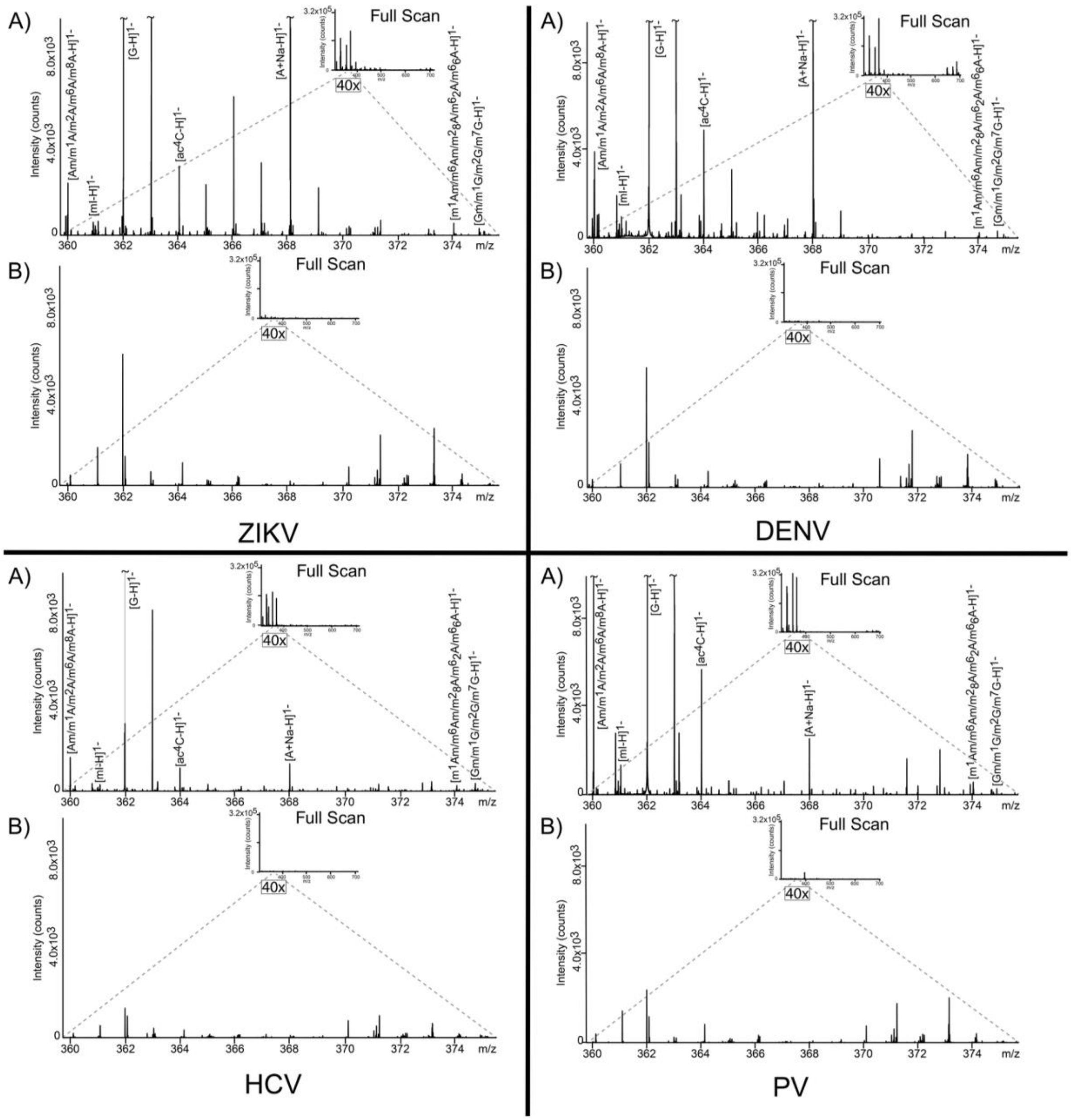


Figure S6: Representative luciferase assay measuring IFN-β promoter activity by poly(I:C). Huh7 cell were co-transfected with pIFN-β-Firefly luciferase and pTK-*Renilla* luciferase. Twenty-four hours later, cells were transfected with transfection reagent alone (mock) or with poly(I:C), and firefly and *Renilla* luciferase activity were measured 24 hours later.



<u>Figure S7.</u> ESI-MS spectra obtained from exonuclease digests of viral RNA captured from **A)** virus- and **B)** mock-infected Huh7 cells, as described in ***Material and Methods***. Signals corresponding to the four canonical bases (C, U, A, and G) and numerous PTMs are readily recognizable in panel **a)**, whereas no such species are present in panel **b)**, thus confirming the specificity of the capture process. Each quadrant shows the affinity purification using the ZIKV, DENV, HCV and PV biotinylated antisense oligonucleotides.

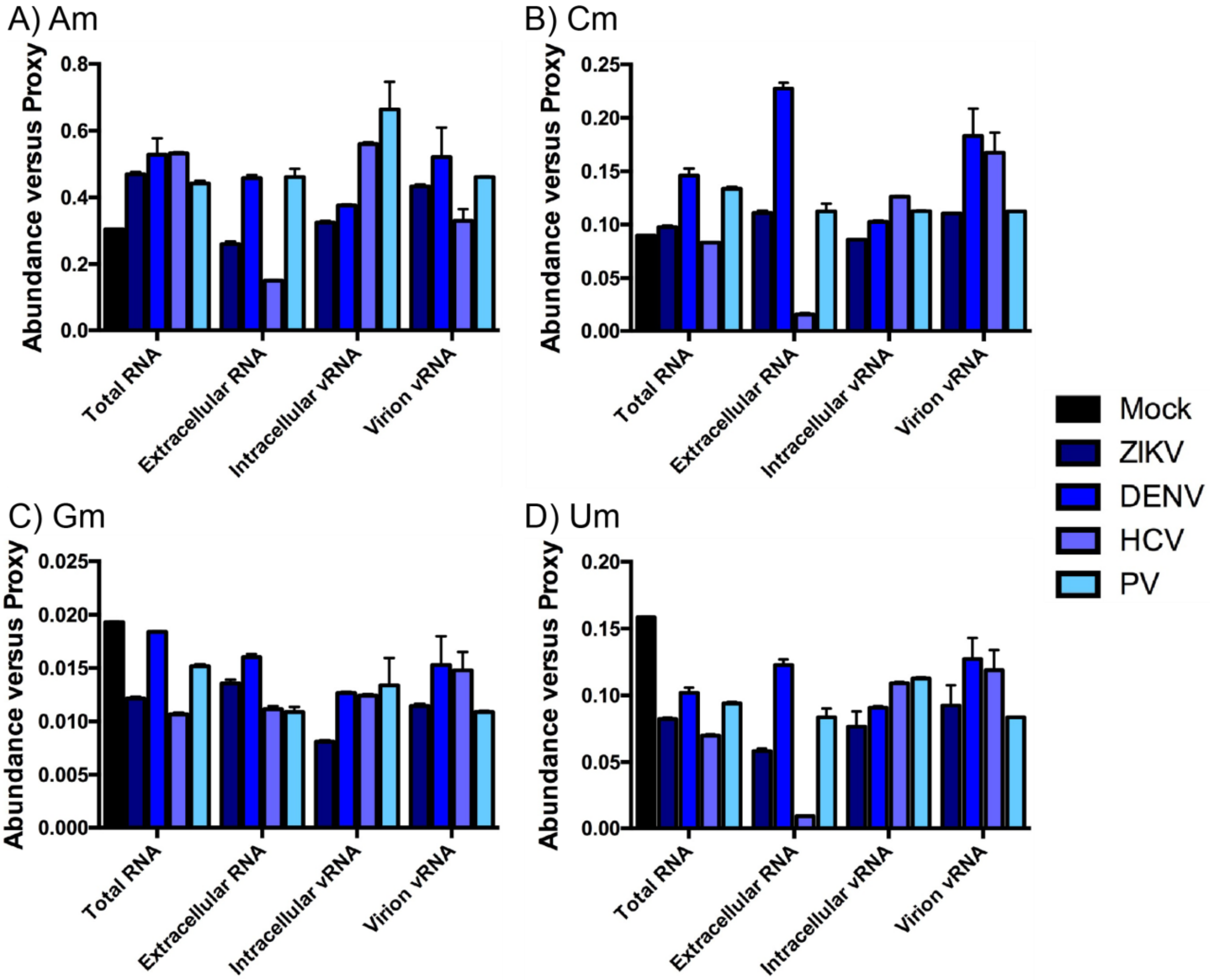


Figure S8. Variations of the relative abundance of 2’ O-methylated ribonucleotides. Relative abundancees were obtained from **A)** 2’ O-methylated adenosine (Am), **B)** 2’ O methylated cytosine (Cm), **C)** 2’ O-methylated guanosine (Gm), and **D)** 2’ O-methylated uridine (Um), as described in ***Materials and Methods***. The samples consisted of total RNA extracts from cell lysates (total RNA), media of mock- or virus-infected cells (extracellular RNA), and from viral RNA that were affinity captured from cell lysates (intracellular vRNA) or virions in the media (Virion vRNA).